ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2009 – December 31, 2009

PROJECT TITLE: Application of Molecular Marker-Assisted Selection to Rice Improvement

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OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES:

The overall objective is to integrate molecular genetic approaches and conventional breeding methods to develop improved germplasm for the California rice industry. Primary emphasis is on the development of molecular (DNA) markers that can be used to predict the presence or absence of a trait of interest (e.g. disease resistance, cold tolerance, grain quality) and the application of these markers via molecular marker-assisted selection to expedite the identification of useful germplasm and streamline the breeding of improved varieties.

In order to employ DNA markers, marker-trait associations must be established (i.e., the value of a DNA marker in predicting a trait must be determined). Basic genetic studies have resulted in the identification of DNA markers for many important traits. Several of these markers are based on differences in the DNA of specific genes. Differences (or polymorphisms) that are directly responsible for the characteristic in question are sometimes referred to as perfect markers as they are always (perfectly) associated with the trait. Genes underlying important traits in rice such as grain quality, yield, grain size, fertility, etc. have been identified. The objective of our research in 2009 was to examine a set of these genes in California varieties as a step towards establishing whether new sources of these genes may be useful for improving yield and/or quality. All work on this project was conducted in the USDA-ARS rice genetics lab and greenhouses in the Dept. of Plant Sciences, UC Davis.

Specific objectives for 2009 included:

1) *Yield-related genes*: Yield is arguably the most important trait to growers. California rice yields are among the highest in the world due in part to highly conducive growing environment and high-yielding semidwarf varieties developed primarily by the RES. Relatively little is known about the genetic basis for high yields due to the complex nature of this trait. However, with advances in rice genetics, genes that contribute significantly to yield are being isolated. Among these genes, the *Gn1a* and *Ghd7* genes have been recently described (Ashikari et al., 2005; Xue et al., 2008). Differences in the sequences of these genes in high and low yielding rice accessions have been reported. In this project, the sequence of these genes will be determined for a set of California varieties. This information will be useful for determining if transfer of these genes from high yielding rice accessions to California germplasm is warranted.
2) *Quality-related genes*: Genes involved in starch biosynthesis are important factors in cooking and eating quality. DNA markers derived from the *Waxy* gene, which encodes granule-bound starch synthase, have been used extensively in rice breeding programs such as those in Texas and Arkansas. The RES currently utilizes one marker (RM 190; Ayres et al., 1997); however, the correlation between this marker and the cooking quality of some rice lines is not sufficient. Other markers within the *Waxy* gene are available that may be more useful than RM 190 (Chen et al., 2008a, b). In addition, DNA markers in other starch biosynthesis genes are available (Bao et al., 2006a, b). DNA sequencing and/or DNA marker analysis will be used to characterize the nature of five of these genes (*Waxy*, *SS1*, *SBE1*, *SBE3*, and *SSIIa*) in California varieties. This information will be useful in identifying additional quality markers that may be used by the RES DNA marker lab.
3) *Wide compatibility gene*: Cross breeding is the cornerstone of any plant improvement program. The ability of different rice varieties to be crossed and produce highly fertile offspring is conditioned by many genetic factors. One gene that is a key regulator of fertility and compatibility of *indica-japonica* hybrids is the *S5* gene. Recently, this gene has been cloned and three forms have been identified (Chen et al., 2008c). They are the *japonica* (*S5-j*), *indica* (*S5-i*) and wide compatibility (*S5-n*) forms. Presence of the *S5-j* and *S5-i* forms in an F_1 results in very high sterility whereas the *S5-n* form in conjunction with either *S5-j* or *S5-i* yields highly fertile hybrids. DNA markers that are able to distinguish the wide compatibility form from the *japonica* and *indica* forms will be used to screen California varieties and other rice germplasm that may be used by the RES in future breeding efforts (such as for hybrid rice studies).
4) *Identification and analysis of additional gene targets*: The genes in this proposal represent high value targets, but are by no means the only genes that have been characterized. In addition to the sequencing and marker analysis, a high priority gene list will be developed as part of this proposal. Genes on this list will be selected in conjunction with the RES and will be based on the various objectives of the breeding programs. Analysis of these genes may be included depending on the availability of funds and personnel. One target of interest is a seedling cold tolerance candidate gene. This gene, *OsGSTZ2*, appears to be involved in the *qCTS12*-associated cold tolerance of M-202 (see 2008 Annual Report for RB-3). Sequence analysis of this gene has revealed two

DNA markers of interest that may be used to screen germplasm for the USDA rice collection.

