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Breeding Rice for Improved Grain Quality

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Abstract

Rice grain quality improvement has become very crucial for most breeding programs around the world. Grain quality is a complex trait which comprises milling, appearance (grain size and chalkiness), cooking and eating (starch properties including apparent amylose content (AAC), gelatinization temperature (GT), gel consistency and paste viscosity measured using rapid visco analyzer measured using rapid visco analyzer (RVA) as well as nutritional quality. Many genes/quantitative trait loci (QTLs) for the various quality traits have been identified/cloned. This has enabled the development of functional markers to facilitate the selection for this complex trait. Functional markers, especially those targeting mutations in the *BADH2*, *waxy*, *alk* and *GS3* genes, are highly associated with aroma, AAC/RVA, GT and grain size, respectively; and thus effective for marker-assisted breeding. Different alleles can be combined through gene pyramiding to improve rice grain quality for various consumers. To be able to meet future needs, rice breeders must exploit modern marker technologies such as genomic selection (GS) to take care of the effects of both major and minor genes for grain quality as well as high yield, abiotic and biotic stress tolerance.

Keywords: rice, grain quality, molecular markers, aroma, *waxy* gene, *alk* gene, *GS3* gene

1. Introduction

Rice is the most important source of calories for at least 50% of the world's population. Consequently, many countries around the world have strategies to achieve self-sufficiency in rice production by expanding the area under cultivation and or increasing yield per unit area. However, for rice, grain quality is as important as yield. This is because unlike other cereals, which are usually processed as food or feed (for animals), rice is mainly eaten as whole cooked grains by humans.

Breeding for consumer-preferred grain qualities have thus become a major goal for breeding programs around the world. To be able to breed for specific consumer preferences, grain

quality must be clearly defined and the genes underlying their control deciphered. The rice grain is basically composed of the lemma and palea which form the hull, the bran, embryo and the endosperm (white rice) (**Figure 1**). Scientists have classified the grain quality of rice as milling, appearance, cooking and eating and nutritional aspects.

The milling quality of rice determines the yield and appearance of the rice after the milling process. It is thus sometimes classified as under appearance quality. The first step in milling involves the removal of the lemma and palea to obtain de-hulled rice called brown rice.

Figure 1. The rice grain. Source: IRRI.

Brown rice has become very important because it has good nutritional value obtained from the presence of the bran. The bran which consists of the aleurone, pericarp and embryo is removed to obtain milled rice. Milling quality thus comprises of brown, milled and head rice recovery (HRY). Brown rice recovery is the percentage of brown rice obtained after de-hulling a sample of paddy. Brown rice absorbs water poorly and does not cook as rapidly as milled rice. Milling recovery is the percentage of milled rice (including brokens) obtained from a sample of paddy. HRY is the percentage of head rice (excluding brokens) obtained from a sample of paddy. It normally includes broken kernels that are 75–80% of the whole kernel. High HRY is one of the most important criteria for measuring milled rice quality. Broken grain has normally only half of the value of head rice. HRY is influenced by genotype (i.e. the potential HRY), production factors and harvesting, drying and milling process. HRY and the degree of milling greatly influence the way the rice appears and thus consumer preference for rice on the market.

Appearance quality is how the rice appears after milling and it is associated with grain length, width, length-width ratio (shape) and translucency/chalkiness of the endosperm. Generally, most markets prefer translucent rice as opposed to chalky ones. Preferences for grain size and shape vary across different countries and cultures. Short grain varieties are preferred in Japan, Korea, Northern China and Sri Lanka while Southern China, India, Pakistan, Thailand, the USA, as well as most African countries prefer long and slender grain rice. Appearance quality has a direct influence on marketability and success of commercial varieties.

Cooking and eating quality is the easiness of cooking as well as texture, springiness, stickiness and chewiness of cooked rice. These characteristics are controlled by starch physicochemical properties comprising of apparent amylose content (AAC), gelatinization temperature (GT), gel consistency (GC) and paste viscosity properties. Starch makes up about 90% of the rice grain.

