The development of molecular maps and the complete sequencing of the rice genome have created many opportunities for the application of DNA markers in breeding and genetics. Marker-assisted selection (MAS) has become a practical tool in cultivar improvement. MAS is time-saving, very efficient, can promote bio-safety (no need for pathogen or insect pest inoculations during screening trials), reliable, and consistent in dealing with traits whose phenotype is affected by the environment [1].

DNA markers are powerful tools in the indirect selection of important traits at the early growth stages before advancing selected lines to the next generation, thereby speeding up conventional breeding. Selection can be made before flowering, thus allowing breeders to manage crossing and generation advance properly and improve the quality of the breeding materials. DNA markers can facilitate the improvement of traits that are difficult to improve or very expensive to evaluate using conventional breeding methods. For instance, restorer genes in hybrid rice breeding are difficult to evaluate, requiring the testing of spikelet sterility in test cross progenies. MAS can also increase flexibility and efficiency in breeding programs. MAS can reach 95 to 96% accuracy in finding homozygous plants, thus reducing the duration of cultivar release by 2 to 3 years [2]. The varieties ‘Cadet’ and ‘Jacinto’ were released in the U.S. in less than 10 years of breeding work, a rarity in traditional rice breeding. DNA markers are very useful in gene transfer and pyramiding to improve germplasm, as it can accelerate the backcrossing of a gene, group of genes, or minor genes called quantitative trait loci (QTL) into an elite breeding line or cultivar. DNA markers are important tools in selecting plants having the gene or combination of genes of interest and those plants that are genetically similar to the recurrent parents in a large-scale backcrossing program. Markers are also very useful in the transfer and tracking of useful genes found in wild rice into cultivated rice. These DNA markers can also provide a safety net to the environment. Screening for pest resistant plants without having to maintain inoculations of pathogen or insect culture, both in the field and in the greenhouse, is a major advantage. Since the plants with the pest resistance gene can be identified without the presence of the insect or pathogen pest, the introduction and proliferation of new races or biotypes that are resistant pathogens and insects can be avoided or minimized.

DNA markers for important traits in U.S. rice have been developed and several other studies on the development of markers are ongoing. Molecular markers are now available for the granule bound starch synthase (waxy) gene that controls grain amylose content and the soluble starch synthase IIa (Alk) gene that controls alkali spreading value (ASV) [3; 4]. Costly laboratory tests for these traits can be deferred or eliminated by using DNA markers during selection.

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Welcome to the latest issue of *Texas Rice*. The lead article is by Dr. Dante Tabien and focuses on the use of molecular markers to improve the speed with which new rice varieties can be developed. Basically, a molecular marker is analogous to a sophisticated fingerprint. In humans, there are DNA markers that identify the presence of genes that make people more prone to specific genetic disorders. Such markers are increasingly used to provide parents and doctors with better diagnostics information so they can provide patients with the best possible chance to counteract the impact of genetic disorders. DNA markers are also increasingly used to determine if patients have been exposed to harmful diseases and parasites so that an appropriate course of medication can be initiated. Instead of waiting until more easily identifiable advanced symptoms develop, molecular markers can provide for a much more rapid identification of the casual organism and rapid initiation of appropriate medical treatments. Rapid identification has the further advantage of rapidly targeting effective medicines, decreasing the rate at which diseases develop resistance to valuable medicines, and increasing the speed with which patients recover.

The use of DNA markers in rice varietal improvement is in some ways much more advanced than how markers are used in human medicine. Rice plant breeders and molecular biologists have joined hands and discovered a number of DNA markers that are associated with the likelihood of a rice plant being prone to infection by a particular pathogen. This part is analogous to specific humans, due to their genetic makeup, being more sensitive to certain types of disease. More importantly, scientists have also identified, in many cases, which genes allow rice to resist injury by particular diseases. By crossing two rice varieties, each possessing desirable genes that confer resistance to either different disease or different races of the same diseases, and then using molecular markers to select the 2nd generation plants from such crosses that contain both sets of desirable genes, rice breeders can quickly identify favorable offspring and discard progeny that lacks either or both sets of genes, thereby greatly speeding up the varietal developmental process. Drs. Anna McClung and Bill Park found that the use of DNA markers could speed the rate of rice varietal development by a number of years. DNA marker selection of improved rice varieties is now standard in Texas rice breeding programs (and programs in many other states as well) and is used to identify the presence of genes that affect disease resistance and grain cooking quality.

