Sequence-tagged sites and low-cost DNA markers for rice

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Sequence-tagged sites (STSs) facilitate the conversion of a genetic map into a physical map, provide a common basis for the comparison of diverse types of mapping data, are stored and disseminated as electronic data, and are amplified from genomic DNA by polymerase chain reaction (PCR). STSs find application as DNA markers in breeding programs and germplasm management because they offer speed, convenience, reliability, and low cost in genomic analysis, but these applications are currently limited by the small number of STSs available. We report here the terminal sequencing of 354 DNA markers of the Cornell-IRRI genetic map of rice and the conversion of 100 of them into STSs by synthesis of pairs of PCR primers. PCR was used to amplify the corresponding loci from genomic DNA of IR36 (indica), Taichung 65 (japonica), and *Oryza longistaminata* (AA genome wild species). More than half of the RZ clones amplified DNA segments that were 0.1-2.0 kbp larger than expected, presumably because of the presence of introns. Amplicon length polymorphisms were detected between *O. sativa* and *O. longistaminata* for about one quarter of the clones. The applications of STSs are illustrated by reference to 1) DNA marker-aided selection for pyramiding of bacterial blight resistance genes, 2) breeding for gall midge resistance, 3) monitoring the inheritance of transgenes, and 4) analysis of genetic variation of AA genome wild species.

Marker-aided selection can increase the efficiency of rice breeding for traits that are difficult to phenotype. Although interest in this approach has been stimulated recently by the construction of genetic maps saturated with DNA markers (Causse et al 1994, Kurata et al 1994), its adoption by breeders will depend on cost, convenience, and reliability when it is applied on the scale of typical breeding. Depending on the dissimilarity of the parental lines, F_2 populations normally range in size from 2,000 to 6,000 plants—large numbers for routine application of most molecular techniques.

Breeders may of course opt to delay DNA marker-aided selection for certain traits until the F_4-F_5 generations, when population sizes decline to several hundred plants, but, in general, DNA marker technology should be expected to cope with thousands of plants per cross and many crosses per season. Furthermore, breeders will need the results by the end of the 3-4 mo breeding cycle and perhaps even more quickly. DNA marker technology based on sequence-tagged sites (STSs) offers a way of meeting these requirements.

Sequence-tagged sites

The STS concept was introduced by Olson et al (1989). In assessing the likely impact of the polymerase chain reaction (PCR) on human genome research, they recognized that single-copy DNA sequences of known map location could serve as markers for the genetic and physical mapping of important genes along the chromosomes. They also recognized that, if the human genome were to have enough markers to be useful in such studies, enormous libraries of mapped DNA clones would have to be stored, amplified, checked, and distributed to interested researchers. Partial sequencing of these clones would, however, provide enough information for the "recovery" of any desired marker by means of PCR. Instead of storing biological material, scientists would store electronic information that could easily be augmented, sorted, and disseminated. These electronically stored markers were called sequence-tagged sites (STSs). The application of STSs in the development of physical maps from genetic maps has indeed gone ahead, not only with the human genome projects but also with other animals and with plants, including rice (Inoue et al 1994).

STS-based PCR and DNA marker technology

DNA maps were originally constructed through the use of restriction fragment length polymorphism (RFLP) analysis and DNA blotting (McCouch et al 1988). Currently, maps are constructed more quickly by random amplification of polymorphic DNA (RAPD) and amplicon fragment length polymorphism (AFLP) analysis (Kurata et al 1994, Vos et al 1995), two techniques that use PCR. PCR is also a more suitable technique than DNA blotting for marker-aided selection: it requires DNA of lower quantity and quality than DNA blots and can therefore tolerate the simplified DNA extraction protocols that are unavoidable with large sample sizes, it is highly automated, and it does not use radiochemical or complex biochemical detection systems (Zheng et al 1995a). STS-based PCR has the further advantage of producing a simple and reproducible pattern on agarose or polyacrylamide gels. The pattern is easily recorded and interpreted, and, being codominant in most cases, these markers allow heterozygotes to be distinguished from the two homozygotes. The equipment needed for STS-based DNA marker technology is relatively inexpensive and robust and requires little bench space or training (Zheng et al 1995b).

STS-based markers have two disadvantages: 1) they require suitable sequence data for each locus, and 2) they are not as polymorphic as some other types of DNA markers, such as microsatellites (Wu and Tanksley 1993). The first disadvantage is

rapidly diminishing in importance, as DNA sequences from rice and other cereals accumulate in the international data bases. In the next section, we report on our contribution to this effort.

The importance of the second problem—low frequency of polymorphism—varies with each locus and each cross. It is, at present, impossible to predict whether STS-based polymorphisms will be found between any two parental lines for any given locus, but in a later section, we look at some of the issues.