Rice starch is composed of two classes of glucose polymers—amylopectin and amylose. Amylose is a lightly branched linear molecule with a degree of polymerization (DP) of 1000–5000 glucose units and amylopectin has a much larger polymer unit containing frequent α -1,6 branching linkages [1, 2].

Amylose content is usually referred to as apparent amylose content (AAC) in literature because the iodine-based assay used for measuring it often detects long-chain amylopectin in addition to the “true” amylose [3]. AAC is the most important element influencing the cooking, eating and processing characteristics of rice [4]. The AAC of rice is known to play a crucial role in determining its cooked texture. It is directly related to water absorption, volume expansion, fluffiness and separability of cooked grains and inversely related to cohesiveness, tenderness and glossiness [5]. Typically, cereal grains contains 20–30% amylose with the remainder (70–80%) being amylopectin [6]. Rice can be classified based on its amylose content: waxy rice (0–2% amylose), very low amylose (3–9%), low amylose (10–18%), intermediate amylose (19–23%) and high amylose (>23%) [7, 8]. Synthesis of amylose is catalyzed by the granule-bound starch synthase (GBSS) protein which is encoded by the *waxy* gene (*Wx*) [9].

There are three alleles of the *waxy* gene—*Wx*, *Wx^a* and *Wx^b* which exist in waxy (sticky) rice, indica and japonica sub-species, respectively [10]. Rice plants with the *Wx^a* allele accumulate

more GBSS protein in the endosperm during grain filling than plants with the Wx^b allele; hence indica rice generally has a higher content of AAC than japonica. The splicing pattern of the first intron of the *waxy* gene is reported to be highly correlated to the level of AAC [11]. The authors reported that transcripts in which the intron is completely spliced out would produce high AAC, ones with introns completely un-spliced would produce grains with no AAC (sticky rice) and those with partially spliced introns would produce intermediate AAC.

Gelatinization temperature (GT) is the range of temperature wherein at least 90% of starch granules swell irreversibly in hot water with loss of crystallinity and birefringence [12]. GT ranges from 55 to 85°C and determines the cooking time of rice [13, 14]. According to Tian et al. [72], “GT is a physical-chemical property which directly reflects the cooking quality of rice grain in terms of energy and time needed for cooking”. Cooking time for rice can be reduced by up to four minutes by lowering the GT of the grain [14]. GT of rice can be classified as low (55–69°C), intermediate (70–74°C) or high (75–79°C) [15]. GT also plays an important role in water uptake, volume expansion and kernel elongation of cooked rice [16]. For example, high GT rice elongates less and is likely to be undercooked when standard cooking procedure is applied but its texture could be softened through overcooking. Although rice grain quality preferences differ around the world, varieties with intermediate GT are generally preferred [15]. GT is predominantly determined by amylopectin structure [17]. Three classes of enzymes including starch synthase, starch branching and starch de-branching enzymes have been implicated in the synthesis of amylopectin [9, 18, 19]. The gene, Starch synthase II (*SSIIa*) has been found to be the major determinant of GT [1, 17].

There two types of rice amylopectin—L- or S-types [19]. For L-type amylopectin the number of short α -1,4-glucan chains with degree of polymerization (DP) of ≤ 10 was less than 20% with DP ≤ 24 . The amylopectin fine structure of japonica has more DP 7–11 chains and fewer $12 \leq DP \leq 24$ chains than indica [17]. Amylopectin side chains of DP ≥ 10 form double helices; the length of these double helices determines gelatinization temperatures of starches [20]. Starch granules with amylopectin containing longer A and B1 chains would, therefore, be more resistant to gelatinization [17]. Consequently, japonica starch granules have lower GT than indica starch granules [21]. GT is highly correlated with alkali spreading value (ASV), which reflects the disintegration of milled rice in dilute KOH [8, 21].

