

Not just a grain of rice: the quest for quality

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A better understanding of the factors that contribute to the overall grain quality of rice (*Oryza sativa*) will lay the foundation for developing new breeding and selection strategies for combining high quality, with high yield. This is necessary to meet the growing global demand for high quality rice while offering producing countries additional opportunities for generating higher export revenues. Several recent developments in genetics, genomics, metabolomics and phenomics are enhancing our understanding of the pathways that determine several quality traits. New research strategies, as well as access to the draft of the rice genome, will not only advance our understanding of the molecular mechanisms that lead to quality rice but will also pave the way for efficient and targeted grain improvement.

Rice quality is a multi-faceted trait

Rice (*Oryza sativa*) is the most important food crop in the world, providing over 21% of the calorific needs of the world's population and up to 76% of the calorific intake of the population of South East (SE) Asia. In countries where rice is consumed, traits of grain quality dictate market value and have a pivotal role in the adoption of new varieties [1–3]. Quality traits encompass physical appearance, cooking and sensory properties and, more recently, nutritional value. The value of each trait, for example the length of the grain, varies according to local cuisine and culture. Physical properties include yield of edible and marketable polished grain, uniform shape, whiteness and, in most countries, translucence. These traits are immediately obvious to consumers and so are major factors defining market value. Cooking and sensory qualities typically include: cooking time [1]; textural properties of cooked rice [2]; aroma and its retention after cooking [3]; and the ability to remain soft for several hours after cooking [4]. Predictable expression of these traits across seasons and years give a variety its reputation.

Our current knowledge of the factors governing quality in different genetic backgrounds is incomplete; this is reflected by the persistence of benchmark varieties for many decades in spite of the yield gains achieved over those same decades. These varieties include Basmati 370, selected around 1920, Khao Dawk Mali 105, selected in 1958, Koshihikari, selected in 1960, and IR64, released in 1985. These varieties, although low-yielding and suscept-

ible to stresses, continue to be grown on many hectares because of their excellent quality.

Data provided by current phenotyping methods have enabled breeders to select for basic quality traits, but two varieties with the same score can be differentiated by consumers [5]. What is needed is (i) enhanced knowledge of the relevant components of the grain that affect quality traits; and (ii) tools that enable rice improvement programs to evaluate them. Here, we review progress through functional genomics for several key traits governing physical and cooking properties, and we explore opportunities for combining genomics with metabolomics to identify new traits of quality.

Advances in functional genomics of rice quality

Deciphering the genotype of an individual is key to both understanding and predicting which traits a plant is likely to express in a given environment, as well as to decreasing the time for the development of new varieties [6]. Here, we discuss recent progress in identifying and characterizing some of the genes responsible for the most important traits affecting grain quality – appearance (shape and chalk), aroma, texture, nutritional properties and cooking time (Box 1).

Appearance

The shape, uniformity and translucence of grains are crucial aspects of grain quality for consumers, millers, wholesalers and retailers. Quantitative trait locus (QTL)-mapping experiments confirm that seed size, shape and weight are all under polygenic control in rice. Numerous QTLs associated with each aspect of grain size and shape have been identified in diverse populations over the past 15 years (<http://www.gramene.org>), and recently three seed size, shape and weight QTLs have been cloned and characterized. These include (i) a grain-length QTL on chromosome 3 (*GRAIN SIZE 3* [*GS3*]; [7]), (ii) a seed width QTL on chromosome 5 (*SEED WIDTH 5* [*SW5*]; [8]) and (iii) a grain weight QTL on chromosome 2 (*GRAIN WEIGHT 2* [*GW2*]; [9]). In all cases, cultivars carrying the recessive allele(s) have longer, wider and/or heavier seeds than those carrying the wild type (WT) alleles [7–9]. The authors each concluded that the genes, directly or indirectly, affect cell division, and recessive alleles at *SW5* and *GW2* were associated with larger numbers of cells in the hull (maternal) tissue [8,9]. This is consistent with observations that grain size (endosperm) is highly correlated with seed

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Box 1. Cooking time: minutes mean millennia

The cooking time of rice is determined by the temperature at which the crystalline structures of the starch begin to melt. This is called gelatinization temperature (GT). In rice, GT ranges from 55–85 °C [82]. Rice with high GT requires more time to cook, and the cooked rice has an unacceptable texture. Recently, *STARCH SYNTHASE IIa* (*SSIIa*) has been identified as a key gene affecting GT [83]. Two haplotypes of *SSIIa* are associated with low GT [84,85] due to mutations in *SSIIa* that affect the binding or the catalytic domain of the enzyme [86].

