Molecular Improvement of Rice Quality

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ABSTRACT

When a new variety of rice is released from a breeding program, it must undergo two major stages to determine whether it will persist. The first is adoption by farmers, and the second is acceptance by consumers. The latter will determine whether a variety becomes popular or not. For this reason, rice improvement programs aim to develop varieties that combine agronomic performance and good grain quality. Grain quality is assessed by a set of routine measurements that are rapid and low cost. The limitation of these methods is that they are not strongly linked to the sensory experience of eating rice. This is complicated by the fact that it is difficult to describe that experience and then link the descriptions to components of the grains. Increasingly, these routine tools are used as phenotyping tools to search for associated genes and mutations. These mutations are then developed into molecular markers that enable breeders to select for quality early in the process. For the physical traits of quality, this is much simpler, because length, shape, chalk, and broken grain can easily be measured and described. Several markers have been developed for some of the traits that impact sensory properties, but there is still a long path ahead to develop a complete suite of markers for both physical and sensory properties of rice.

Background

Rice quality is becoming more important in rice-consuming countries as progress is made on the Sustainable Development Goals set by the United Nations, and people have more discretionary income. Studies have shown that when discretionary income increases, some of those additional resources will be directed toward the purchase of higher quality rice (19,39). The quality of rice is assessed by a number of low-cost, high-throughput tests that provide a certain amount of information, but the tests do not always differentiate varieties (11). Our previous research investigated samples of rice from many countries and found that varieties considered by consumers to be different are not separated by classic tests of quality (10). Therefore, it is important to understand the sensory differences that are important to consumers in different markets and then develop tools to screen for these qualities reliably and robustly.

The rice quality traits that are evaluated in both domestic and global markets are physical, cooking, and aromatic properties. The most important physical traits are grain size, chalkiness, and milling properties. Grain dimensions are highly heritable (12,39,60), as are chalkiness and the propensity to break (61,64). While chalk and broken grain both have elements of heritability, they are both heavily impacted by environment. For chalk, high temperature and humidity during grain-filling is the key factor (61,62), and for broken grains, postharvest treatment has the largest impact (44). Currently, physical properties of rice grains are measured on a range of instruments, such as the newly released Q-Sorter (4), which uses image analysis and artificial neural network technology to provide the metrics of the physical qualities of a sample of rice.

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The cooking quality of rice is measured in most programs using routine, low-cost methods that indicate different sensory properties. For example, amylose content indicates grain firmness and stickiness (30,31) and is measured by the iodine binding test. The aromatic compound, 2-acetyl-1-pyrroline (2AP), is quantified by smelling the rice or by gas chromatography (5,37).

Molecular Breeding Strategies

Over the last decade, increased interest in understanding the molecular and genetic basis of grain quality traits in rice has led to identification of many genes that regulate and control these traits. The discovery of these genes and the polymorphisms that are widely distributed in different rice backgrounds has made it possible to develop functional markers that can readily be used and incorporated in marker-assisted selection (MAS) in breeding programs.

Molecular methods used to screen for rice quality include the detection of genetic mutations that alter the way a gene is expressed and, therefore, translate to a measurable difference in the phenotype. These mutations can include single nucleotide polymorphisms (SNPs), markers for small insertions or deletions (InDels), and simple sequence repeats (SSRs). The utility of using genetic polymorphisms to measure rice quality relies on the robustness and relevance to the trait of the phenotyping method that is used to identify the associated genotype. The benefit of possessing a suite of molecular tools to measure rice quality is that DNA is best extracted from young leaf tissue, whereas assessment of grain quality can only be done on mature grain after harvest. Because they provide a quick selection tool during early stages of plant development, access to a portfolio of robust and trait-specific molecular markers can save months of field work. The most commonly used PCR-based markers are summarized in Table I.