Amylose content is the primary measure of the texture of cooked rice, but it fails to describe precisely the texture of certain types of rice [2]. Gel consistency (GC) is, therefore, used to complement AAC. Rice with the same amylose content can be classified as hard gel consistency (26–40 mm); medium gel consistency (41–60 mm); or soft gel consistency (61–100 mm) [22]. The length of gel flow is inversely proportional to GC; therefore, the long gel length corresponds to soft GC and the short gel length means hard GC. Amylopectin, rather than AAC, has been reported to be the major determinant of GC [23]. GC is reported to be affected by milling (lipid content), protein content, aging of milled rice (fat oxidation) and rice flour particle size (efficiency of dispersion) [24].

GC measurements are reported to have poor repeatability, and some laboratories have replaced them with starch paste viscosity parameters [8]. Starch viscosity curves are useful for breeding because the shape of the curve is unique to each class of rice [25]. Their use for

breeding purposes was however limited because the traditional equipment used for measurement, Brabender Viscoamylograph (Brabender OHG, Duisberg, Germany), has many disadvantages. It requires a large amount of rice flour (40–50 g) and a long period (80–94 min) is required to run a sample [8, 25]. It has now largely been replaced by Rapid Visco Analyser (RVA, Newport Scientific Pty Ltd., Warriewood, Australia). RVA is popular because it is easy to operate, gives rapid results and requires a small sample (3 g) to run [25]. The rice starch viscosity profile, tested on the Rapid Visco Analyser, is usually called RVA profile. The RVA profile is generated by subjecting rice flour to a “heat-hold-cool-hold” temperature cycle [25]. This cycle (RVA) mimics the process of cooking and monitors the changes to slurry of rice flour and water, during the test [8]. The primary RVA parameters include peak viscosity, PV (first peak viscosity after gelatinization); trough or hot paste viscosity, HPV (paste viscosity at the end of the 95°C holding period) and final or cool paste viscosity, CPV (paste viscosity at the end of the test) [26]. Secondary parameters derived from the primary ones include breakdown (BD = PV – HPV); setback (SB = CPV – PV); consistency (CS = CPV – HPV); set back ratio (SBR = CPV/HPV) and stability (ST = HPV/PV) [26–28]. Other parameters include peak time (time required to reach peak viscosity), and pasting temperature (temperature of initial viscosity increase) [29].

Breeding for improved grain is complex because many of the quality traits are phenotyped using subjective and or expensive biochemical methods. Consequently, the scientific community has map/clone many quantitative trait locus (QTLs)/genes for various quality traits and developed molecular marker to facilitate selection for specific grain quality types.

In this chapter, the major breakthroughs in studying the genetics of grain quality will be highlighted and its usefulness in grain quality improvement as well as future trends in rice breeding is discussed.

2. Genetic analyses of grain quality in rice

2.1. Genetics of milling and appearance quality

2.1.1. Genetics of milling quality

Milling quality comprises of brown, milled and head rice recovery. It is a complex trait and its genetics has not been fully deciphered. A good review of studies carried out on milling quality has been done by Bao [30]. At least 20, 19 and 34 QTLs have been identified for brown, milled and head rice recovery, respectively [30].

2.1.2. Genetics of appearance quality

Many QTLs associated with grain size and length have been identified, including one QTL on chromosome 3 with a major effect on grain length/size [31–36]. The QTL on chromosome 3 was mapped to a region of 93.8 kb in length [31, 32]. Wan et al. [40] established that a major QTL controlling grain length, *qGL-3a*, was a single Mendelian gene—long grain was controlled by a recessive gene, *gl-3*. They further mapped the *gl-3* gene to a region of 87.5 kb

and suggested that the *gl-3* gene could be the same as a grain size gene mapped for rice grain weight [31]. This gene, subsequently referred to as *GS3*, was cloned through comparative sequence analysis [37] and position cloning confirmed by transformation [38]. The researchers found that all the varieties with large grains had a nonsense mutation, in the second exon of the *GS3* gene which caused a 178-aa truncation in the C-terminus of the putative protein [37–39]. They found that the *GS3* locus was a major QTL for grain length and weight, and a minor QTL for grain width and thickness. Slender shape grain (large length:width ratio) was also reported to be controlled by the *GS3* locus (*gl-3* gene) [38, 40].

