

Generation and characterization of novel genetic variation in rice for the enhancement of grain quality and agronomic performance

Thomas H. Tai^A

^AUSDA-ARS Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA

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Introduction

Over the past fifteen years, my program has been involved in the generation and characterization of induced mutant populations of rice for forward and reverse genetics applications. The development of powerful, high throughput sequencing-based strategies for mutation detection has increased the value of mutants generated by traditional chemical mutagens. This has enabled functional genomics research and the development of breeding germplasm to be performed using the same genetic resources. One such functional genomics approach is the reverse genetics method known as Targeting of Induced Local Lesions in Genomes (TILLING). We have used the TILLING approach to identify mutations in genes involved in phytic acid content, starch biosynthesis and arsenic uptake and accumulation. In addition, various mutants of interest have been identified by visual evaluation and other forward genetics screens.

Materials and Methods

Rice mutant populations in the varieties Nipponbare, Kitaake, and Sabine were generated using various chemical mutagens including ethyl methanesulfonate, sodium azide, and methyl nitrosourea (Till et al. 2007; Monson-Miller et al. 2012). Mutation detection was performed by TILLING (Till et al. 2007; Tsai et al. 2011), exome capture (Henry et al. 2014) and a reduced representation sequencing strategy (Monson-Miller et al. 2012; Tai et al. 2016).

Rice grain mutants were identified by visual inspection of brown rice samples, the alkali spreading value test, and I₂/KI staining. Mutants with low phytic acid content were identified using an indirect colorimetric assay that detects high inorganic phosphate (Larson et al. 2000). Screening with germanium (Ma et al. 2002) was performed to identify mutants with putative alterations in silicon/arsenic uptake and accumulation.

Results and Discussion

Using chemical mutagenesis, rice mutant populations suitable for forward and reverse genetics have been developed. A publicly available TILLING resource was generated using the variety Nipponbare and this has used to successfully identify mutations in an array of target genes. Mutation detection by sequencing has also facilitated the characterization of the mutation density and type in rice mutant populations derived from the variety Kitaake, enabling the comparison of different chemical mutagenesis protocols. Forward genetics of the Nipponbare, Kitaake and Sabine mutant populations has also resulted in the identification of a number of interesting mutant phenotypes for germplasm enhancement in addition to gene discovery and functional studies.

Conclusion

The generation and exploitation of rice populations using traditional chemical mutagenesis remains an important approach particularly in light of consumer and industry concerns over biotechnological approaches such as genetic transformation and gene/genome editing. Advances in non-GMO technologies such as high throughput sequencing have increased the value and utility of these mutant populations.

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Posters:

Genetic analysis of seedling vigor in temperate japonica rice (*Oryza sativa* L.)

Karla Cordero-Lara^{A,B}, HyunJung Kim^A and Thomas H. Tai^C

^ADepartment of Plant Sciences, UC Davis, Davis, CA 95616 USA

^BINIA CRI Quilamapu, Casilla 426, Chillán 3780000, Chile

^CUSDA-ARS Crops Pathology and Genetics Research Unit, Davis, CA 95616 USA

Presenting author: Thomas Tai (thomas.tai@ars.usda.gov or thtai@ucdavis.edu)

Abstract: A quantitative trait loci (QTL) analysis of seedling vigor traits was conducted under dry-seeded conditions using recombinant inbred lines derived from a cross of the California varieties M-203 and M-206. Height at early seedling (HES) and height at late seedling (HLS) stage, growth rate (GR), and fresh weight (FW) were evaluated under controlled environmental conditions. HES, HLS, and GR were also evaluated in outdoor basins over two years. M-203 was significantly better for all traits in the growth chamber, however, no significant differences were observed between the parents in the outdoor basins with the exception of HES in 2015. A map consisting of > 6,000 single nucleotide polymorphisms derived from genotyping by sequencing was constructed to facilitate QTL analysis. Six QTL were identified in the controlled environment, one each on chromosomes 2, 9, 10, and 12 and two at the same position on 1. qFW1 and qHES1 explained 34.6% and 8.7% of the variation observed for their traits and this locus corresponds to previously reported shoot length QTL. All positive effects were contributed by the M-203 parent with the exception of qHLS12. For the outdoor basin environment, the QTL qHES8 (12.3%; M-206 allele) and qHES9 (9.4%; M-203 allele) were detected in 2016, but no QTL were identified in 2015. RIL-187 was consistently among the highest vigor lines in all environments examined. The molecular and genetic resources developed here will allow further investigation of seedling vigor and breeding of improved temperate japonica varieties.