PCR-based markers are commonly used worldwide due to their simplicity and the lesser need for instrument investments. However, accelerated development in genotyping strategies has amplified the efficiency of MAS while reducing the cost (48). Therefore, numerous breeding programs are now shifting to multiplex genotyping and developing custom in-house assays that incorporate trait-based markers such as stress and disease resistance, physiological and agronomic traits, and grain quality (1,38).

Molecular Improvement of Physical Quality

Physical quality traits include

- The dimensions of length, width, and breadth of white grain
- Grain transparency
- Presence of chalk in the grain
- Grain color on the yellowness spectrum
- Propensity of the grain to break

The physical attributes of the grain are the things first noticed by consumers, and this information is encoded by consumers

Table I. PCR-based functional markers developed for molecular screening of rice quality traits in breeding programs[a]

Trait	Target Gene/ Allele	Type	Forward Primer Sequence (5′ to 3′)	Reverse Primer Sequence (5′ to 3′)	Allele Specific Primer Sequence (5′ to 3′)/Restriction Enzyme[b]	Reference
Grain length						
	GS3 exon 2 C/A SNP	SNP	TGCCCATCTCCCT CGTTTAC	GAAACAGCAGGCTG GCTTAC	*Pst*1 RE	Fan et al. (23)
		SNP	AGGCTAAACACATG CCCATCTC	CCCAACGTTCAGAA ATTAAATGTGCTG	IRSP: AACAGCAGGCTGGCT TACTCTCTG IFLP: ACGCTGCCTCCAGAT GCTGA	Gandhimani et al. (28)
Grain width						
	GW2 1 bp deletion	InDel	CTACACAATGTCC ATTCTGCAAAT	CCACGATACTCCAC AGCATAACT	*ApoI* RE	Ya-dong et al. (57)
	GW5 1,212 bp deletion	InDel	GCGTCGTCAGAGGT AGA	GTGGGATAGGATG AAACC	N/A	Weng et al. (54)
Aroma						
	Fgr exon 7 3 SNP 8 bp deletion	InDel, SNP	TTGTTTGGAGCTT GCTGATG	AGTGCTTTACAAAG TCCCGC	IFAP: CATAGGAGCAGCTGAA ATATATACC INSP: CTGGTAAAAAGATTAT GGCTTCA	Bradbury et al. (8)
	Fgr exon 2 7 bp deletion	InDel	TACTTGGCCCGGA CGGCGCC	TACTTGGCCCGGAC GGCGCG	GAGGCGCTGAAGAGGAACCG	Li et al. (32)
Amylose						
	GBSSI CT repeats	SSR	CTTTGTCTATCTC AAGACAC	TTGCAGATGTTCTTC CTGATG	N/A	Ayres et al. (3)
	GBSSI Exon 1 G/T SNP (Wx^b)	SNP	CAGGAAGAACAT CTGCACGG	TTTCCAGCCCAACA CCTTAC	ATCAGGAAGAACATCTGC ACGT	Chen et al. (14)
	GBSSI Exon 2 duplication	InDel	TGCAGAGATCTTC CACAGCA	GCTGGTCGTCACGC TGAG	N/A	Wanchana et al. (52)
	GBSSI Exon 4 A/G SNP (Wx^{op})	SNP	TGCTACAAGCGTGG AGTGGA	ACCAGTACAAGGAC GCTTGG	*Acc*I RE	Fitzgerald et al. (25)
	GBSSI Exon 6 A/C SNP (Wx^{in})	SNP	CCCATACTTCAAA GGAACATA	GGTTGGAAGCATCA CGAGTT	TCTTCAGGTAGCTCGCCAGT	
Gel temp						
	SSIIa Exon 8 A/G SNP	SNP	AGAACGACTGGAAG ATGAACG	GATGTCCACACCTT TCTGCC	CTTGCACCGCGGCTTGCC	Cuevas et al. (18); Waters et al. (53)
	SSIIa Exon 8 A/G SNP	SNP	GGGTGGGTGGGGTT CTCG	CACCATTGGTACTT GGCCTTGAC	GCGGGCTGAGGGACAGCA	
	SSIIa Exon 8 GC/TT SNP	SNP	GGGTGGGTGGGGTT CTCG	CACCATTGGTACTT GGCCTTGAC	GCCGCGCACCTGGAAA	
Chalk						
	Chalk5	SNP	TCAAAAACCACCAT TCGAAATGA	TGTGTATAAACATTT TTGTTGTTTTTGCA	C-F: CATTTTCAGTGCGTCC AAAACC C-R: AAGTTATATACGGTGC GTCTCATGGA	Tao et al. (46)
Gel consistency						
	GBSSI exon 10 SNP	SNP	GCATCACCGGCAT CGTC	GCTCCGGCCATGAT GAGATG	*Apa*I RE	Tran et al. (49)