A seed width (*SW5*) QTL on chromosome 5 [41, 42] and a grain width and weight (*GW2*) QTL on chromosome 2 [43] have also been cloned. In all these cases, the authors reported that genotypes with the recessive allele(s) have longer, wider and/or heavier seeds than genotypes with the wild type allele and concluded that, in each case, the genes affect cell division.

More recently, a gene on chromosome 3, that controls grain length (*GL3*) and encodes protein phosphatase with Kelch-like repeat domain (*OsPPKL1*) has been identified and cloned [44–46]. The novel *qGL3-1* allele increased in a new variety [46]. Two other genes for grain width chromosomes 5 [47] and 8 [48] have been cloned.

The cloning of these genes provides a basis for marker-aided selection and QTL pyramiding in breeding for appearance-quality. Transgressive segregation for grain dimensions has also been reported by several authors [49–51]. This could make gain from selection very feasible.

2.2. Genetics of cooking and eating quality

2.2.1. Genetics of amylose content

Conventional genetic studies have revealed that AAC is controlled by one major gene with several modifiers [52–54]. High amylose content was reported to be dominant over low and intermediate amylose content [52, 53]. Other studies concluded that AAC was under the control of two complementary genes [54]. Using diallel analysis, the gene action for AAC was found to be mainly additive although dominance effects were also involved [55]. The authors found no evidence of maternal effects in their study. However, He et al. [60] said that the “inheritance of grain quality is more complicated than that of other agronomic traits in cereals due to epistasis, maternal and cytoplasmic effects, and the triploid nature of endosperm”. The complex nature of inheritance of amylose content is supported by various reports [56, 57].

The advent of molecular marker technology is helping scientists to better understand complex quantitative traits [58]. Grain quality traits, including AAC, have been extensively studied using molecular marker-based quantitative trait locus (QTL) [49, 59–62]. AAC is reported to be mainly controlled by the *waxy* gene locus (*Wx*) on chromosome 6, which encodes the granule-bound starch synthase (GBSS) [9, 11, 60]. Minor QTLs for AAC have also been detected on chromosomes 1, 3, 4, 7, 8 and 11 [49, 62–64]. Using association analysis, the *Wx* gene was reported to act additively with five minor genes—*AGPlar*, *PUL*, *SSI*, *SSII-3* and *SSIII-2*—to affect AAC [65]. In at least three studies, QTLs for AAC were not associated with the *Wx* locus [32, 66, 67]. This is presumably because the parents shared the same *Wx* allele, making it easier to detect other loci associated with the trait [32].

2.2.2. Genetics of gelatinization temperature

Monogenic [54], digenic [68] and polygenic inheritance [69] for gelatinization temperature or ASV, have been reported. Using an 8×8 diallel cross, the inheritance of GT was found to fit into additive-dominance with dominance effects being predominant [70]. Crosses in which one parent has high GT have been found to produce many high GT F₂ individuals [2, 71]. High GT individuals in an F₂ population are essentially homozygous and do not segregate in subsequent generations. F₂ individuals with low and intermediate GT will continue to segregate into all three classes of GT for several generations.

Many molecular marker-based QTL analyses have been done for GT [49, 51, 59, 60, 67]. GT is reported to be mainly under the control of the *alk* locus on chromosome 6 that encodes soluble starch synthase II (*SSIIa*) [1, 17]. The *SSIIa* locus was reported to explain 25.5% of the variation in GT [1]. The authors found that, gene interactions as well as the *Wx* gene (4.7%) were important contributors to the variation in GT. The contribution of the *Wx* locus to the control of GT has also been reported by other researchers [29, 58, 62]. However, Tian et al. [72] reported that the control of GT was independent of the *Wx* locus. Other minor QTLs or modifier genes have been found to contribute to variations in GT [49, 58, 63]. Four minor genes including the *Wx* gene—*Wx*, *SBE3*, *ISA* and *SSIV-2*—have been reported to act additively with *SSII-3* (also referred to as *SSIIa* by other authors), the major for GT, to affect the trait [65].