Lowering the GT of the rice grain could decrease average cooking times by up to 4 min. Although this might initially seem entirely insignificant, by computing the number of times rice is cooked in any one day by millions of households around the world, a decrease of just 4 min for each cooking event could save >10 000 years of cooking time each day. This represents massive potential for global savings of carbon and is of particular relevance to poor, rural households that depend on scarce local supplies of fuel.

size (hull) [10] and supports the conclusion that the size of the edible endosperm in rice is largely determined by the dimensions of the lemma and the palea (hull) [8]. A fourth gene, (*GRAIN INCOMPLETE FILLING 1* [*GIF1*] [11]) on chromosome 4, cloned from a population of induced mutants, is a cell-wall invertase gene that is not associated with cell division, but rather influences grain weight by affecting the rate of grain-filling. The mutant form of *GIF1* showed slower grain filling than the WT, and this was accompanied by markedly more grain chalkiness as a result of abnormally developed and loosely packed starch granules [11].

The suite of recessive alleles conferring large, heavy grains [7–9] is not present in all cultivated forms of *O. sativa*; in many cases, small and/or slender grains are preferred, such as the short slender variety Samba Mahsuri, which is extremely popular in Southern India [12]. Indeed, each of the five sub-populations (*indica*, *aus*, *tropical japonica*, *temperate japonica* and *basmati*) carries different combinations of grain size and shape alleles,

and some sub-populations contain significantly more genetic variation for grain size and shape than others (Hui Jiang, Cornell University, Ithaca, NY, USA, personal communication). The power to obtain different combinations of alleles conferring particular grain shapes and sizes has implications for yield improvement, potentially enabling breeders to develop high-yielding varieties with specific morphological characteristics of grain to satisfy diverse quality requirements of the growing number of rice consumers.

Chalk is the other trait of appearance that affects consumer acceptance of rice. Chalky grains have opaque spots in the endosperm that range in size (Figure 1) [13]. Chalk predisposes the grains to break during polishing [14], decreasing the amount of edible rice. Even if the chalky grains resist breakage, their presence lowers the overall market value [15]. All markets dictate the value of rice based on just two traits: the proportion of chalk and the proportion of broken grain. Chalk directly and indirectly contributes to both traits.

Starch granules in translucent areas of grains are bigger and more tightly packed than the small loosely packed granules in chalky areas of the grain [13] (Figure 1). Because of this, many studies approach chalk by focusing on processes of starch synthesis [13,15–20]. However, none provide clear guidance towards genetic or biochemical processes underlying chalk.

Chalk is induced by high temperature [13], but a genetic component is illustrated by several varieties that consistently produce chalky grain [15]. Several QTLs associate with chalk [19,21], several of which are stable across multiple environments [19]. Two have been fine-mapped to regions on chromosomes 9 and 1, and allelic variation at the region on chromosome 9 associates with chalk values [21]. While heritability has been demonstrated, the genes associated with chalk have not. Incomplete starch granules implicate processes of grain-filling. The endosperm is