[a] SNP: single nucleotide polymorphism; SSR: simple sequence repeat; InDel: marker for small insertions or deletions; IRSP: internal reverse short primer; IFLP: internal forward long primer; IFAP: internal fragrant antisense primer; INSP: internal nonfragrant sense primer.
[b] Digestion with a restriction enzyme (RE) after amplification.

and decoded in their decision to purchase or not. Both visual and semantic encoding is used to make decisions about food quality (29). If the rice is broken, the consumer knows it will be sticky due to starch leaching during cooking and forming a glue around the grains. If the rice is yellow, the consumer could conclude that the rice is old. It is important, therefore, that the physical quality of rice is good and that breeding programs can select for those physical traits that can be measured. It would be a breeder's dream to have a panel of molecular tools available for assessing the complete physical quality of rice grains and to be able to do this at the first and second leaf stages.

There are several molecular tools that have been identified for assessing the physical quality of rice, and these include markers for grain length and width and for chalk. The lack of markers for transparency, yellowness, and broken grain is due to the lack of a phenotyping tool, lack of significant variation in the trait, or multiple causes of the phenotype. The percentage of broken grain can also be affected by harvesting techniques, postharvest handling, and moisture transitions in the grain (40,55). Yellowing, on the other hand, increases throughout storage and aging (6). Therefore, it is difficult to find genes that are associated with broken and yellow grain.

Chalkiness in rice is one of the simpler physical traits to phenotype due to the advances in image analysis tools that provide good phenotyping data for gene-mapping studies. Using biparental mapping, six stable quantitative trait loci (QTLs) have been reported (61). Individually, the presence of each QTL leads to a small and measurable difference in chalkiness, but the combination of all six leads to a significant decrease in chalk. One of the QTLs, found on chromosome 5 and termed Chalk5 (33) contains a *vacuolar proton translocating pyrophosphorylase* gene. This QTL positively contributes to low chalk in the genetic backgrounds studied by Zhou et al. (63). However, this QTL in other backgrounds is not sufficient to reduce chalk completely (61). Research to identify and clone genes for chalk did not progress after about 2016, and this is most likely due to the loss of phenotyping capacity. Recently, phenotyping capacity for the degree of chalk has been restored due the launch of a new instrument, the Q-Sorter (4), which is able to provide excellent data on the distribution of chalk (Fig. 1). The deployment of this instrument to rice research programs should enable new research into the genetics of physical quality to commence again.