2.2.3. Genetics of gel consistency

Some conventional genetic analyses have reported that GC is under the control of one major gene and several minor genes [73–75]. The difference between hard and soft gel, hard and medium as well as medium and soft was reported to be under monogenic control [73]. Hard gel was dominant over medium and soft gel; medium gel was dominant over soft. The authors concluded that it was possible to select for GC in early segregating generations. However, multigenic control of GC with genes acting additively has also been reported [76]. Using an 8×8 diallel cross, GC was found to fit into an additive-dominance model of inheritance with dominance being predominant [70]. Transgressive segregation has been observed for GC [63, 70, 77]. Using a genetic model for studying quantitative traits of triploid endosperm, cooking quality traits were found to be controlled mainly by genetic effects—seed, maternal and cytoplasmic. Cytoplasmic effects were the main components of GC [78]. Genotype by environment interactions as well as epistatic effects has also been found to play important roles in the control of GC [58, 78].

Many QTLs for GC have been mapped using molecular markers [1, 29, 61–63]. Some researchers reported that a single locus in the *Wx* region and some modifier genes control GC [62, 63]. He et al. [1] also reported that the *Wx* locus is the major determinant of GC but it explained only 38.9% of the phenotypic variation. Using association analysis, the *Wx* was confirmed as the major gene controlling GC [65]. A major QTL for GC at the *Wx* locus has been cloned [79].

In addition to the *Wx* gene, GC is reported to be affected by three other minor genes in the starch biosynthesis pathway—*AGPiso*, *SBE3* and *ISA* [65]. Two QTLs with minor effects on GC had earlier been reported [60, 67] and the *alk* locus has also been found to make minor contributions to GC [29].

2.2.4. Genetics of paste viscosity

The genetics of rice paste viscosity has not been studied as widely as AAC, GC and GT. RVA was reported to be controlled by a single locus [80]. The authors' finding was based on both $F_{2:3}$ segregation and diallel analyses. Subsequently, QTL mapping has been used to locate the chromosomal positions of genes controlling various RVA parameters in rice [29, 67, 81, 82].

In general, the major QTLs for most RVA parameters related to eating quality including HPV, CPV, BD, SB and CS were found at the *waxy* locus [29, 81–83]. In at least three studies, QTLs for PV were not found at the *Wx* locus [29, 67, 81]. Recently, a major QTL for PV was detected at the *Wx* locus [82].

QTLs related to the cooking process such as pasting temperature and peak time have generally been found at the *alk* locus on chromosome 6 [29, 82]. Minor QTLs for the various RVA parameters have been found on all 12 chromosomes of rice [29, 81–84].

2.2.5. Association between RVA parameters and AAC

RVA parameters including HPV, CPV, SB and CS have been found to be positively correlated with AAC while BD is negatively correlated to AAC [28, 29, 85]. AAC has been reported to be negatively [13, 86], positively [87] and not significantly [28, 29, 85] correlated with PV. It has been suggested that the SNPs in Ex 10 of the *waxy* gene has a confounding effect that influences the relationship between PV and AAC [88]. The authors found that the effect of AAC on PV would be different depending on the amount of TAC and GAT *waxy* gene haplotypes in the germplasm used by the various researchers. The significant correlations between AAC and most RVA parameters is not surprising because both traits are mainly controlled by the *waxy* gene [81, 89]. An SSR marker in the *Wx* gene (RM 190) explains a large portion of the variation in AAC and most RVA parameters [86, 90, 91]. The RVA parameters and AAC are also highly associated with three SNPs—intron 1 (G→T), exon 6 (A→C) and exon 10 (C→T) substitutions—in the *Wx* gene [86, 91, 92]. Using the three SNPs together, four *waxy* SNP haplotypes were found in a collection of world germplasm [86]. These *waxy* SNP haplotypes include TAC, GCC, GAC and GAT for low, intermediate, high and high AAC plus high RVA, respectively [86, 93].