SUMMARY OF 2009 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1) *Yield-related genes*: DNA primers for *Gn1a* gene, which encodes a cytokinin oxidase enzyme, were obtained (Table 1) and used to amplify parts of this gene (Fig. 1) from a small set of California cultivars (Calrose, S-102, M-206, L-202). Initial analysis indicates that the primers for the *Gn1a* gene appear to work well although the *Gn1a*-3 fragment did not amplify from the Calrose and L-202 DNA. This is probably a minor technical issue that will be resolved upon repeating the amplification. The amplified fragments will be sent to the UC Davis sequencing facility to determine the DNA sequence. Sequence data will be compared to determine the nature of these genes in each accession. If sequence differences are observed, DNA markers based on these differences will be developed and tested.

Table 1. DNA primers used for analysis of yield-related and wide compatibility genes.

Gene	name	Forward Primer	Reverse Primer	Fragment size in bp
GW5 (*qSW5*)	GW5-1	AGTACGACCATGATGTTTCCC	GACCTAACCCATCTCATTCCA	none (775)
	GW5-2	GCGTCGTCAGAGGTAGA	GTGGGATAGGATGAAACC	697 (1897)
GW2	GW2-1	GGTACCACCAGCATCCTCAT	AGAGAAAAAGCTGAAGTGGAAGG	877
	GW2-2	TGACAACCACTCCTGTCCTG	TCATTTGCTTCCCTTCAACA	963
	GW2-3	CGTCAGCTTAGTGCAGTGCT	ACCTGACCCAACAAATCAGC	482
	GW2-4	TTCCCCAATAAGTTGCTTGC	GGTGAACAAAAAGGCCAAGA	917
Gn1a	Gn1a-1	CAAACCAAATCTTTCAGTCCTTG	CGCGTGTCTTAGTAGATGTGATG	911
	Gn1a-2	CTCGCCGTGTCAGTGGAG	CCAAAAGTTTTTGCAGCTTAGTT	877
	Gn1a-3	GTGCTGGCAAAATTAATAACTCG	AAAAATCCACCCCGTAAAAATTA	443
	Gn1a-4	AAAAACAATGTCCGTTCTCTTGA	ATGCATGGAAAAATATTGCAAAC	1032
Ghd7	Ghd7-1	CAATGAGGAGTCGCCAAATTAT	GCAGCAGAAATGAAGAGTTGTTT	985
	Ghd7-2	TGGGGCACAAAATCATGTC	AATGGGCCATCGATCACTAA	784
S5	S5-1a	CTGAGCAAGCAAGAAAGAAAGAA	TCTACCACTAGGAGCAGTTTTCG	460 (324)
	S5-1b	CTGCCCCTGAGCAAGCAAGAAAG	ATGTGTAGGATCTGCCGGGATCGA	884 (748)
	S5-2	CAATCAACAGGCCAACCTACTCAC	TTGGCACGAACGGTTCAAGAG	968
	dCAPS	GCATGGATGTCAAGTACAGCG	CGTCAGTGGGCAAGCAGAAG	140

Alternate fragment sizes are indicated in parentheses. These may be scored on the basis of the differences in size, whereas in the case where the primers amplify one fragment size, additional analysis (e.g., sequencing) is needed to detect differences between cultivars.