Sources of STSs suitable for DNA marker-aided selection

The first set of STSs for rice was identified by Williams et al (1991) who sequenced the ends of 30 of the mapped clones that had been used to prepare the Cornell-IRRI genetic map (McCouch et al 1988). They showed that PCR primer pairs for eight of these loci amplified genomic DNA to yield single bands of the expected sizes. An additional 63 STSs were reported by Inoue et al (1994) for clones mapped by the Rice Genome Research Project in Japan (Kurata et al 1994). In these two cases, clones were first mapped and then partially sequenced.

It is also possible to derive STSs by sequencing clones first and then mapping them. This order of events is obligatory for microsatellites and is widely followed for other types of cloned DNAs that are converted into STS, such as cDNAs, cloned genes, and cloned PCR products. The large number of expressed tagged sites (ETSs) obtained from rice cDNA clones by single-pass sequencing and mapping (Kurata et al 1994) also constitute STSs. Single-pass automated sequencing provides information (350-450 bases) from only one end of each clone. This is enough information for ETSs to be used in the development of a physical map or for similarity searches in data bases, but the PCR amplicons derived from ETSs are generally too short to reveal polymorphism between parental lines by amplicon length polymorphism (ALP) or PCR-based RFLP (Ghareyazie et al 1995). It is possible, however, to detect polymorphisms between such short amplicons through special electrophoretic procedures that detect single-stranded conformational polymorphism (Fukuoka et al 1994). Microsatellites form another class of STS marker: the ends of the clones are unique even though the satellite sequence appears in multiple locations around the genome (Wu and Tanksley 1993).

Terminal sequencing of RFLP markers from the Cornell-IRRI map

To increase the number of STS markers available for rice, we sequenced both ends of 354 of the 726 RFLP markers placed on the Cornell-IRRI genetic map by Causse et al (1994). Some of the markers (136 clones of the RZ series) were cDNA clones derived from mRNA of etiolated leaves of IR36. Other markers (183 clones of the RG series) were random *Pst*I clones of genomic DNA of IR36. Of the remaining 35 STSs, 27 were derived from oat cDNA library clones (CDO series) and 6 were from barley cDNA clones (BCD series).

The choice of about 350 loci as targets for sequencing was dictated by the need to achieve a useful density of markers at an affordable cost. This number of markers