2.2.6. Relationships between AAC, GC and GT

Since the rice grain is basically composed of starch (approximately 90%), genes involved in starch biosynthesis are naturally expected to affect cooking and eating qualities. Starch biosynthesis is a complex system composed of multiple subunits or isoforms of four classes of enzymes: ADP-glucose pyrophosphorylase (*AGP*), starch synthase (*SS*), starch branching enzyme (*SBE*) and starch debranching enzyme (*DBE*) [94, 95]. The effect of 18 genes involved in different steps of starch synthesis on AAC, GC and GT was investigated through association analysis [65]. These genes include: *AGP*, ADP-glucose pyrophosphorylase; *AGPlar*, *AGP* large subunit; *AGPiso*, *AGP* large subunit isoform; *AGP_{sma}*, *AGP* small subunit; *GBSS*, granule-bound starch synthase; *SS* (*SS-I*, *SS-II-1*, *SS-II-2*, *SS-II-3*, *SS-III-1*, *SS-III-2*, *SS-IV-1* and *SS-IV-2*) soluble starch synthase; *SBE* (*SBE1*, *SBE3* and *SBE4*) starch branching enzyme; *ISA*, isoamylase; *PUL*, pullulanase; *ISA* and *PUL* belong to starch debranching enzyme (*DBE*). The authors found that genes related to starch synthesis cooperate with each other to form a fine regulating network that controls the eating and cooking quality of rice.

Tian et al. [65] confirmed earlier reports that *Wx* gene affects AAC, GC and GT (“three in one” function of the *Wx* locus)[29, 58, 62] and found strong evidence that the *Wx* gene does not only have a major effect on AAC, but also regulates GC as a major gene and GT as a minor one. Some authors have proposed that the *Alk* locus (*SSIIa*) only affects GT and GC (a “two in one function”) [29, 58]. However, Tian et al. [65] found that the *Alk* locus (referred to as *SSII-3* in their report) plays an essential role not only in controlling GT and GC but also AAC. The authors also showed that some other starch synthesis related genes affect additively AAC, GC and GT as minor genes resulting in the fine complex network controlling cooking and eating qualities of rice grains.

AAC, GC and GT were found to be highly correlated: AAC is negatively correlated with GC (−0.91) and GT value (−0.46), whereas GC is positively correlated with the GT value (0.50) [65]. The negative correlation between AAC and GT was due to the natural occurrence of different haplotype combinations of *Wx* and *SSII-3* in rice germplasm. The *Wx* gene has three haplotypes: *Wx-III* is the wild type allele results in high AAC rice, *Wx-II* is associated with medium level of AAC and *Wx-I* has a loss-of-function mutation that produces waxy rice varieties [11]. Since AAC is negatively correlated with GC, varieties with *Wx-I* show high GC values, those with *Wx-II* show medium GC values, and those with *Wx-III* have low GC values [65]. The *SSII-3* has two allelic states: *SSII-3-I*, which leads to varieties that have higher GT values and *SSII-3-II* which leads to phenotypes with lower GT values.

The effect of either *Wx* or *SSII-3* on AAC and GT values was found to fall into a consistent pattern: *SSII-3-I* contributed to higher AAC under the same *Wx* background, whereas *SSII-3-II* led to lower AAC. *Wx* also combined with *SSII-3* to influence GT. For varieties that had the *SSII-3-I* background, *Wx-I* caused lower AAC and GT. GT value were however increased by *Wx-II* and *Wx-III*. They found five natural haplotype combinations between AAC and GT in their panel—*Wx-I/SSII-3-I*, *Wx-II/SSII-3-I*, *Wx-II/SSII-3-II*, *Wx-III/SSII-3-I* and *Wx-III/SSII-3-II*. Varieties belonging to *Wx-II/SSII-3-I* had medium AAC and high GT values and varieties belonging to *Wx-III/SSII-3-II* had high AAC and low GT values, these two haplotype combination form 71% of the germplasm. At the same time, varieties with *Wx-III/SSII-3-I* haplotype had high AAC and high GT values, and those with *Wx-II/SSII-3-II* had low AAC and low GT values. The *Wx-II/SSII-3-II* haplotype combination would be most useful for rice breeding in many parts of the world especially if it is combined with long-grain and fragrant/aroma alleles.