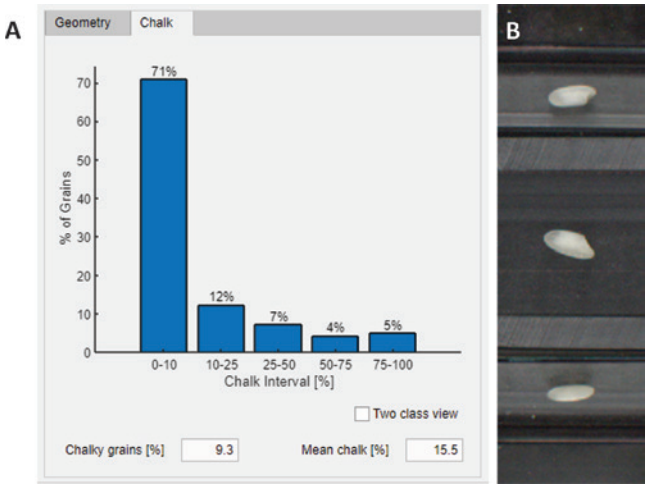

Fig. 1. A, Analysis of rice chalkiness using the Qualysense Q-sorter. Percentage of grains with chalk (chalky grains %), mean chalkiness values, and distribution of chalk in the grains, are shown. **B,** Capturing three angles of a single rice grain increases the precision of the image analysis algorithm.

Like chalk, the length and width of the grain can be measured quite accurately, enabling the phenotypic data to be used in association mapping. Several QTLs have been identified for length and width, and these have recently been reviewed (16,63). The two major genes associated with grain length and width are *GS3* (22), *GW5* (34,54), and *GW2* (45), but many others have been identified (23,28,35,57). There is a wide diversity in the range of grain length and width, illustrating that these traits must be controlled by combinations of genes, rather than a single mutation (24). Zhou et al. (63) reviewed the genetic evidence and proposed that grain shape is controlled by three major biochemical processes: the hormones brassinosteroid, auxin, and cytokinin; G-protein signaling; and proteasomal degradation. The many steps in all these pathways suggest a rich resource for determining the combination of mutations that lead to each class of shape, and those types that are between the major length classes of short, medium, and long and width classes of slender and bold.

Molecular Improvement of Cooking and Eating Quality

Cooking quality refers to the amount of time the rice takes to cook, in terms of energy consumption, and in some countries, the amount of water that is absorbed. The more water that is absorbed, the larger the resulting bowl of rice. Markers for water absorption in terms of extending the food value of rice have not been studied, but there are several studies that have investigated water absorption in terms of grain elongation during cooking of basmati rice (2). Arikit et al. (2) discovered three QTLs that are associated with elongation of cooked rice: two of these are on chromosome 6, and one is on chromosome 4. The elongation of rice is a quality factor for consumers of basmati rice, but the absorption of water to extend the serving size of the rice is a quality factor for poor rice consumers. The viscosity curves for two varieties of rice with the same amylose content (high) are shown in Figure 2A; the height of the curves differ significantly between the two varieties. In Figure 2B, the same data, normalized to remove the differences in height, shows that the shape of the two curves is the same, except for an offset in