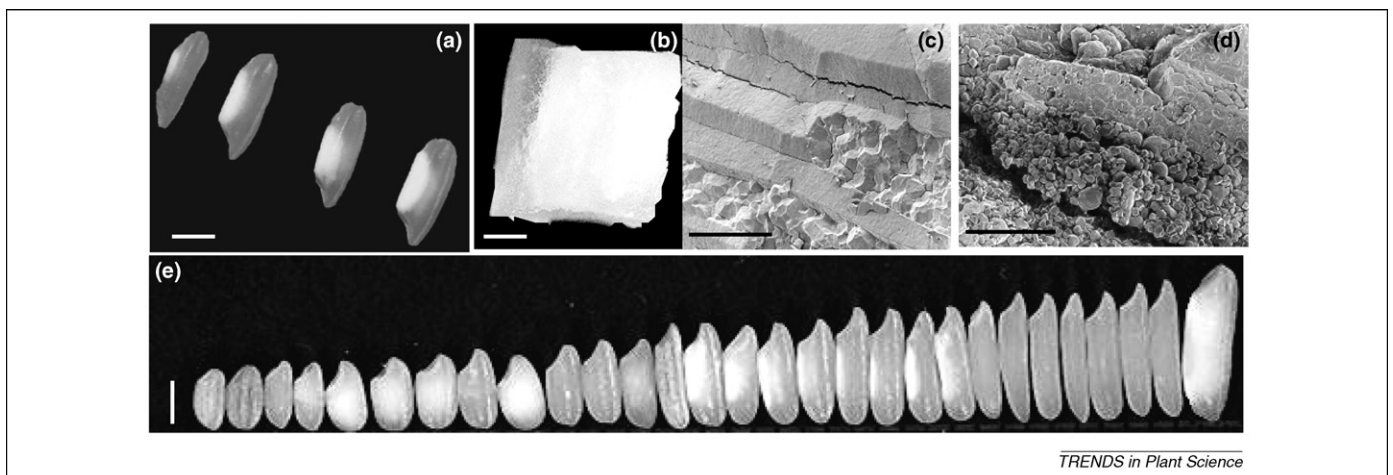


Figure 1. Polished but not perfect. The first stage of rice processing is to remove the hull (lemma and palea), exposing the ‘brown’ rice, which consists of the endosperm and embryo encased in the bran layers. In the next stage of processing, the bran layers, including the pericarp, aleurone, sub-aleurone layers and the embryo are removed during a polishing process, which results in white rice, the most commonly consumed form. However, not all white rice is perfect; chalkiness can at best devalue the grain or at worst cause it to break or be pulverized during polishing. (a) The size of the opaque (chalky) areas in the grain varies from small to large (scale bar represents 1 mm). (b) A section through the chalky area showing different refractive properties between the chalky and translucent portions of the piece (scale bar represents 250 μ m). (c) Scanning electron microscope (SEM) micrograph of cells in translucent areas of the grain showing starch granules tightly packed into cells (scale bar represents 50 μ m). (d) SEM micrograph of cells from the chalky region of the grain showing space in and between cells and small and disorganized starch granules (scale bar represents 50 μ m). (e) Maximum diversity in grain shape within *Orzya sativa*. Length ranges from 3–11 mm and width from 1.2–3.8 mm. Grains were supplied by T.T. Chang Genetic Resources Centre, IRRI, the Philippines and the hull and bran layers were removed at the Grain Quality, Nutrition and Postharvest Centre, IRRI, the Philippines (scale bar represents 2 mm).

Box 2. The issue of fragrance

The genetic and biochemical bases of fragrance both in cooked and uncooked grains are highly complex. 2AP is the major aromatic compound in both the SE Asian jasmine varieties and the South and Central Asian Basmati rices. Consumers can readily differentiate between the aroma and flavour of these two styles, but so far science has not been able to explain the differences. In a set of 464 traditional varieties chosen to represent all major sub-populations of *O. sativa* and regions of Asia, no clustering was observed for 2AP content, suggesting that there is no distinct difference between 2AP contents of jasmine and Basmati rices [3]. Between 70 and as many as 140 different volatile components have been detected in (un)cooked rice grains [72–74]. These include compounds derived from biochemically distinct pathways, leading to a highly diverse mixture involving hydrocarbons, organic acids, alcohols, aldehydes, ketones, esters and phenols, among others. Many of these compounds are chemically synthesized and stored during grain development but, equally importantly, many others result from chemical breakdown of, for example, fatty acids during the storage of polished grains. The appearance of catalysis products is particularly associated with the required storage period (up to 1 year) for Basmati varieties, during which time the typical Basmati fragrance develops [75]. However, catalysis products (e.g. hexanal) are also of major importance in the development of undesirable off-flavours during incorrect storage of non-Basmati varieties [76,77].