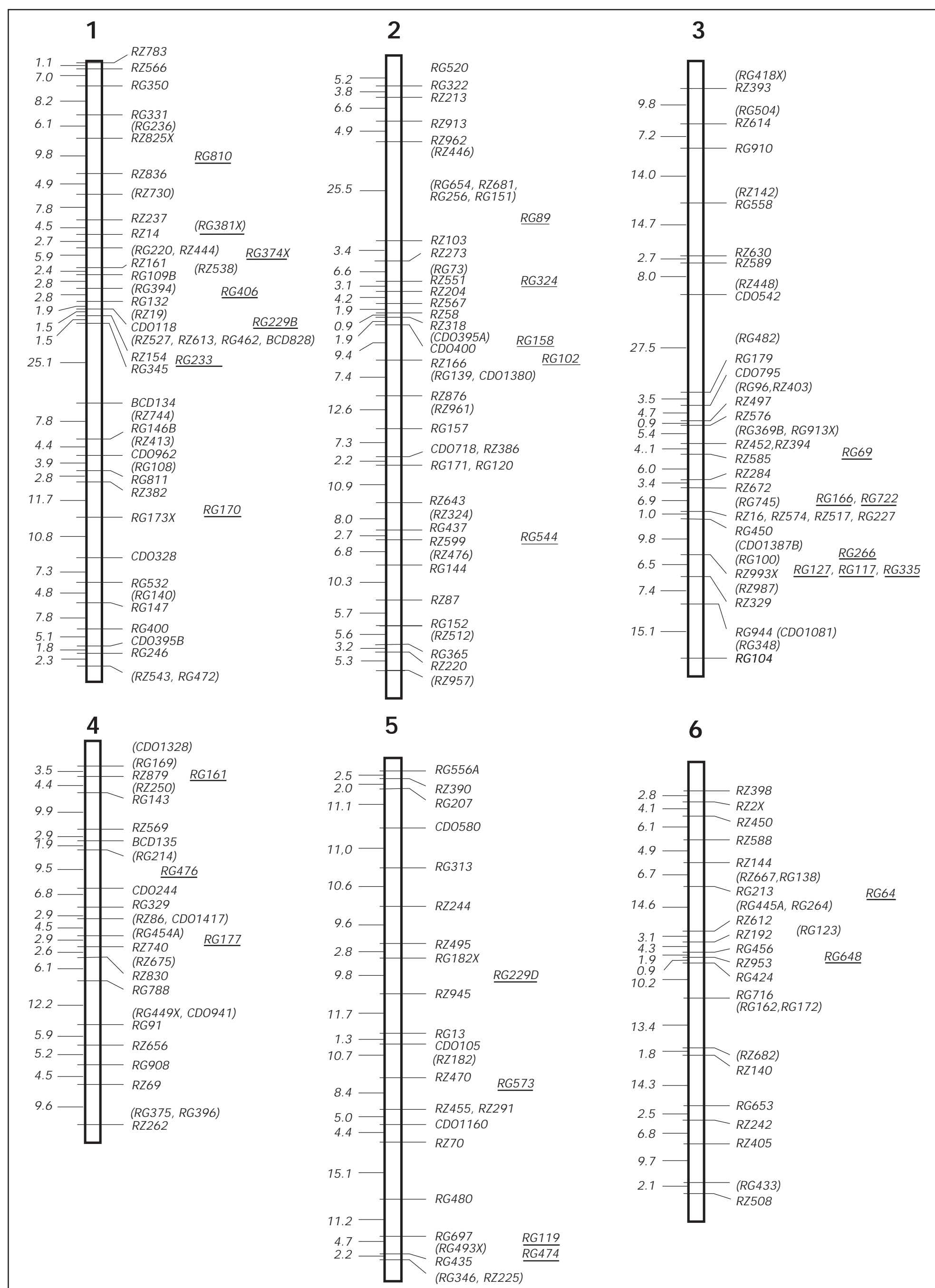
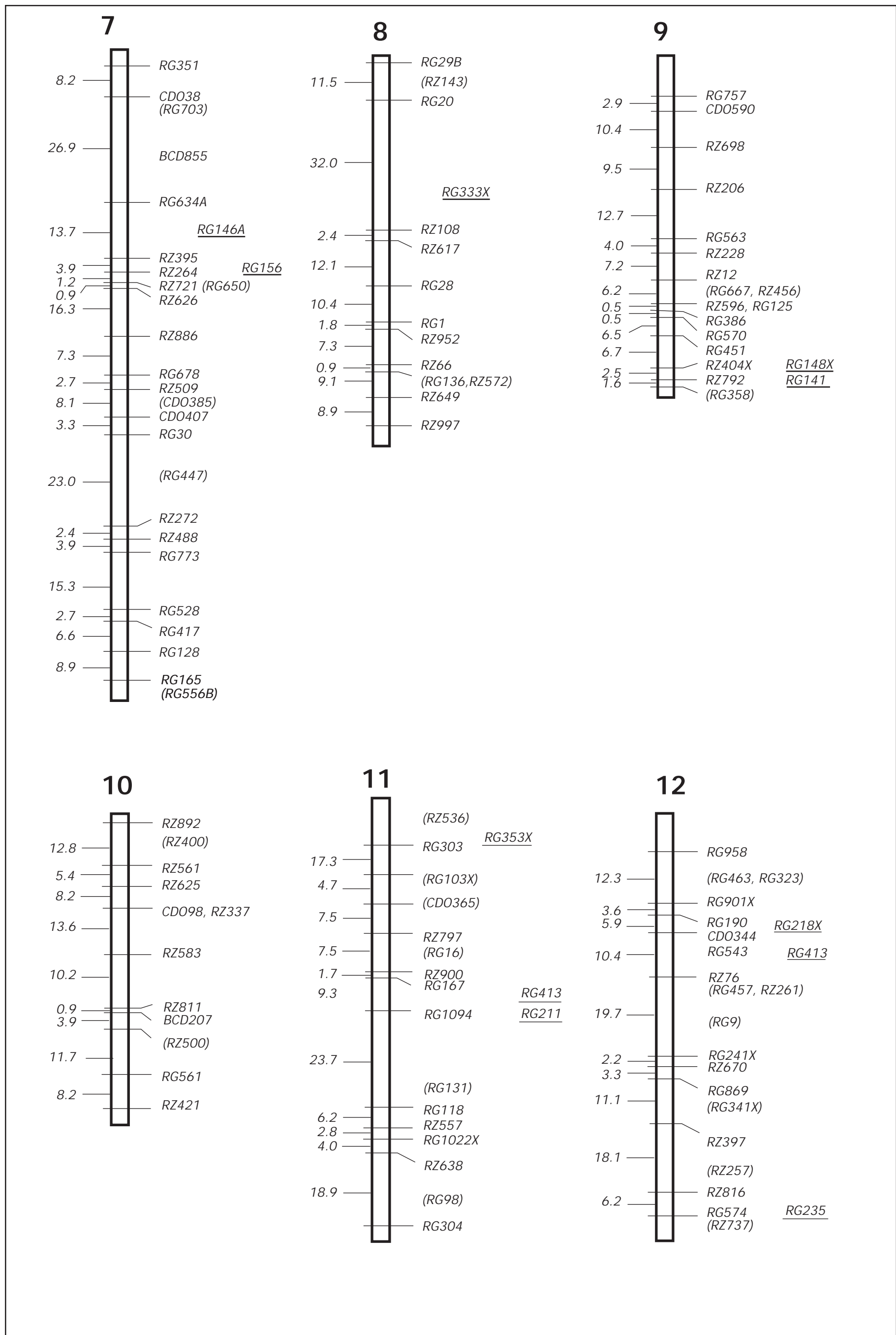


Fig. 1. Sequence-tagged sites on the Cornell-IRRI genetic map of rice. Cloned markers were mapped at Cornell University (McCouch et al 1988, Causse et al 1994) and sequenced at IRRI and Texas A&M University.