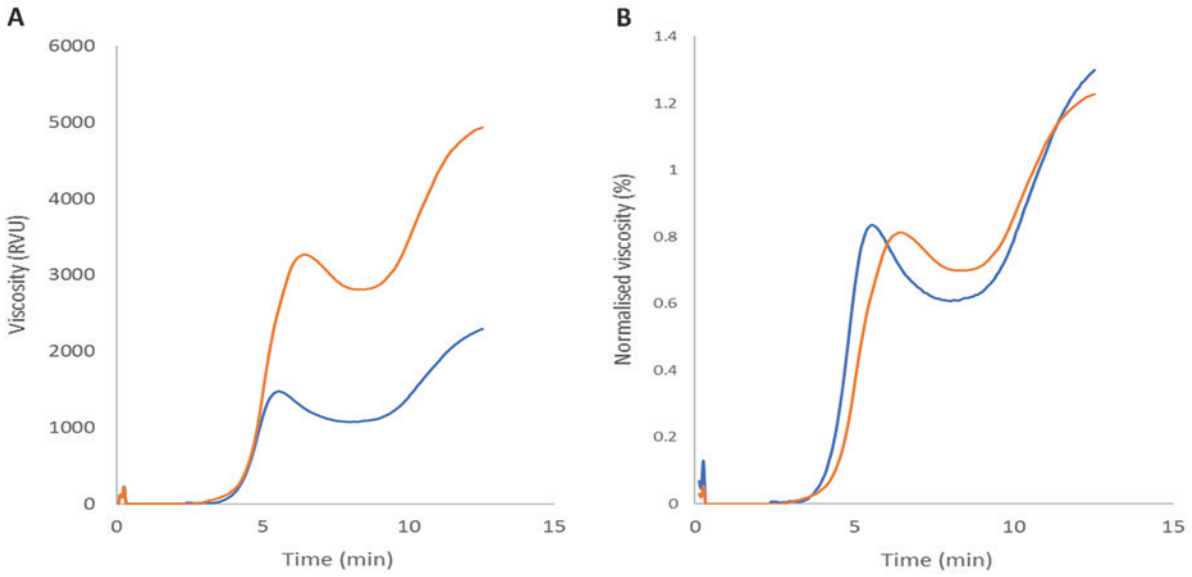

Fig. 2. Rapid visco analyzer curves for two varieties of rice with the same amylose content (**A**) and normalized curves with swelling removed (**B**).

peak time, which is related to differences in gelatinization temperature (GT). The difference in the height of the curves is likely due to the amount of water the starch absorbs while climbing to the peak (Fig. 2A); this is an area warranting future investigation. Several studies report a negative association between peak height with amylose content (15,43) and amylose structure (47). These studies suggest that as amylose leaches from granules, the amylose polymers inhibit the swelling of the amylopectin molecules and starch granules. Therefore, any effort to identify genes for peak swelling must account for both amylose content and amylose structure.

Amylose content and structure are both strongly related to the stickiness and texture of rice (26,31,41). The *Waxy* gene encodes Granule Bound Starch Synthase, which is the enzyme responsible for the synthesis of amylose (42). There are five alleles of the *Wx* gene (13). The association between amylose content and *Wx* haplotype, using 110 diverse varieties of rice, is shown in Figure 3A. The haplotypes are determined by a range of SNPs in different coding regions of the gene (13). With the development of CRISPR (clustered regularly interspaced short palindromic repeats) technology, whereby the DNA can be altered, several groups have worked on the *Wx* gene to fine-tune the amount of amylose in the grain and save several years of plant breeding trials (56,59).

In the untranslated region of the *Wx* gene, there is a region of small sequence repeats of C and T that range from 8 to 22 repeats (3). Many rice breeding programs use these CT repeats to determine the *Wx* gene that is in their breeding material. In a diverse set, amylose content is not associated with the CT repeat (Fig. 3B), but within a narrow breeding program, the CT repeat has been used. For example, in evaluating the progeny from a cross between two parents that differ in the CT repeat, the amylose content can be evaluated at an early stage of the breeding program. One perplexing fact about the CT repeat is that the benchmark varieties from each germplasm class for high quality: Koshihikari, Khao Dawk Mali 105, IR64, and Basmati 370, all carry 17 CT repeats. It would be an interesting endeavor, using current biotechnology tools, to insert 17 CT repeats into the same genetic background in each germplasm class to determine whether there is something unique and special about the amylose that is synthesized when the *Wx* gene contains 17 CT repeats.

Retrogradation, or hardening of the cooked rice upon cooling, is another trait linked to amylose. As the rice cools, the amylose polymers aggregate and form a network. A SNP on exon 10 of the *Wx* gene was associated with retrogradation, and this was explained by the difference in hot water-soluble and -insoluble amylose molecules (49).

GT is an important trait of cooking quality. GT ranges from 55 to 80°C (18), and this has a major impact on the time it takes to cook rice and, thereby, the energy required to cook it. When GT is high, the texture of the cooked rice is soft, and the grains break (18). GT is usually measured by differential scanning calorimetry, which determines the temperature at which the starch crystals melt and the energy required to melt the starch. The major gene that governs GT is *Starch Synthase 11a* (*SS11a*) (50). There are four haplotypes of this gene: two explain low GT, and the other two explain high GT. The phenotypic impact of the haplotypes is the length of the chains in the clusters of the amylopectin molecule. If the chain length within the clusters is long and uniform, the crystals are considered perfect, and they require more energy to melt. Two of the haplotypes impact the activity of the enzyme encoded by *SS11a*: the chains within the cluster differ in length, and the crystals are imperfect (17,18,50).