The second article in this issue of *Texas Rice* is by Dr. Omar Samonte and largely focuses on how the agronomic management of rice can influence how much methane is released by rice into the environment. In a nutshell, rice grows best in flooded fields. Flooded rice fields absorb less oxygen from the atmosphere, through the water into the soil. As a result, flooded rice soils tend to develop anaerobic or oxygen deficient environments. An anaerobic environment in the root zone of a flooded rice field results in the rice plants developing specialized cells called aerenchyma, that are basically hollow tubes that allow transport of oxygen from the atmosphere through the outer leaf sheaths to the roots. Unfortunately, the aerenchyma cells also allow transport of methane from the roots, to the atmosphere. Omar discusses how different management approaches can reduce the amount of methane produced by a rice field. Equally interesting, Omar provides data showing that different rice varieties vary tremendously in terms of the amount of methane they transmit to plant leaves.

Please continue to send us your suggestions.

Sincerely,

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Methane and Greenhouse Gases

Nitrous oxide (N\textsubscript{2}O), methane (CH\textsubscript{4}), ammonia (NH\textsubscript{3}), and other trace constituents in the earth’s atmosphere that have infrared absorption bands in the spectral region of 7 to 14 μm contribute to the atmospheric greenhouse effect. A doubling of N\textsubscript{2}O, CH\textsubscript{4}, and NH\textsubscript{3} concentrations causes additive increases in surface temperature by 0.7°, 0.3°, and 0.1°, respectively [1]. Among these greenhouse gases, CH\textsubscript{4} is an end-product of anaerobic fermentation that accumulates in flooded rice soils and is transported to the atmosphere by diffusion, ebullition (emission through bubbling), and plant-mediated transport.

About 86.7% of the CH\textsubscript{4} emission from the paddy soil to the atmosphere is due to plant-mediated transport (diffusion through the stems of the rice plant), while 13.3% is due to ebullition and diffusion [2]. Based on field experiments conducted at the Texas AgriLife Research and Extension Center at Beaumont, total seasonal CH\textsubscript{4} emissions range from 4 to 16 g/m\textsuperscript{2}/season [3] and 22 to 37 g/m\textsuperscript{2}/season in Texas [4], respectively. Understanding the factors that affect methane emission rates is necessary in developing strategies that mitigate the emissions.

Plant-Mediated Methane Transport

The roots of rice plants require oxygen for respiration, and the aerenchyma (well-developed system of air spaces) provides a pathway through which atmospheric oxygen is transferred to the roots. The aerenchyma also provides the pathway for CH\textsubscript{4} emissions from the rhizosphere to the atmosphere. The transport of CH\textsubscript{4} from the rhizosphere through rice plants to the atmosphere [5] starts with the uptake of CH\textsubscript{4} by the roots through dissolution (dissolving of methane in water) and diffusion. Methane in the soil water dissolves into the surface water of the roots, then diffuses into the cell-wall water of the root epidermis cells, and then diffuses through the cell-wall water of the root cortex. The CH\textsubscript{4} is then gasified in the root cortex and transported to the shoots through intercellular spaces (space formed after cells die) and aerenchyma, which are specialized oxygen transport vessels in rice plants. Methane is released to the atmosphere through micro pore openings in the aerenchyma in the leaf sheath of the lower leaf position and through the stomata in the leaf blade [5]. It is also released to the atmosphere through cracks and porous structures found in nodes (not the internodes) of the culms, with CH\textsubscript{4} emission rates increasing linearly as the number of culm nodes increases [7]. Furthermore, panicles can also emit CH\textsubscript{4}, but their contribution is minor [7]. Methane diffuses through rice plants depending on the concentration gradient between the soil and the atmosphere, and on the factors that enhance CH\textsubscript{4} diffusion rates (e.g. increased temperatures and concentration gradient) [6].

Seasonal and Diel Methane Emission Dynamics

Within a rice growing season, methane emissions are higher in later growth stages than in earlier stages, even at the same soil temperatures. This may be due to the larger root systems and larger number and size of tillers at later growth stages, which in turn increase aerenchyma size and facilitate the diffusion CH\textsubscript{4} through the rice plants [8]. It was approximated from a study by Wang et al. [9] that maximum methane emission rates from different cultivars ranged from 0.117 to 0.171 kg/ha/h at tillering stage, 0.508 to 1.660 kg/ha/h at heading stage, and 0.284 to 0.991 kg/ha/h at ripening stage.

Diel CH\textsubscript{4} emission variation is determined by
the diffusion coefficient for CH₄, which in turn is temperature-dependent. In fact, soil temperature is a major factor that explains 78% of the variation in CH₄ emissions, with less CH₄ dissolving in floodwater at higher temperature [8]. Soil temperatures ranging from 30 to 35°C are optimum for CH₄ production in paddy soils [10]. As a result, under ambient temperature, CH₄ emission rates increase as soil temperature rises in the morning, reach a maximum in the early afternoon, and decrease and level off at night while soil temperature declines until sunrise the next day [8; 9]. Methane emission rates also decline when soil temperature remains high for more than two hours, indicating that CH₄ source strength (or CH₄ concentration in soil solution) becomes limited at high CH₄ emission rates [8; 9].