7
8.2
26.9
13.7
3.9
1.2
0.9
16.3
7.3
2.7
8.1
3.3
23.0
2.4
3.9
15.3
2.7
6.6
8.9
RG351
CDO38
(RG703)
BCD855
RG634A
RG146A
RZ395
RZ264
RG156
RZ721 (RG650)
RZ626
RZ886
RG678
RZ509
(CDO385)
CDO407
RG30
(RG447)
RZ272
RZ488
RG773
RG528
RG417
RG128
RG165
(RG556B)
8
11.5
32.0
2.4
12.1
10.4
1.8
7.3
0.9
9.1
8.9
RG29B
(RZ143)
RG20
RG333X
RZ108
RZ617
RG28
RG1
RZ952
RZ66
(RG136,RZ572)
RZ649
RZ997
9
2.9
10.4
9.5
12.7
4.0
7.2
6.2
0.5
0.5
6.5
6.7
2.5
1.6
RG757
CDO590
RZ698
RZ206
RG563
RZ228
RZ12
(RG667, RZ456)
RZ596, RG125
RG386
RG570
RG451
RZ404X
RG148X
RZ792
RG141
(RG358)
10
12.8
5.4
8.2
13.6
10.2
0.9
3.9
11.7
8.2
RZ892
(RZ400)
RZ561
RZ625
CDO98, RZ337
RZ583
RZ811
BCD207
(RZ500)
RG561
RZ421
11
17.3
4.7
7.5
7.5
1.7
9.3
23.7
6.2
2.8
4.0
18.9
(RZ536)
RG353X
RG303
(RG103X)
(CDO365)
RZ797
(RG16)
RZ900
RG167
RG413
RG1094
RG211
(RG131)
RG118
RZ557
RG1022X
RZ638
(RG98)
RG304
12
12.3
3.6
5.9
10.4
19.7
2.2
3.3
11.1
18.1
6.2
RG958
(RG463, RG323)
RG901X
RG190
RG218X
CDO344
RG543
RG413
RZ76
(RG457, RZ261)
(RG9)
RG241X
RZ670
RG869
(RG341X)
RZ397
(RZ257)
RZ816
RG574
RG235
(RZ737)

would give an average spacing of about 5 cM for a genome that displays a total genetic length of 1,200-2,000 cM, depending on the relatedness of the parents of the mapping population (McCouch et al 1988, Causse et al 1994, Kurata et al 1994). Our goal was to be able to identify a pair of flanking markers less than 5 cM distant from each gene of interest. This proximity would give a 99.75% chance of avoiding false positives and false negatives in DNA marker-based selection.

STS map of rice

A map of the sequences is provided in Figure 1. Manual sequencing was successful for 294 clones, but the remaining 60 clones could be sequenced manually from only one end. Automated sequencing at Texas A&M University was able to provide the missing sequences. The sequence data from the 354 loci were used for 1) blast searches with the aim of identifying clones with homologues in the international data bases (Benson et al 1993) and 2) the design of PCR primers to amplify the corresponding loci from genomic DNA. The information obtained from blast searches will be provided in detail elsewhere.

PCR primer synthesis for recovery of STSs

The PCRPlan program of the PC/Gene software package was used to design suitable primers for amplification of the corresponding genomic loci. The two terminal sequences for a given clone were combined in inverse orientation and treated as a single sequence for computer analysis. More than 300 primer pairs have now been synthesized and validation has been completed for the majority. The validation test requires that each pair of primers should amplify:

- a single product of the expected size when the sequenced plasmid clone is used as template, and either
- a single product of the same size when genomic DNA from IR36 is template, or
- for RZ clones only, a single product of a larger size if the genomic DNA contains introns missing from the corresponding RZ clone.

Of 100 loci (50 RZ and 50 RG) analyzed in detail (Table 1), all RG clones and 42% of RZ clones satisfied the first and second criteria, while 58% of RZ clones satisfied the first and third criteria.

Amplicon length polymorphisms (ALPs)

Of the 100 primer pairs referred to in Table 1, 70 pairs amplified bands of identical size from IR36 (indica), Taichung 65 (japonica), and *O. longistaminata* (AA genome wild species). However, at 30 loci, ALPs were seen among these three DNAs. Figure 2 shows the amplifications obtained for five representative RG and RZ loci. RG369B and RZ452 were monomorphic, amplifying a product of the same size from plasmid DNA and the three genomic DNAs. With RG100 and RG450, the amplicon from *O. longistaminata* was different from the other three amplicons. RZ142 showed evidence of an intron but the intron length was different in IR36 compared with

Table 1. Comparison of amplicon lengths among IR36 (indica), Taichung 65 (japonica), and *Oryza longistaminata* at 100 sequence-tagged sites.[a]

Amplicon lengths for IR36, Taichung 65, and *O. longistaminata*	Type of marker				
	RG	RZ +intron	RZ -intron	Total RZ	Total
Same length	33	16	21	37	70
O. longistaminata different	14	12	0	12	26
IR36 different	0	1	0	1	1
T65 different	2	0	0	0	2
All three different	1	0	0	0	1
Total	50	29	21	50	100

[a]The sites correspond to 50 RG and 50 RZ markers (see text). The RZ markers were divided into two groups based on whether the primer pairs amplified a band from IR36 genomic DNA that was larger than (+intron) or equal in size to (-intron) the amplicon from the corresponding RZ clone.