The four haplotypes do not account for intermediate GT, suggesting there are additional loci that impact GT (58). Additionally, the energy required to gelatinize the starch is likely related to different structures of the amylopectin molecule. This feature has not been studied, and this could relate to cooking time and cooked rice texture.

It is important to understand the genetics of GT for any rice improvement program that transitions completely to using marker-assisted selection, because GT has a such a large impact on cooking and sensory properties, and in our recent work, high temperatures during grain-filling raised GT by 6–10 degrees Celsius (unpublished data).

Molecular Basis of Fragrance

Aromatic rice is highly prized by many consumers and fetches the highest prices in the international rice market (9). The major gene for aroma is the *amino aldehyde dehydrogenase* gene (*AADH*) (7,51). Mutations in *AADH* lead to the accumulation of 2AP, so the presence or absence of aroma can be determined using a marker for the mutation in *AADH* (8). However, this

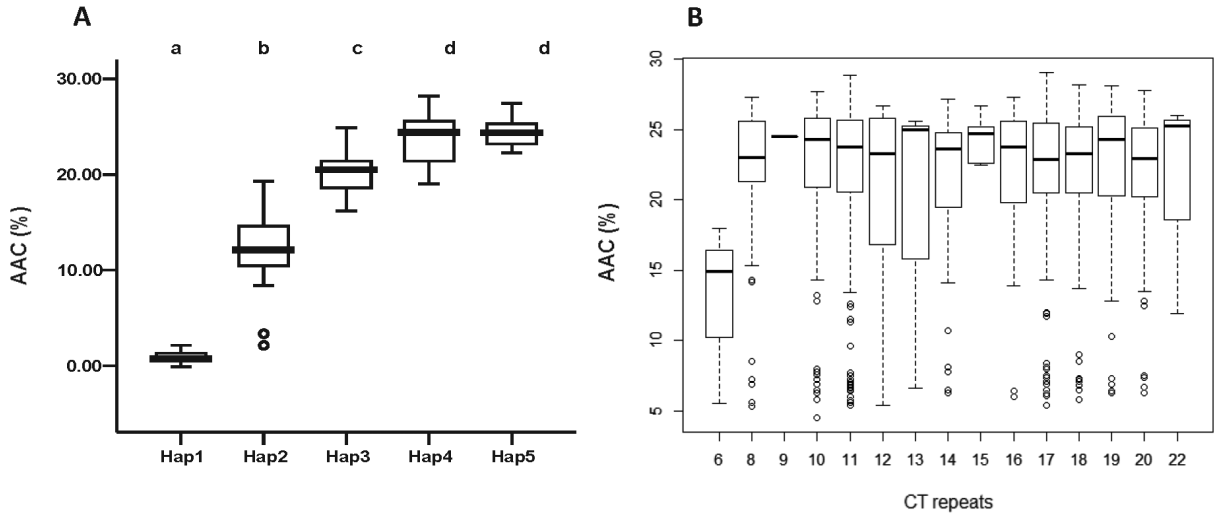

Fig. 3. Association between amylose content and **A,** *GBSSI* haplotypes of the *Waxy* gene (*N* = 110); and **B,** *GBSSI* CT repeats (*N* = 1,059).