**Rice Establishment Effects on CH₄ Emission**

The method by which a rice crop is established in the field affects CH₄ emission rates. Rice fields established through direct-seeding on dry soil emit the lowest amount of CH₄ (26.9 g/m²/season), compared to direct-seeding on wet soil (37.1 g/m²/season), transplanting of 30-day old seedlings (40.3 g/m²/season), and transplanting of 8-day old seedlings (42.4 g/m²/season) [11]. Rice straw decomposes more rapidly to produce CH₄ in submerged soil (anaerobic condition) than in dry soil (aerobic condition). Direct-seeded rice on dry soil has an aerobic condition during the early growth stages resulting in lower CH₄ production from organic matter, such as rice straw [11]. The methane emission rate is 75% lower in a dry-seeded rice field than in a transplanted field, even when both fields are initially flooded at the same time [12].

**Irrigation Effects on CH₄ emissions**

Compared with continuous flooding, intermittent irrigation (in which there are a few days of no standing water between two irrigations) has a 25% lower CH₄ emission rate, while constant moisture (the field had no standing water, but remains saturated) has a 58% lower emission rate [12]. Methane emissions are lower by 44% with midseason drainage compared with continuous flooding, while emissions are lower by 30% with intermittent irrigation compared to midseason drainage [13]. Intermittent irrigation appears to be a promising method for mitigating CH₄ emissions from rice fields, since it does not affect rice yield (when weeds are properly controlled) and it can be easily applied at the farm level [12]. Flooding decreases soil redox potentials to -150 to -210 mV and methane evolution continues until fields are drained and soil reduction potentials increase [14].

**Cultivar Effects on CH₄ emissions**

Methane emissions rates vary by up to 56% comparing different cultivars [12], and since emission rates are not correlated with grain yield [12], then selecting for cultivars with relatively lower emission rates will not reduce cultivar grain yields. Cultivars with high dry matter production are associated with big root systems, from which high amounts of carbon, which is an important source for CH₄ production, are released. As a result, root dry weight is correlated with both the total amount of carbon released from roots and CH₄ emission rates [12; 15]. Furthermore, cultivars with high tiller numbers at the tillering stage are associated with high CH₄ emission rates, even when plant roots are exposed to the same CH₄ partial pressure. Methane emission rates also increase with leaf area [7]. The development of cultivars screened for high harvest index and high panicle-to-tiller ratio may be one of the strategies in mitigating CH₄ emission from rice fields [15]. Another strategy would be to breed for cultivars with reduced root exudates and litter, but with increased root mass most of which growing in the oxidized soil layer, and to select for cultivars with less CH₄ transportation ability [16]. This latter desirable characteristic might not be possible if reduced CH₄ transportation also means reduced O₂ transportation, since reduced oxygen transport could reduce root survival.

**Nutrient Effects CH₄ emission**

Methane emissions from plots treated with 120 kg/ha N using potassium nitrate, ammonium sulfate and urea were 90, 100, and 220 kg/ha, respectively
Ammonium sulphate and potassium nitrate, may be useful as \( \text{CH}_4 \) emission-mitigating compounds, since they reduced \( \text{CH}_4 \) emissions by 55 and 59\%, respectively, compared to that of urea [14]. Single superphosphate, when applied to a flooded rice field, supplies P to the rice crop and also mitigates \( \text{CH}_4 \) production and emission through its S content [17].

Plant nutrients applied as fertilizer affect the total \( \text{CH}_4 \) emission from flooded rice paddies by their direct or indirect effect on plant growth [18]. Potassium applied as muriate of potash significantly reduces \( \text{CH}_4 \) emission from flooded alluvial soil planted to rice, with the highest \( \text{CH}_4 \) emissions from control plots (125 kg/ha \( \text{CH}_4 \)) and the lowest emissions in field plots receiving 30 kg/ha K (64 kg/ha \( \text{CH}_4 \)), with the latter treatment representing a 49\% reduction in \( \text{CH}_4 \) emission [18]. Potassium application prevents the decrease in the redox potential and reduces the contents of active reducing substances and \( \text{Fe}^{2+} \) content in the rhizosphere soil. Potassium also inhibits methanogenic bacteria (bacteria that produce \( \text{CH}_4 \) by the fermentation of simple organic carbon compounds under anaerobic conditions) and stimulated methanotrophic bacteria (bacteria that grow using methane as their only source of carbon and energy). In addition to being an effective option in mitigating \( \text{CH}_4 \) emission from flooded soils, K fertilization produces higher plant biomass (both above- and underground) and grain yield, especially in K-deficient soils.