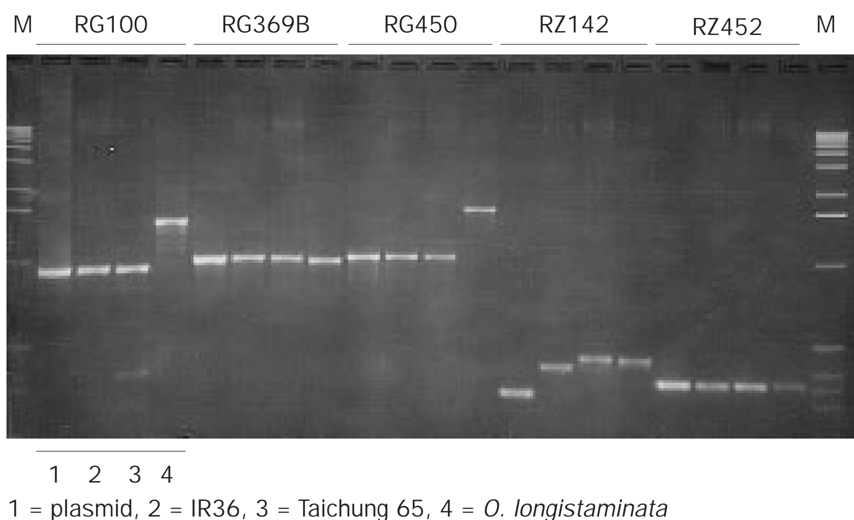


Fig. 2. Comparison of amplicon lengths obtained for plasmid DNA and genomic DNA from IR36, Taichung 65, and *O. longistaminata* at five sequence-tagged sites (RG100, RG369B, RG450, RZ142, and RZ452).

Taichung 65 and *O. longistaminata*. The additional length of amplicons attributable to introns varied from 0.1 to 2.0 kbp.

Most of the ALPs were between *O. longistaminata* and the two *O. sativa* cultivars. Only 4% of the loci showed ALPs between IR36 and Taichung 65. The percentage of ALPs was higher for RZ loci with introns (45%) than for RG loci (34%). No ALPs were found for RZ loci that lacked introns between the primer annealing sites. This result reflects the greater evolutionary constraints placed on coding sequences compared

with introns in respect of the insertions and deletions required to register an ALP. We are now studying the frequency of RFLPs obtained by digestion of these amplicons with a range of restriction endonucleases.

Applications of STSs

We turn now to four applications of STSs: two examples of DNA marker-aided selection, the use of multiplexing in the search for transgenes, and germplasm analysis within the AA genome wild species.

Pyramiding of major genes for durable resistance to bacterial blight

Durable pest and disease resistance continues to be a major goal of rice breeding (Khush 1995). Although it is recognized that major resistance genes are unlikely to offer durability when used singly, it is possible that combinations of major genes could achieve this goal. To test this hypothesis, IRRI staff are pyramiding major genes for blast and bacterial blight resistance using DNA marker-aided selection. These diseases are found in most countries and ecosystems where rice is grown but are especially prominent under rainfed cultivation. The pyramiding process was initiated with RFLP/DNA blotting, but now about half of the markers have been converted into STS markers. Table 2 shows the current situation with four genes for bacterial blight resistance. STS markers are available for *xa5* and *Xa21* but DNA blotting is still required for *Xa4* and *xa?* (the latter on chromosome 8). For a population of 200 lines, STS marker-aided selection for two loci is accomplished in less than a week, but selection by DNA blotting takes up to 2 mo and consumes more leaf material, more chemicals, and more labor.

Breeding for gall midge resistance

Gall midge is the major dipteran pest of rice. It is found from South China to Sri Lanka and in parts of tropical Africa. Asian rice gall midge (*Orseolia oryzae*) exists as a number of biotypes. For some of these biotypes, excellent sources of major gene resistance are known, but changes in biotype distribution and the evolution of new biotypes pose a threat to rice production in certain areas. The absence of gall midge

Table 2. Sequence-tagged sites for pyramiding genes for bacterial blight resistance.

Gene (chromosome)	Marker	Genetic distance (cM)	RFLP/DNA blot or STS	Restriction endonuclease
Xa4 (11)	Npb181	1.7	RFLP	*Hind*III
xa (5)	RG556	0.5	STS	*Dra*I
Xa? (8)	RG136	3.8	RFLP	*Dra*I
	RZ28	5.1	RFLP	*Xho*I
Xa21 (11)	pTA248	1.2	STS	ALP[a]
	RG103	0	STS	*Mva*I

[a]ALP = amplicon length polymorphism.