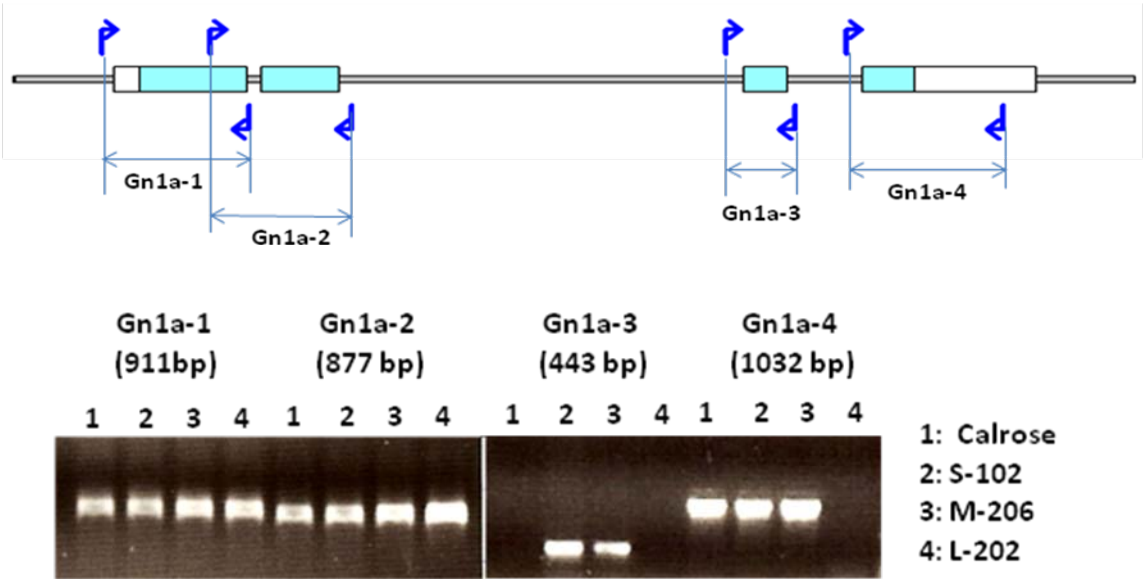

Fig. 1. Analysis of the *Gn1a* gene. Simplified *Gn1a* gene structure diagram shown at the top. Light blue boxes represent part of the gene encoding the protein and open boxes represented untranslated regions. The blue arrows represent the DNA primers used to amplify the DNA fragments as indicated. DNA fragments are shown at the bottom. Columns (numbered 1 – 4) indicate the cultivars from which the fragments (white bands) have been amplified. The fragments have been loaded onto an agarose gel and visualized by staining with a dye that fluoresces under UV light.

DNA primers for *Ghd7* gene, which encodes a CCT domain protein, were obtained (Table 1, Fig. 3) but failed to amplify fragments of this gene (data not shown) from a small set of California cultivars (Calrose, S-102, M-206, L-202). New amplification conditions and/or primers must be tried. Once amplification is achieved, sequencing and analysis will be performed.

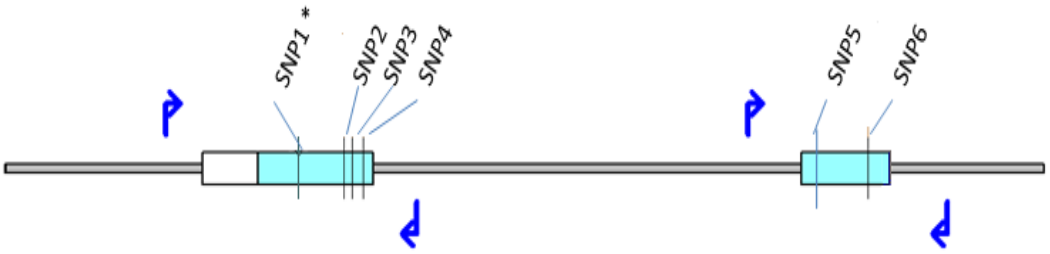

Fig. 3. Simplified *Ghd7* gene structure diagram. The blue boxes represent part of the gene encoding the protein. The blue arrows represent the DNA primers used to amplify the DNA fragments as indicated. SNP stands for single nucleotide polymorphism. The locations of SNP that have been previously identified by sequence analysis of various

rice accessions are shown. The asterisk (*) indicates a SNP that causes early termination of the encoded protein.

In addition to the *Gn1a* and *Ghd7* genes, we have initiated analysis of two genes involved in grain size, *GW2* and *GW5* (Song et al., 2007; Shomura et al., 2008; Weng et al., 2008). In the case of *GW2*, which encodes a RING-type E3 ubiquitin ligase, primers for amplifying fragments of the gene for sequencing were obtained (Table 1) and conditions are being optimized to produce fragments for analysis as shown in Fig. 4.