In summary, the mitigation of \( \text{CH}_4 \) emissions may require a combination of cultural management and breeding strategies. In a study that ranked several practices for their reduction of greenhouse gasses (\( \text{CO}_2 \), \( \text{CH}_4 \), and \( \text{N}_2\text{O} \)), upland rice was the most effective, followed by shallow flooding, ammonium sulfate, midseason drainage, off-season straw (application of straw two months before planting to reduce the availability of dissolved organic carbon released from fresh straw to methanogenic bacteria), slow-release fertilizer, and continuous flooding [19]. The choice of which greenhouse gas mitigation strategy to use becomes more complicated when the issue of economic sustainability of the rice production system is integrated into the decision process.

For more information, please consult the following references:


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DNA Markers continued ...

in early generation breeding materials. These markers are currently being used in the breeding programs at the Texas AgriLife Research and Extension Center at Beaumont, TX, and several elite lines with the traits were identified without having to conduct the wet laboratory analysis. Resistance to blast disease, which is caused by *Pyricularia grisea* Saccardo, is another important trait for rice cultivars in the U.S. and in the major rice areas of Asia. Markers for *Pita*, one of the most important resistance genes for rice blast, was developed at Arkansas [5], while the markers for 4 genes (*Pi-k, Pi-b, Pi-ta2 and Pi-i*) that are effective against 8 to 10 blast races were identified in Texas [6; 7]. A number of other markers have also been identified. Mapping of QTLs for seedling cold tolerance, which is important in early planting, is complete and markers have been developed in California [8; 9]. Herbicide tolerance genes from Louisiana have been used in several breeding programs for the development of Clearfield rice cultivars, while private companies have been focusing on Liberty link herbicide resistance.

Mapping of traits that are associated with yield and seedling vigor has been done, but the gene mapping for nutrient use efficiency and milling quality have just been initiated. Recently, the submergence gene for flood-prone areas was mapped and the necessary DNA markers were developed through the collaborative work of scientists from the International Rice Research Institute in the Philippines and the University of California at Davis. A list of several important traits in rice and their markers can be found in a review article by Jena and Mackill [1].

The ongoing five-year multi-state and multi-institution program *A Coordinated Research, Education, and Extension Project for the Application of Genomics Discoveries to Improve Rice in the United States*, known as RiceCAP (Rice Coordinated Agricultural Project), focuses on the development and use of DNA markers for rice varietal improvement. The project aims to bridge the gap between basic research on rice genomics and applied research of rice breeding. Specifically, the project will identify and verify the gene(s) for milling traits and resistance to sheath blight, a destructive disease of rice worldwide.

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DNA Markers continued ...

(http://www.uark.edu/ua/ricecap/index.html). It also aims to train conventional rice breeders in using biotechnology-based tools, such as DNA markers, and educate students and other users on the advantages of using these tools in the development of rice cultivars. Scientists from the Texas AgriLife Research and Extension Center and the USDA-ARS unit at Beaumont are currently working on sheath blight resistance and milling traits as part of RiceCAP.

The cost of using the DNA markers and the availability of DNA markers that are tightly linked to the trait or gene of interest are two of the major constraints to the large-scale and wide-spread application of DNA markers in crop breeding. However, the cost of DNA markers is decreasing very quickly and is increasingly becoming less of a constraint. The development of user-friendly, high throughput and automated methodology in using DNA markers will improve efficiency and cost-effectiveness. At the Texas AgriLife Research and Extension Center at Beaumont, in addition to screening breeding lines for the waxy gene, MAS is also used to screen for resistance to rice blast and for semi-dwarfness (Fig. 1). Breeding lines selected from the observational nursery are evaluated as to whether they contain these markers. For blast resistance, different markers confer resistance to different races and the markers being used include those linked to the Pi-b, Pi-ks, Pi-kh, Pi-ta2, and Pi-z blast resistance genes. For semi-dwarfness, breeding lines are evaluated to determine whether they contain the marker for the sd-1 gene, which is typical of most U.S. cultivars.

For more information, please consult the following references:


Fig. 1. Marker-assisted selection being conducted at Texas AgriLife Research and Extension Center at Beaumont, TX. (Photo by Patrick Frank)
DNA Markers continued ...


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As of September 5, 2008, 100% of the rice acreage in Texas had reached the heading stage, while 90% had been harvested.