Aromatics can be directly linked to consumer preference in both positive terms (flavour and fragrance components such as 2AP and the aromatic alcohols) and negative terms (off-flavours of which hexanal and 2-pentylfuran have significant importance [77]). Understanding such complexity is not simple, as not only the concentration but also the aroma threshold of different components are of importance. Components such as 2AP, which represents just a single component in the cocktail of volatile metabolites, might, however, in sensorial terms, be of major importance due to their low odour threshold (human detection limit) [74]. Therefore, concentration and aroma threshold of aromatics need to be taken into account and several groups consequently include sensory panel analysis in their experiments [59,78], or alternatively use GC-olfactometry [79]. Targeted analytical methods for key components such as 2AP and hexanal [75,80], as well as ‘unbiased’ methods [3,81], have regularly been described. Building a solid information base, and perhaps exploring other technology platforms, to characterize this trait fully is essential for future success in meeting market and consumer requirements in this area.

heterotrophic, generating the hypothesis that source–sink interactions are involved in chalk. Current climate models predict that mean global temperature will continue to increase [22], and unless chalk is solved, it poses a significant threat to food security as the world becomes warmer. Thus metabolomic technologies could provide pointers to the pathways that underlie chalk and clues to the genes that regulate its appearance.

Aroma

The aroma of ‘fragrant rices’ is an issue of particular importance as it is not only a factor determining market price but also a trait with clear local and national identity. For example, many different varieties of the jasmine style of rice are consumed in countries of SE Asia and many Basmati styles of rice are consumed in countries of South and Central Asia [3,23] (Box 2). The flavour and aroma of these two types of rice can be discriminated easily by consumers, even though the major aromatic component in both types is 2-acetyl-1-pyrroline (2AP) [24].

Several studies have suggested that the presence of 2AP is due to an allele of the *BETAINE ALDEHYDE DEHYDROGENASE 2* (*BADH2*) gene defined by deletion

on exon 2 or 7 [3,23,25]. This was recently confirmed by the absence of 2AP in an aromatic variety after transformation with functional *BADH2* [26]. *BADH* genes usually oxidize betaine aldehyde in the pathway of glycine betaine synthesis [27], but glycine betaine has not been detected in rice [28]. When expressed in *E.coli*, *BADH2* shows a greater affinity for 4-aminobutyraldehyde than for betaine aldehyde [26,29], and it is capable of oxidizing 4-aminobutyraldehyde to γ -aminobutyric acid (GABA) [30]. In *Bacillus cereus*, Δ^1 -pyrroline is the precursor of 2AP [31]; it is the cyclic form of, and exists in spontaneous equilibrium with, 4-aminobutyraldehyde [26]. Thus, it was concluded that *BADH2* participates in the pathway of GABA synthesis by oxidizing 4-aminobutyraldehyde, produced from putrescine, to GABA. However, varieties carrying *badh2* cannot carry out the oxidation step, and accumulate both 4-aminobutyraldehyde and Δ^1 -pyrroline, thus activating the pathway of 2AP synthesis [26,29]. However, in another study, increased expression of Δ^1 -pyrroline-5-carboxylate synthetase in fragrant varieties compared with non-fragrant varieties, as well as concomitant elevated concentrations of its product, led to the conclusion that Δ^1 -pyrroline-5-carboxylate, usually the immediate precursor of proline synthesized from glutamate, reacts directly with methylglyoxal to form 2AP [32], with no direct role proposed for *BADH2*.

Several varieties have been identified that accumulate 2AP but do not carry the deletion on exon 2 or 7 of *BADH2* [3]. Although it is possible that those varieties carry other mutations that could affect the function of *BADH2*, several varieties with consistently high values of 2AP led the authors to suggest that more than one pathway leads to 2AP in those varieties [3]. This is consistent with the evidence for two pathways described above. Thus the full genetic and biochemical stories of 2AP synthesis are yet to be fully written.

Texture

The major components of a polished rice grain are starch (up to 95% dry weight), protein (5–7%); and lipids (0.5–1%). The amount of both proteins and lipids affects sensory properties [4,33], but most genomic research has focused on the starch components of the grain – amylopectin (see Box 1) and amylose.

Amylose is considered the most important predictor of sensory quality in rice, and grains are classified according to amylose content as waxy (0–2%); very low (3–9%); low (10–19%); intermediate (20–25%); or high (>25%) [5].