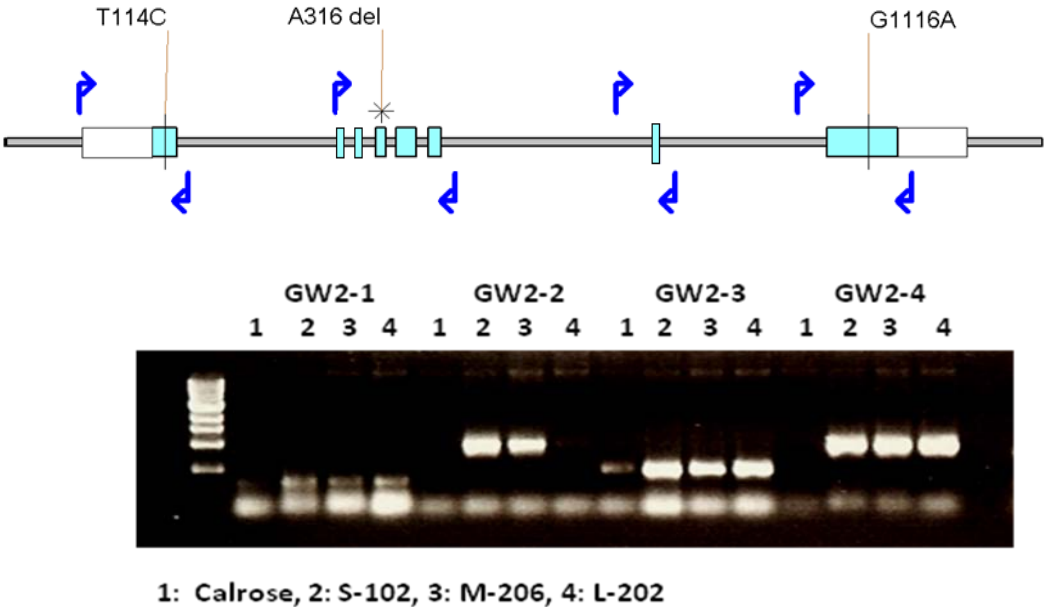

Fig. 4. Analysis of the *GW2* gene. A simplified diagram of the gene structure is shown at the top. Notations above the diagram refer to SNP (T114C and G1116A) and indel (A316del) markers. The numbers indicate the position along the length of the gene where the SNP or indel occurs and the letters indicate the nucleotide differences. In the case of the indel, the difference is the presence or absence of the A nucleotide at position 316. The bottom image is of DNA fragments of the *GW2* gene amplified from four California cultivars. Fragments correspond from left to right to the regions marked by the blue arrows (primers) in the diagram above.

Previous work resulting in the identification of the *GW5* gene, which encodes a novel nuclear protein, showed that a 1.2 kb deletion which removes part of the gene (Fig. 5) is associated with increased grain width and weight, leading to increase yield. Primers were obtained for detection of this difference and were used to analyze four California cultivars (Calrose, S-102, M-206, and L-202). Interestingly the preliminary results indicate that only the S-102 cultivar appears to have the deletion. Confirmation of these results and analysis of additional California cultivars and breeding lines is in progress.

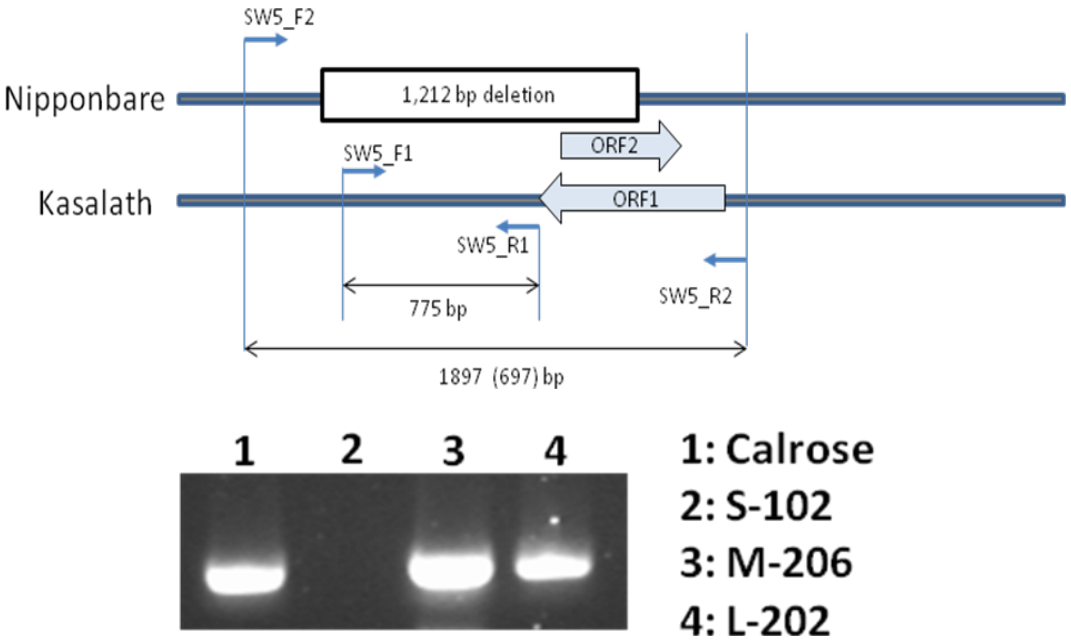

Fig. 5. Analysis of the *GW5* gene which is involved in grain width and weight. A simplified diagram of the region containing the *GW5* gene (ORF1, open reading frame 1) in two rice cultivars Nipponbare (temperate japonica) and Kasalath (aus) is shown at the top. Blue arrows indicate the location of primers used to detect the 1.2 kb deletion event. Predicted sizes in base pairs (bp) of fragments amplified by primers are as noted. Using primers SW5_F1 and SW5_R1, analysis of the GW5 genes in four California cultivars was performed as shown at the bottom. Results indicate that only S-102 appears to have the deletion found in Nipponbare, which is characteristic of *japonica* rices.