2.2.7. Inheritance of aroma

Monogenic, digenic and trigenic control of aroma has been reported by various authors [96–100]. However, some authors believed that aroma was quantitatively inherited [15]. The lack of agreement among researchers appears to be related to the different aromatic varieties and methods used in evaluating aroma [101].

However, the use of molecular markers to study the inheritance of fragrance appeared to favor monogenic recessive inheritance of fragrance [102–107]. A gene associated with fragrance was originally mapped by Ahn et al. [102] to rice chromosome 8, where it was associated with the RFLP marker, RG28. Bradbury et al. [105] identified a gene encoding betaine aldehyde dehydrogenase 2 (*BADH2*) as the likely cause of aroma in Basmati and Jasmine styled rices.

An eight base pair deletion (8-bp) and three SNPs in exon 7 of the *BADH2* gene distinguished fragrant from non-fragrant rices in that study. These polymorphisms served as the basis for developing an allele-specific marker for fragrant (Bradbury et al. [104]). A new fragrance allele with sequence identical to that of the *BADH2* allele in exon 7, but with a 7-bp deletion in exon 2 was identified as the cause of fragrance in some varieties [107]. Based on this information, the authors developed functional markers which can distinguish non-fragrant from fragrant rice and differentiate fragrance caused by 8-bp deletion on exon 7 from that caused by the 7-bp deletion on exon 2 of chromosome 8. In addition, 8 new alleles were discovered at the *BADH2* locus, all of which conferred fragrance in 24 accessions that did not carry any of the previously identified alleles [108, 109]. Another molecular marker study reported that three genes, located on chromosomes 3, 4 and 8, caused fragrance in Pusa 1121 [51]. The authors identified a *BADH1* gene in the aroma QTL on chromosome 4 and also mapped the QTL on chromosome 8 to the *BADH2* region. The *BADH2* gene is known to code for 2-acetyl-1-pyrroline, or 2AP [110, 111]. The accumulation of 2AP has been explained by the absence of *BADH2* activity leading to increased levels of 4-aminobutyraldehyde/ Δ^1 -pyrroline, the immediate precursor of 2AP [112]. However, in another study, it was concluded that *BADH2* had no direct role in the synthesis of 2AP [113]. The authors found that Δ^1 -pyrroline-5-carboxylate, usually the immediate precursor of proline, synthesized from glutamate, reacts directly with methylglyoxal to form 2AP. Fitzgerald et al. [14] declared that “the genetic and biochemical stories of 2AP synthesis are yet to be fully written”.

2.3. Marker-assisted breeding for grain quality in rice

Breeding for improved grain is complex because many of the quality traits are phenotyped using subjective and or expensive biochemical methods. Consequently, the scientific community has map/clone many QTLs/genes for various quality traits and developed molecular markers to facilitate selection for specific grain quality types.

Most fragrant rices including Jasmine and Basmati types have the 8-bp deletion on exon 7 of the *BADH2* gene and an allele-specific marker has been developed for selecting rice with this mutation [104]. This marker is being used widely for selecting for aroma. It is a co-dominant marker (**Figure 2**) and thus very useful for marker-assisted backcrossing for recessive trait such aroma because selection of lines carrying the aroma gene can done in the heterozygote state without progeny testing.

Other researchers have also developed markers for the 8-bp deletion in exon 7 of chromosome 8 [51, 115]. Functional markers have also been developed for other alleles in the *BADH2* gene including a 7-bp deletion in exon 2 [107] and a 3-bp insertion in exon 13 found in aromatic rice varieties from Myanmar [116].