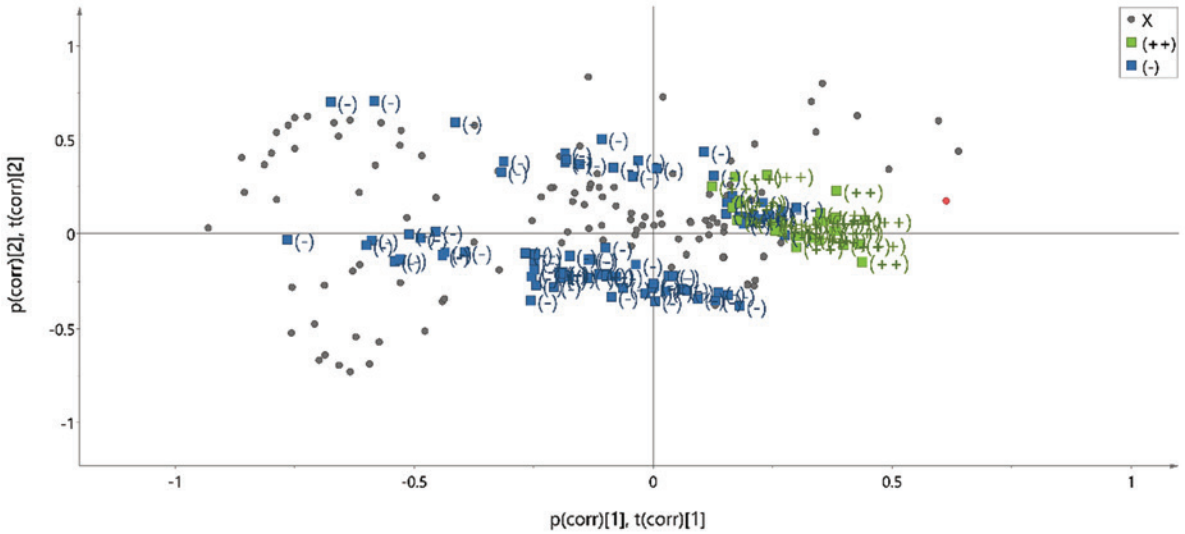

Fig. 4. Principal component analysis biplot of volatile compounds (circles) versus fragrant rice varieties (boxes) showing the accumulation of 1-pyrroline (red circle) in a rice variety carrying the 2AP quantitative trait loci in chromosome 1 (green squares, marked ++). PC 1 = 18.2%; PC2 = 13.

gene only indicates that the rice is aromatic. There is significant variation in the amount of 2AP in different varieties of rice (21), which suggests that other loci contribute to the accumulation. A second QTL for aroma has been described that potentially participates in the acetylation of 1-pyrroline to create 2AP (20). This QTL is located on chromosome 1, and the candidate genes around the QTL regions are *thiamine pyrophosphate kinase* (*TPK1*), *glycerol-3-phosphate acyltransferase* (*G3PAT*), and *acyltransferase* (*AT*). A positive haplotype ('T' SNP) in this QTL was reported to accumulate higher levels of 1-pyrroline, but lower levels of 2AP (Fig. 4). Accumulation of 1-pyrroline in rice reduces the amount of rice fragrance in several ways: 1) 1-pyrroline is not fragrant; 2) direct acetylation of 1-pyrroline to 2AP is unlikely to occur in rice; and 3) 1-pyrroline volatilizes to the air, reducing the amount of substrate (4-aminobutanal) for 2AP production (20).

Conclusions

The capacity of science to undertake genomic discovery has been improving exponentially for a number of years (36), and we have reached the summit, with full sequencing of multiple genomes now possible (27). The task of finding new genes for rice quality is impeded by two issues. First, our capacity to phenotype traits of quality is not as advanced as the available biotechnology tools. Recently, a new instrument was launched that enables the phenotyping of a range of physical attributes of the grain. However, our ability to phenotype many of the indices of cooking quality is not at the level where the metrics explain sensory quality. Herein lies the second limitation. Our knowledge of how the components of the grain explain sensory quality is quite limited. Until we are able to describe how a change in the amount or structure of any of the parts of the grain impacts the sensory experience of eating the grain, we are limited in our ability to use the full potential of the molecular toolbox to improve rice quality.

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