2) *Quality-related genes*: Starch is the primary constituent of milled rice and its chemical properties directly affect the cooking, eating, and milling quality of rice grains. Starch consists of amylose and amylopection. Genes involved in the synthesis of starch are well-known. A number of markers based on these genes have been used in previous studies (Ayres et al., 1997; Bao et al., 2002, 2006a, b; Larkin and Park, 2003; Larkin et al., 2003; Akagi et al., 1996; Han et al., 2004). Markers for the *Waxy* (granule-bound starch synthase), *SS1* (soluble starch synthase 1), *SBE1* (starch branching enzyme 1), and *SBE3* (starch branching enzyme 3) genes (Table 2) were used to assess a set of 49 cultivars (Table 3). In addition to these genes, analysis of the *SSIIa* or *alk* (soluble starch synthase IIa) gene has been initiated. According to Bao et al. (2006a), peak gelatinization temperature is strongly associated with two adjacent SNPs (GC/TT) in the *SSIIa* gene, which can be detected by PCR amplification using specific primers (Fig. X). A preliminary experiment with the cultivars Colusa and Calamylow-201 indicated that Colusa has the TT allele, which is generally associated with low gelatinization temperature, while Calamylow-201 has GC allele, which is generally associated with

high to intermediate gelatinization temperature. Work is in progress to confirm these results and extend the analysis of this marker to the remaining California cultivars and breeding lines of interest.

Table 2. Starch synthesis gene-based markers used for analysis of California cultivars.

Gene	Primer combination	Marker Type	Polymorphism Type	Reference
Wx	RM190f-snpf [484]/ RM190r [485]	SSR	size	Bao et al. (2006); Ayres et al. (1997)
Wx	RM190f-snpf [484] / Wx snpr [W2R]	SNP	T or G	Bao et al. (2006); Ayres et al. (1997)
SBE3	SEB3(SNP)f [492] / SEB3(SNP)r [493]	SNP	C or G	Bao et al. (2006); Han et al. (2004)
SBE1	SBE1f [486] / SBE1r [487]	SSR	size	Bao et al. (2006b, 2002); Akagi et al. (1996)
SBE1	SBE1(STS)f [490] / SBE1(STS)r [491]	STS	insert or no insert	Bao et al. (2006b); Han et al. (2004)
SS1	SS1f [488] / SS1r [489]	SSR	size	Bao et al. (2006b, 2002); Larkin et al. (2003)
SSIIa	F7, F17, F18 & F22 / R1, R6, R18, R20 &R21	SNP	sequence variation	Bao et al. (2006a)

Primer combination: DNA primers used to amplify marker DNA for analysis
Marker type: SSR – simple sequence repeat, SNP – single nucleotide polymorphism, STS – sequence tagged site
Polymorphism type: Indicates basis for differences (size = fragment/band size; letter = nucleotide difference; insert/no insert = presence or absence of transposon; sequence variation = multiple sequence differences)
References: Indicates studies where markers were developed and/or applied.

Table 3. Starch synthesis gene marker analysis of 49 cultivars.

Cultivar	Wx* (RM 190, SSR)	Wx CTn	Wx (SNP)	SBE3 (SNP)	SBE1* (SSR)	SBE1 (STS)	SS1* (SSR)
Colusa	115	**18 (19)**	T	G	208	I	187
Caloro	116	**19**	T	G	208	I	186
Calrose	116	**19**	T	C	208	I	187
CS-M3	117	**19**	T	C	208	I	187
CS-S4	117	19	T	G	208	I	186
S6	117	19	T	C	208	I	187
M5	117	20	T	C	208	I	187
Calrose76	117	**19**	T	C	209	I	186
M7	115	19	T	C	210	I	186
M9	117	19	T	C	226	NI	185
Calmochi-201	117	20	T	C	208	I	186
L-201	119	**20**	G	C	224	NI	185
M-101	111	17	T	C	209	I	185
M-301	115	18	T	C	208	I	185
S-201	116	19	T	C	208	I	185
Calmochi-202	117	20	T	C	208	I	185
M-302	117	20	T	C	208	I	185
M-401	116	19	T	C	224	NI	187
M-201	115	**19 (18)**	T	C	208	I	185
L-202	119	**20**	G	C	225	NI	185
Calmochi-101	112	**17**	T	C	210	I	185
M-202	118	20	T	C	223	NI	185
A-301	120	21	G	C	224	NI	185
M-102	118	20	T	C	209	I	187
M-203	116	19	T	C	225	NI	185
S-101	116	19	T	C	209	I	185
M-103	118	20	T	C	209	I	185
S-301	118	20	T	C	209	I	186
L-203	120	21	G	C	220	NI	186
M-204	114	18	T	C	209	I	187
A-201	119	21	G	C	224	NI	187
L-204	119	21	G	C	219	NI	188
S-102	117	20	T	C	209	I	(208)
Calmati-201	119	21	G	G	220	NI	193
Calhikari-201	117	20	T	G	210	I	193
L-205	104	13	G	C	220	NI	194
M-402	118	20	T	C	225	NI	190
M-205	115	19	T	C	226	NI	192
M-104	118	20	T	C	210	I	187
M-206	117	20	T	C	225	NI	191
M-207	116	19	T	C	225	NI	189
M-208	116	19	T	C	226	NI	191
L-206	120	21	G	C	221	NI	188
Calmati-202	120	21	G	C	221	NI	190
Calamylow-201	116	19	T	G	211	I	190
Terso	115	19	T	C	227	NI	190
Kokuhorose	116	19	T	C	227	NI	189
Akitakomachi	114	18	T	G	211	I	191
Koshihikari	114	18	T	G	211	I	(215)