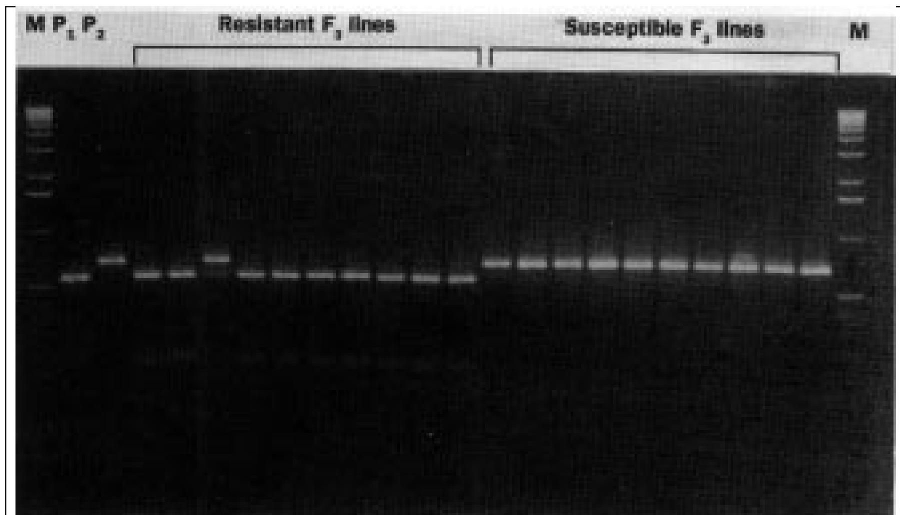


Fig. 3. Cosegregation of *Gm6(t)* with STS marker RG476. DNA was extracted from the two parental lines and from 10 F_3 lines breeding true for resistance and 10 F_3 lines breeding true for susceptibility. M = marker DNA, P_1 = Duokang #1 (resistant parent), P_2 = Feng Ying Zhan (susceptible parent). Note a single recombination event giving a heterozygote in the third resistant F_3 line.

from the Philippines, the existence of biotypes, and the sporadic occurrence of the high-humidity conditions favoring the insect have created difficulties for IRRI in developing a breeding program for gall midge resistance. DNA markers linked to resistance genes offer a way forward, not only for IRRI's breeders but also for breeders in affected countries wishing to pyramid genes for more durable resistance. Figure 3 shows the use of STS marker-aided selection for the *Gm6(t)* gene that provides resistance to gall midge biotypes 1-4 in southern China (Tan et al 1993). The marker 476 is located on chromosome 4, about 4 cM from the *Gm6(t)* gene. Note in Figure 3 the single heterozygote, representing one recombination event among the 20 lines breeding true for either resistance or susceptibility.

Multiplexing for studies on the inheritance of transgenes

PCR-based methods are used to follow the inheritance of genes inserted into plants by transformation. In most cases, it is easier to follow the inheritance of these "transgenes" by PCR than by any other method. Of course, transgenes may be amplified directly: it is not necessary to identify flanking markers for them. Transgenes are therefore usually not mapped and they do not constitute STSs. However, in all other respects, they may be analyzed as if they were STSs.

Figure 4 illustrates the application of the method to a backcross population. The transgene in this example is a synthetic *CryIA(b)* gene acquired from Plantech in Japan in a Nipponbare background (Fujimoto et al 1993). The backcross was part of

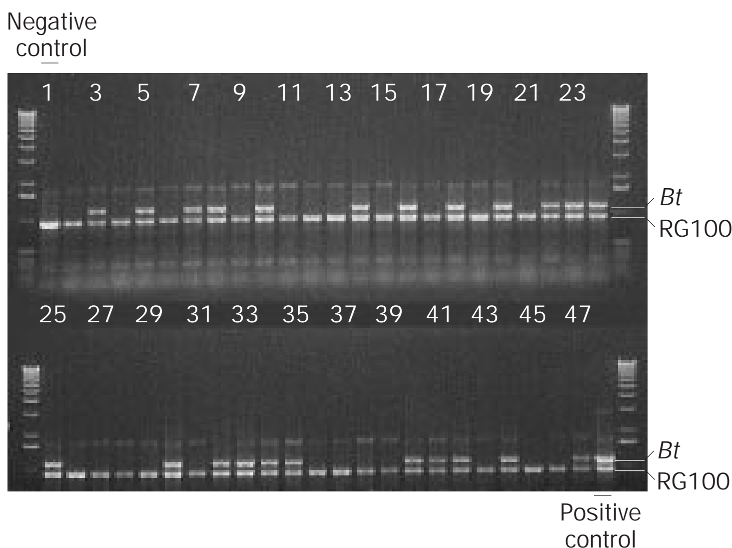


Fig. 4. Segregation of synthetic *CryIA(b)* gene in BC_2 population. *Bt* rice from Fujimoto et al (1993) crossed and backcrossed with line A as recurrent parent. PCR with primer pairs specific for *Bt* gene and RG100. End lanes: DNA markers, lane 1: negative control (DNA from nontransgenic plant), lane 48: positive control (DNA from nontransgenic plant plus DNA of plasmid *Bt* 1291 (Fujimoto et al 1993) equivalent to one gene copy per haploid rice genome, lanes 2-47: DNA from BC_2 plants.

a program to introduce the gene into the IRRI breeding line A3049. As expected, the *CryIA(b)* gene segregated 1:1 (PCR positive:PCR negative) in the BC_1 population.