The enzyme granule bound starch synthase I (GBSSI) is required for amylose synthesis, and several alleles are encoded by the *Wx* locus [34,35]. A single nucleotide polymorphism (SNP) at the splice site of intron 1 differentiates low amylose varieties from intermediate and high varieties and defines the *Wx^a* and *Wx^b* alleles for high and low amylose, respectively [36]. A recent association study shows that an SNP in exon 6 (identified as the *Wxⁱⁿ* allele [35]) results in an amino acid substitution from serine to tyrosine that separates high and intermediate amylose varieties [34]. Using near isogenic lines, it was shown that the SNP on exon 6 decreases the expression of the GBSSI protein, shifting the amylose content of the grain from high

to intermediate levels [35]. Varieties with very low amylose (<9%) have an opaque phenotype and show even less activity of GBSSI than low amylose varieties [37]. An SNP in exon 4, resulting in an amino acid substitution from aspartate to glycine, associates with the opaque phenotype and defines the Wx^{op} allele [35]. The *waxy* mutants carry a deletion in the *Wx* gene that is fatal to activity of GBSSI [37], and consequently they contain no amylose.

Although five alleles of the *Wx* gene that associate with the five classes of amylose are known [35], it is likely that we do not yet fully understand the genetics of amylose content. Each class is characterized by a range of amylose contents, suggesting that additional regulatory elements or environmental conditions affect expression.

Factors, other than amylose, that affect the texture of cooked rice are gel consistency (GC) and gelatinization temperature (GT – see Box 1), which is a function of amylopectin structure. Generally, as amylose content increases, firmness does too, but the texture of high amylose rices can be soft or hard, and this is differentiated by the GC test [38]. When GC is evaluated in a range of mapping populations, QTLs for GC are consistently associated with the *Wx* locus (for a review see [39]), providing further support for the hypothesis that more allelic variation at this locus awaits discovery to explain more about the sensory properties of grain quality.

Nutrition

Opportunities for improving the nutritional value of rice grains have become increasingly evident. Hidden hunger (micronutrient deficiency) has been recognized in developing countries where rice is the staple, and technology to address particular deficiencies has emerged [40]. Rice is generally consumed as a polished grain. Nutritional components such as minerals and vitamins are either absent, or present at low levels in polished grains [41]. In recent years, transgenic techniques have been successful in (i) elevating the Fe content of rice endosperm by introducing ferritin genes into rice [42], and (ii) introducing nutritionally valuable amounts of β -carotene into rice endosperm (Golden Rice 2) by introducing the carotene desaturase gene from *Erwinia uredovara* and the phytoene synthase gene from maize (*Zea mays*) [43]. Although neither of the enhanced rices have left the laboratory yet, both iron and vitamin A deficiency are known to have a major impact on human health in developing countries. Biofortification of staple foods is one part of a long-term strategy for addressing these problems (<http://www.harvestplus.org>).

The main component of rice grains is starch. Digestion of the starch provides the energy to sustain activity. Studies in other cereals show that starch is digested at different rates (glycaemic index [GI]). Low GI starch provides energy for prolonged periods, with benefits of more stable blood sugar levels resulting in sustained concentration and activity [44]. Furthermore, manipulating the rate at which starch is digested could become a tool for managing diseases that are dependent on blood sugar levels, such as Type II diabetes [44], which is prevalent in the developed world and a growing pandemic in developing countries [45]. Another fraction of the starch, resistant starch

(RS), escapes digestion in the small intestine, fermenting in the large bowel [46]. RS leads to an increase in absorption of minerals and other nutrients, resulting in more rapid recovery from conditions like diarrhoea, and a decrease in mortality and negative health impact [46,47]. Work on other cereals has identified several genes of starch synthesis that, when mutated, lead to variability in both the amount of RS and the GI [48,49]. Synteny suggests that exploring homologous genes in rice could provide a vehicle for improving the nutritional value of the major component of rice grains.