Asterisks (*) indicate that these markers detect differences in fragment sizes. The numbers represent base pairs (bp) of DNA. Values in parentheses indicate the fragment

produced was weak with regard to visualization/detection. The *Wx* CTn column indicates the number of CT repeats as calculated from the *Wx* RM190 marker sizes in the previous column. The CTn values in bold font indicate cultivars analyzed by Ayres et al. 1997. CTn values presented in parentheses are from the Ayres et al. study while the others are in agreement.

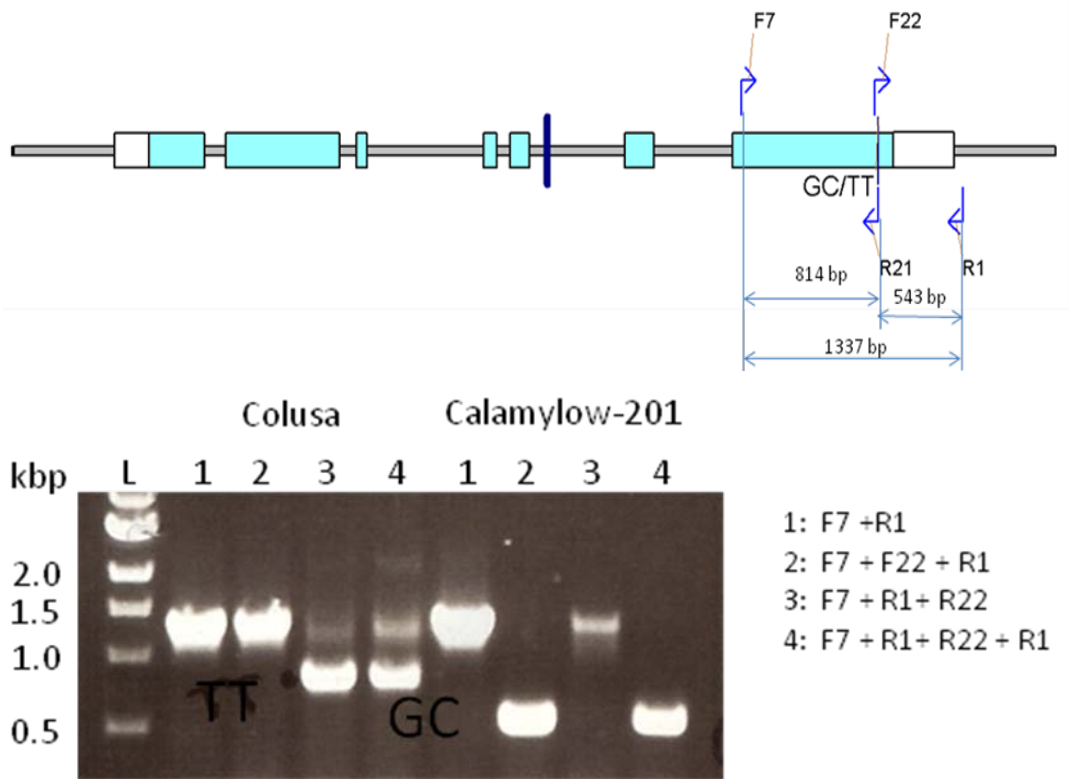

Fig. 6. Analysis of the *starch synthase IIa* gene. Simplified gene structure diagram is shown at the top. Light blue boxes indicate parts of the gene encoding the protein. Blue arrows indicate the position and orientation of primers for PCR amplification. Fragment sizes are indicated in base pairs (bp). Amplified fragments from Colusa and Calamylow-201 are shown at the bottom. Fragments have been loaded onto an agarose gel for separation by size and visualized by staining with a dye that fluoresces under UV light. Primers used for amplification of products in lanes 1 through 4 are indicated to the right of the gel picture. Lane L is a size standard. Fragments observed in Colusa lanes 3 and 4 are indicative of the TT allele of *SSIIa* and fragments in Calamylow-201 lanes 2 and 4 are indicative of the GC allele.