One way of reducing costs and increasing the convenience of DNA marker technology is to use duplexing or multiplexing: the mixing of two or even more pairs of primers to give more than one amplicon per incubation. Of course, this is only possible when the amplicons differ in mobility and do not require digestion with restriction endonucleases to detect polymorphisms. We use duplexing in the PCR analysis of transgenic plants (Fig. 4). The purpose here is to provide a positive control for the DNA extraction and the PCR reaction. One primer pair corresponds to the transgene, the other to an endogenous rice DNA sequence (usually RG100 from chromosome 3). This control is a useful safeguard when rapid minipreps (Zheng et al 1995b) are employed for extraction of leaf DNA: the DNA is used for PCR without quality check or quantity check. Using duplexing for quality control is cheap, fast, and convenient.

Application of STS markers to the study of relations within the AA genome species

We are using STSs to develop rapid procedures for the analysis of AA genome species of the genus *Oryza*. Table 3 shows that, with just 14 STS primer pairs and three

Table 3. Evolution of a DNA marker-aided selection program.

Stages in marker-aided selection program	Location of stage[a] Present	Future
1 Priority setting and identification of breeding objective	B	B
2 Identification of donor and recipient varieties	B	B
3 Development of mapping populations	B	B
4 Phenotyping of mapping populations	B	B
5 DNA extraction from mapping population	M	B
6 RAPD/bulk segregant analysis to tag gene	B	
7 Mapping of gene	M	M
8 Identification of closest flanking markers (<5 cM)	M	M
9 Identification of polymorphisms between donor and	M	B
recipient varieties	B	B
10 Development of breeding populations	M	B
11 Application of DNA marked-aided selection		

[a]B = breeding station, M = specialist DNA marker laboratory.

accessions of each species, it is possible to infer that *O. meridionalis* is the AA genome species most distantly related to *O. sativa*, with *O. longistaminata* the second most distantly related. Wang et al (1990) came to the same conclusion about affinities among AA genome species through an extensive study using RFLP/DNA blots. Table 3 shows also that *O. longistaminata* is the AA genome species with the greatest intraspecific genetic diversity, a result consistent with its outbreeding habit (Vaughan 1994).

From such studies on AA genome species, non-AA genome species, and the cereals in general, we shall form a data base that will simplify the molecular characterization of these materials. The data base will identify STS markers that are appropriate to use within taxa of different degrees of divergence. The results in Table 3 indicate that STSs will simplify the study of gene flow between *O. sativa* and weedy AA genome relatives found in Asia, such as *O. nivara* and *O. rufipogon*. Such studies will be relevant to the current debate on biosafety of transgenic rice.

Conclusions

Molecular breeding with STS markers as a multidisciplinary activity

Zheng et al (1995a) describe in detail the methodology of PCR-based DNA marker-aided selection using STS. Here, we emphasize the multidisciplinary nature of molecular breeding. Table 4 presents a breakdown of molecular breeding into 11 steps. About half of these steps are routine for the expert staff of rice breeding stations. Relatively few steps require the expertise and infrastructure of a specialized DNA marker laboratory.

Agreement on step 1 is important for effective collaboration between the breeding station and the DNA laboratory and may require considerable multidisciplinary activity in itself. The staff of the breeding station would certainly be able to accomplish steps 2-4 and step 10 with conventional resources. Initially, the staff of the DNA marker

Table 4. PCR-based comparison of the eight AA genome species of the genus *Oryza* at 14 sequence-tagged sites (STSs).

Species	Number of STSs showing species-specific ALP[a]	Number of STSs showing intraspecific ALP
O. sativa	1	3
O. rufipogon	0	4
O. barthii	0	2
O. nivara	0	7
O. glaberrima	0	5
O. glumaepatula	1	4
O. longistaminata	3	11
O. meridionalis	5	4

[a]ALP = amplicon length polymorphism.

laboratory would have to accomplish steps 5-9 and collaborate with the breeding station on step 11.

If step 11 involves PCR-based STS analysis, this step would be one of the first steps transferred to the breeding station, along with step 5. Step 6 would be transferred next, followed perhaps by step 9. Because of their resource requirements and their difficulty, steps 7 and 8 would probably remain the responsibility of the DNA marker laboratory. Nevertheless, over a period of about 5 yr, it should be possible for major rice breeding stations in Asia to develop basic skills in DNA marker technology and experience the benefits of the approach.