Identification of useful grain quality characteristics in rice mutants using TILLING and forward genetics

HyunJung Kim^A, Areum Chun^B, Mira Yoon^B, and Thomas H. Tai^C

^ADepartment of Plant Sciences, UC Davis, Davis, CA 95616 USA

^BCrop Post-harvest Technology Research Division, National Institute of Crop Science, Rural Development Administration, Suwon, 16429, Korea

^CUSDA-ARS Crops Pathology and Genetics Research Unit, Davis, CA 95616 USA

Presenting author: Thomas Tai (thomas.tai@ars.usda.gov or thtai@ucdavis.edu)

Abstract: Rice (*Oryza sativa* L.) is unique among major cereal crops as the vast majority is used directly for human consumption as whole milled kernels. In this form, rice starch provides the bulk of the daily calorie intake for billions of people worldwide. Major differences in local, eco-regional, or social-cultural preferences require the development of a wide array of grain quality characteristics to meet the needs of diverse consumers. Forward and reverse genetic approaches were applied to identify mutants of interest from populations derived from chemical mutagenesis of the varieties Nipponbare and Kitaake. Forward screens based on visual evaluation of grains for altered translucence resulted in the identification of about 30 mutants which are currently being evaluated for changes in starch properties. Targeting of Induced Local Lesions in Genomes (TILLING) and targeted sequencing by exon capture have been employed to identify mutations in starch synthesis genes. A total of 63 putative mutations in eight target genes were identified by TILLING. Of these mutations, 29 in six genes were confirmed by Sanger sequencing and lines carrying homozygous mutations were isolated. Most of these mutations are novel based on comparison to the natural variation present in the *O. sativa* germplasm of the 3,000 rice genomes project. Scanning electron microscopy and physicochemical tests are being performed to evaluate the effect of the mutations on grain quality. Genetic studies are underway to identify the mutations underlying the mutants identified in forward screens and to examine the effects of combining mutant alleles of starch synthesis genes.

Identification and characterization of reduced epicuticular wax mutants in rice (*Oryza sativa* L.)

Thomas H. Tai^A and HyunJung Kim^B

^AUSDA-ARS Crops Pathology and Genetics Research Unit, Davis, CA 95616 USA

^BDepartment of Plant Sciences, UC Davis, Davis, CA 95616 USA

Presenting author: Thomas Tai (thomas.tai@ars.usda.gov or thtai@ucdavis.edu)

Abstract: In land plants, epicuticular wax works together with other components of the plant cuticle to prevent uncontrolled loss of water and to protect against various environmental challenges. In this project, chemically-mutagenized populations of rice (*Oryza sativa* L.) derived from more than 5,000 M2 families were screened for adhesion of water droplets resulting in a wet leaf/glossy (wlg) phenotype. Mutants were identified in > 15 independently-derived M2 families. SEM analysis confirmed the association of the wlg phenotype with changes in the epicuticular wax crystals of these plants. The phenotypes of five of these mutants have been confirmed to be due to single gene recessive mutations. Evaluation of mutants from three M2 families revealed significant reductions (> 50%) in surface wax content and increased cuticle membrane permeability. Progress on characterization of the mutant phenotypes and mapping the underlying mutations will be presented.