3) *Wide compatibility gene*: The *S5* gene, which encodes an aspartic protease, plays a major role in regulating wide compatibility and the sterility of *indica-japonica* hybrids. DNA markers that can distinguish the *indica* (*S5-i*), *japonica* (*S5-j*) and wide compatibility (*S5-n*) forms of this gene are available. The *S5-n* form is easily distinguishable from the other forms based on size (there is a 136 bp deletion in the *S5-n* allele; Fig. 7).

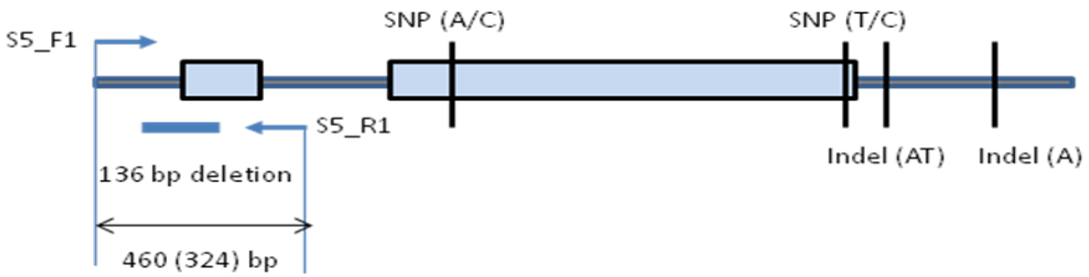

Fig. 7. The *S5* gene is a major factor in regulating wide compatibility and the sterility of *indica-japonica* hybrids. Three alleles (*S5-n*, *S5-i*, and *S5-j*) can be distinguished using DNA markers. The *S5-n* allele has a 136 bp deletion (blue bar) that can be detected as a size difference in amplified DNA products. The *S5-i* and *S5-j* alleles can be distinguish based on single nucleotide polymorphism (SNP) and insertion/deletion (Indel) markers.

Initial analysis of the *S5* gene in 45 California cultivars indicates that seven of these cultivars (L-201, L-202, L-203, L-204, L-205, L-206, and A-201) have the *S5-n* wide compatibility allele (324 bp) while the other 38 cultivars all produced fragments indicative of the *S5-i* or *S5-j* alleles (460 bp). It has previously been demonstrated that the *S5-n* allele is present in the U.S. ancestral line CP SLO17 and cultivar Lemont. It is not clear to us from the available pedigrees of L-201 (CI 9701/3/R-134-1/R48-257//R50-11) and L-205 (M7/R660//M7/R1588/3/82-Y-52/4/Rexmont/83-Y-45) what the original donors of the *S5-n* allele are for these cultivars. CP SLO17 and L-201 are found in the

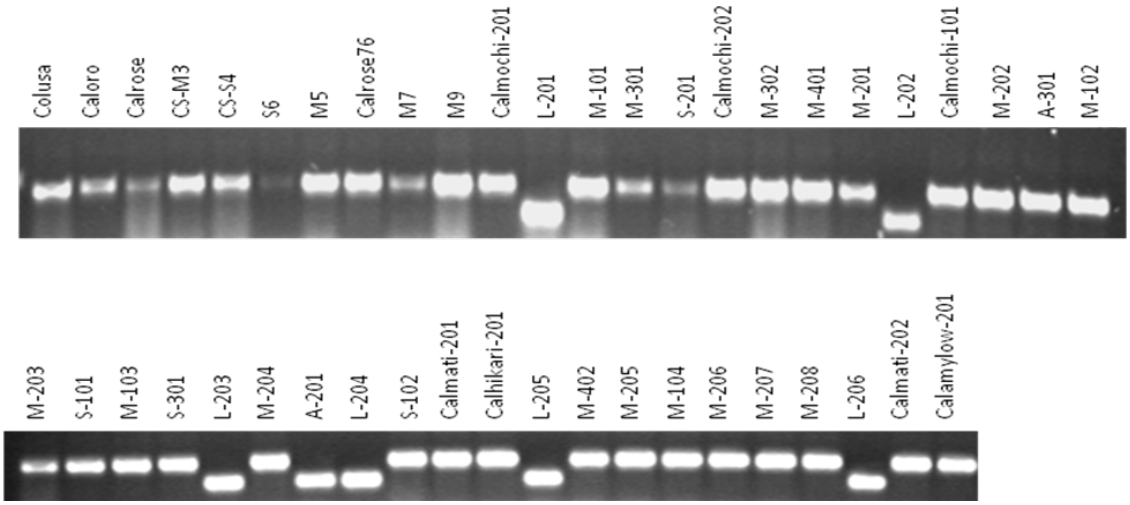

Fig. 8. *S5-n* DNA marker analysis of California cultivars. The lower band is indicative of the wide compatibility allele (*S5-n*) and the upper (larger) band (fragment) may either be *S5-i* or *S5-j*.