Maximizing the probability of finding intraspecific STS polymorphisms

The frequency of STS polymorphisms between and within the indica and japonica subspecific groups of *O. sativa* will determine the utility of STS-based markers for rice breeding. ALPs will be uncommon (Table 1) and it will be necessary to use RFLP analysis on the STS amplicons. In their study of STS polymorphisms between ecotypes of *Arabidopsis thaliana*, Konieczny and Ausubel (1993) used up to 83 restriction enzymes to look for polymorphisms. This reliance on restriction endonucleases can become very expensive and is not certain to be effective.

Strategies are needed for maximizing the probability of detecting STS polymorphisms and for deciding when to move on to alternative approaches. These strategies will emerge as experience with STS markers and other markers increases. For this reason, it is desirable that an international STS data base be set up to record the successes and failures that are encountered in attempts to find polymorphisms for STS markers between various cultivars.

STS data base

A data base has been initiated at IRRI to record the results of STS marker studies in a systematic manner. Data base entries will draw attention to a range of helpful points.

For example, Table 1 suggests that cDNA clones for genes lacking introns should be avoided when searching for ALPs and probably also PCR-based RFLPs. The data base will indicate whether RZ clones (and other cDNA clones) correspond to gene segments with or without introns. The data base will also identify which restriction enzymes have been used to digest each amplicon and how many bands are produced. It should be possible to correlate these data with the lineages of breeding programs to understand how ancestral genes have been inherited by elite breeding lines. Inclusion of these data in the RiceGenes relational data base (Paul et al 1994) would be highly desirable.

Cited references

Benson D, Lipman DJ, Ostell J. 1993. GenBank. Nucleic Acids Res. 21(13):2963-2965.

Causse MA, Fulton TM, Yong G-C, Ahn S-N, 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.

Fujimoto H, Itoh K, Yamamoto M, Kyozuka J, Shimamoto K. 1993. Insect-resistant rice generated by introduction of a modified delta-endotoxin gene of *Bacillus thuringiensis.* Bio/Technology 11:1151-1151.

Fukuoka S, Inoue T, Miyao A, Monna L, Zhong HS, Sasaki T, Minobe Y. 1994. Mapping of sequence-tagged sites in rice by single strand conformation polymorphism. DNA Res. 1:271-277.

Ghareyazie B, Huang N, Second G, Bennett J, Khush GS. 1995. Classification of rice germplasm. I. Analysis using AFLP and PCR-based RFLP. Theor. Appl. Genet. 91:218-227.

Inoue T, Zhong HS, Miyao A, Ashikawa I, Monna L, Fukuoka S, Miyadera N, Nagamura Y, Kurata N, Sasaki T, Minobe Y. 1994. Sequence-tagged sites (STSs) as standard landmarkers in rice genome. Theor. Appl. Genet. 89:728-734.

Khush GS. 1995. Modern varieties—their real contribution to food supply and equity. GeoJournal 35:275-284.

Konieczny A, Ausubel FM. 1993. A procedure for mapping *Arabidopsis* mutations using codominant ecotype-specific PCR-based markers. Plant J. 4(2):403-410.

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin S-Y, 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 Z-X, Momma 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.

McCouch S, Kochert G, Yu Z-Y, Wang Z-Y, Khush GS, Coffman WR, Tanksley SD. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815-829.

Olson M, Hood L, Cantor C, Botstern D. 1989. A common language for physical mapping of the human genome. Science 245:1434-1435.

Paul E, Goto M, McCouch S. 1994. RiceGenes—an information system for rice research. Probe 4:3-4.

Tan Y, Pan Y, Zhang Y, Lixia Z, Xu Y. 1993. Resistance to gall midge (GM) *Orseolia oryzae* in Chinese rice varieties compared with varieties from other countries. Int. Rice Res. Notes 18(4):13-14.

Vaughan DA. 1994. The wild relatives of rice. Manila (Philippines): International Rice Research Institute.

Vos P, Hoges R, Bleeka M, Reijans M, van de Lee T, Homes M, Freijters A, Pot J, Pelemam T, Kaiper M, Zabeau M. 1995. AFLP: a new concept for DNA finger printing. Nucleic Acids Res. 23:4407-4414.

Williams MNV, Pande N, Nair S, Mohan M, Bennett J. 1991. Restriction fragment length polymorphism analysis of polymerase chain reaction products amplified from mapped loci of rice (*Oryza sativa* L.) genomic DNA. Theor. Appl. Genet. 82:489-498.

Wu K-S, Tanksley SD. 1993. Abundance, polymorphism, and genetic mapping of microsatellites in rice. Mol. Gen. Genet. 241:225-235.

Zheng KL, Huang N, Bennett J, Khush GS. 1995a. PCR-based marker-assisted selection in rice breeding. IRRI Discussion Paper Series No. 12. Manila (Philippines): International Rice Research Institute.

Zheng KL, Subudi PK, Domingo J, Magpantay G, Huang N. 1995b. Rapid DNA isolation for marker-assisted selection in rice breeding. Rice Genet. Newsl. (in press)

Notes

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