Functional markers for a *waxy* gene SSR called RM 190, and *waxy* SNPS on intron (In1), exon 6 (Ex6) and exon 10 (Ex10) are used to select for AAC and RVA around the world [93]. The haplotype across these three SNPs in the *waxy* gene (*waxy* SNP haplotypes) have been found to be more efficient in selecting for AAC and RVA than the RM 190 [86, 88]. Across the *waxy* SNP haplotypes (In1-Ex6-Ex10) TAC, GCC, GAC and GAT is highly associated with low AAC, intermediate AAC, high AAC and high AAC accompanied with high RVA paste viscosity.

Figure 2. Agarose gel showing allele-specific marker for the 8-bp deletion in the *BADH2* gene. Lanes 1 and 17 = 100 bp DNA ladder. Fragrant individuals (F), non-fragrant individuals (N) and heterozygote types (H) [114].

The *alk* gene has been cloned [117] and validated as being the major gene for GT through genetic transformation [118]. Two SNPs (GC/TT and G/A) in the *alk* gene was found to be highly associated with GT [118]. These two functional SNPs have been used to developed DNA markers for selection of GT [118, 119].

Functional markers have also been developed for grain size [39, 120, 121]. These markers are very highly associated with the C-A SNP mutation in exon 2 of the *GS3* gene which is responsible for 80–90% of the variation in kernel length.

These validated markers will facilitate marker-assisted breeding for grain quality in rice. Various alleles of these important genes can be pyramided together to obtain the different consumer preferences for grain quality across countries and regions.

2.4. Future trends in rice breeding and grain quality improvement

By 2050, rice production must double in order to keep pace with population growth. Population growth will come with income growth so consumers will demand even higher quality rice. In addition to this challenge, new biotic and abiotic stresses are merging to due to climate change. Consequently, Rice breeders have to consider a huge number of simple and quantitative traits in combination when developing new lines while, at the same time, maintaining and improving grain quality.

Even though, MAS has been successfully used to improve some biotic, abiotic and quality traits in rice it is based on large effect QTLs/genes and does not take care of epistatic and genetic background effects. Most traits of interest to rice breeders are not controlled only by a few large-effect genes, but by a combination of many genes of small effect and/or major genes.

Genomic selection (GS) has been projected as alternative to conventional MAS. GS has huge potential to enhance breeding efficiency by increasing gain per selection per unit time [122]. GS breeding allows breeders to select the most desirable parents for the next generation using genome-wide DNA marker data. These parents are selected based on the relationship between the genome-wide markers and phenotypes of the individuals undergoing selection. The major advantage of GS over MAS is that genotyping is not restricted to selected markers that target genes with large effects, but rather all available marker data are used to predict breeding value. This helps to prevent loss of information. Genes with small effect can be tracked and selected for using information on all the marker data. GS would become more effective tool for increasing the efficiency of rice breeding as the costs of genotyping continue to decline [122].

2.5. Conclusion

Since rice is eaten mainly by humans as whole grain in cooked form, its grain quality is extremely important. The quality of the rice grain can be classified as milling, appearance, cooking and eating as well as nutritional quality. Different consumers around the world demand very specific measurements and combinations of the various aspects of rice grain quality. Breeding for these specific consumer demands can be challenging because grain quality is phenotyped using subjective, biochemical analyses that can be very expensive. Marker-assisted selection is thus a very good option for breeding for grain quality. The sequencing of the rice genome over a decade ago has made it possible for researchers to identify genes for the various grain quality traits. Functional molecular markers have been developed that are highly efficient in selecting for grain size, aroma, AAC, GT and paste viscosity parameters. These markers are increasingly being used for breeding for consumer-preferred grain qualities around the world. Modern genome-wide marker technologies which will take care of genes with small effect and allow breeders to simultaneously select for grain quality, yield and stress tolerance are recommended for future rice breeding work.

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