pedigree of L-202. At least one of the cultivars L-201, L-202, and Lemont is found in the pedigrees of L-203, L-204- L-206, and A-201. Additional analysis will be performed to confirm which of these alleles are present in the remaining cultivars.

4) *Identification and analysis of additional gene targets*: Due to time constraints, development of a high priority gene list for future analysis has been postponed until January 2010 at which time analysis of the current set of genes should be completed and discussions can be held with RES breeders concerning analysis of additional germplasm accessions and genes.

Additional 2009 research activities related to RF-1 and RB-3:
During the 2009 growing season, a number of populations and lines were grown although advancement of these populations was somewhat hampered by the discovery of panicle rice mite in the UC Davis greenhouses in January. Following the recommended host-free period, seeds (treated with phostoxin and by freezing) were planted starting in June. This effectively reduced the generation advance from two to one generation for 2009.

Population	Description	Size	Use
Terso mutants	Early flowering/maturing lines (earliest 78 days)	87 families (about 4 M3 generation lines per family)	Breeding germplasm
M-204/S-301	F1 plants (seed from Dr. V. Andaya)	7 plants ranging from 700 to 1000 F2 seeds per plant	Mapping population development (milling yield)
S-301/M-204	F1 plants (seed from Dr. V. Andaya)	8 plants ranging from 800 to 1000 F2 seeds per plant	Mapping population development (milling yield)
S-301/M-206	F2 plants (seeds from Dr. V. Andaya)	290 F2 plants, harvested single panicles of F3 seeds	Mapping population development (milling yield)
M-203/M-206	F2 plants (seeds from Dr. V. Andaya)	294 F2 plants, harvested single panicles of F3 seeds	Mapping population development (milling yield)
Milling yield lines	Advanced medium grain breeding lines (provided by Dr. J. Lage)	135 lines (seeds from same source as used in pilot milling yield test; RF-1 project)	Mapping population development (milling yield)

In addition to the gene markers analyzed, we also assessed a recombinant inbred mapping population derived from the cross of L-202/Lemont (253 lines) developed by Dr. F. Jodari for analysis of grain quality (fissuring) using microsatellite markers. The purpose of this work was to determine the degree of heterozygosity remaining in these lines and facilitate the selection of lines that are most likely to be fixed for further phenotypic (trait) and genetic evaluation. Of the 253 lines, 56 exhibited one or more heterozygous loci (out of 8 loci tested) and four lines exhibited at least one marker locus that was not the same as either parent (L-202, Lemont).

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Weng et al. (2008) Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. Cell Res 18: 1199-1209

Xue et al. (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nat Genet 40: 761-767

PUBLICATIONS OR REPORTS:

None at this time.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

We have initiated the analysis of ten genes involved in yield (*Gn1a*, *Ghd7*, *GW2*, and *GW5*), quality (*Waxy*, *SBE1*, *SBE3*, *SS1*, *SSIIa*), and wide compatibility (*S5*). Optimization of experimental conditions is underway for genes requiring analysis by DNA sequencing (*Gn1a*, *Ghd7*, and *GW2*). At least two of these genes (*Gn1a* and *GW2*) will be sequenced from an ancestral cultivar (Calrose) and representatives of the three major grain types/breeding programs (S-102, M-206, and L-202) by the end of 2009. Analysis of the GW5 gene for grain weight and width in four California cultivars (Calrose, S-102, M-206, and L-202) indicates that only S-102 appears to have the form of the gene that is associated with increased grain width. A larger set of California cultivars will be assessed with the *GW5* DNA markers by the end of 2009. Grain quality markers based on four starch synthesizing genes (*Waxy*, *SBE1*, *SBE3*, and *SS1*) have been employed to analyze a small set of cultivars including all the released varieties from the RES. In addition, one marker for the *starch synthase IIa* (SSIIa) gene has been successfully tested and will be used to characterize a larger set of California cultivars by the end of 2009. Our results indicate that there is significant variation present in California cultivars for the genes examined. This variation will be analyzed in conjunction with trait data to determine the utility of specific gene markers for directing selection efforts during the breeding process.