Genetical metabolomics – new opportunities

Although genomics approaches have helped to identify and characterize several genes that determine important grain quality traits, we also seek to understand the regulatory

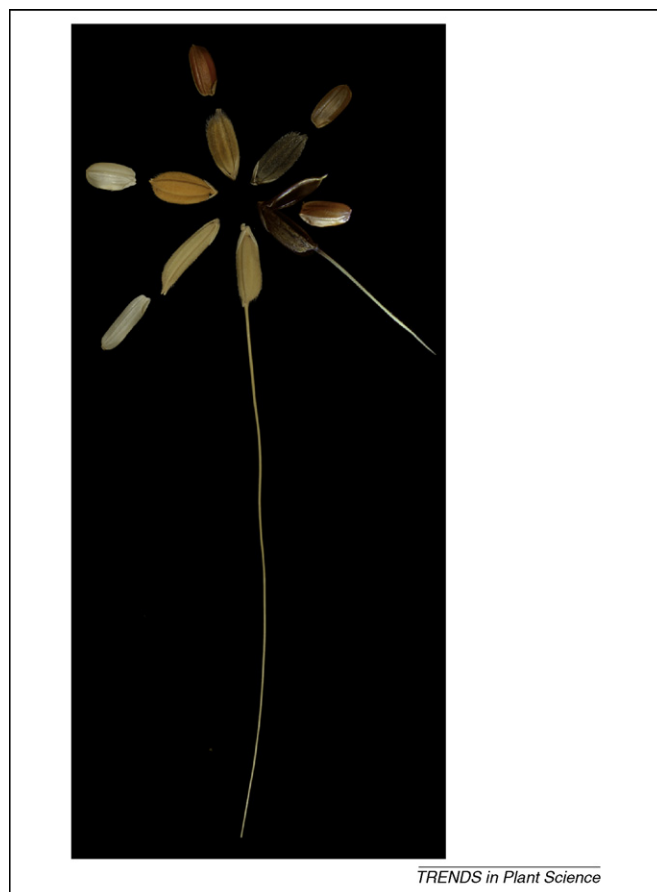


Figure 2. Morphological diversity in seeds and whole grains of rice. Diversity of colour, size and shape of rice seeds (or caryopses; inner circle) and corresponding grains (edible portion of the seed which is visible only after the outer glumes, or hulls, are removed; outer circle). Grains consist of endosperm and embryo and are covered by the pericarp, tegumen and aleurone cell layers, collectively referred to as ‘bran’. Before the advent of electricity, the bran layer was removed manually, which left remnants of the bran and most of the aleurone layer on the rice grain. Today, rice is mainly polished by machine, resulting in completely white grains free of bran and with no aleurone layer remaining. Minerals, phenolics, sterols, vitamins such as thiamine, niacin, tocopherol, tocotrienol, β -carotene and lutein, and many other phytochemicals with potential health benefits are found in the bran layers of rice [67–71], and coloured bran layers can often contain elevated amounts of some of these compounds [69]. Despite clear health benefits, campaigns to increase the consumption of brown rice have been unsuccessful because of short shelf-life and different sensory properties of brown rice compared with white rice of the same variety. Open hull (on right) shows the two, hinged glumes that encase and protect the rice grain during the growth and development of seeds on a rice plant; the lemma is slightly larger and has an extended awn in this example, while the palea represents the smaller, inner portion of the hull. Photo provided by Raluka Iorga, Cornell University.

and biochemical pathways that are involved in trait expression and that are responsible for subtle qualitative and quantitative differences in each quality trait. For fragrance, we know that the concentration of 2AP is affected by temperature and pre- and post-harvest management [50,51], but we know little about the underlying genetic, biochemical or metabolic reasons for this variability. We can predict that incorporating alleles for greater grain width or grain weight (and correspondingly greater yield) is likely to increase the amount of chalk in the grains (Figure 1). We can also speculate that the larger, heavier grains will have altered signalling between sink and source, which could affect the ability of all the starch granules to grow equally. Environmental conditions during grain-filling are known to affect the expression of at least two alleles of *GBSSI*, leading to lower levels of amylose in the grain. This again suggests interplay among the genes, the environment and the biochemical processes that regulate grain-filling, and it implies a key role for technologies that are able to identify signalling compounds that are associated with genetic variability.

The International Rice Information System (IRIS; <http://www.iris.irri.org>) recognizes around 5000 released varieties. This vast repertoire of biodiversity (see Figure 2), together with what is available in wild relatives and traditional landraces, must be explored and better utilized if we are to address constructively the limitations to rice production [52,53]. Advanced genetics and genomics approaches are creating new opportunities in large-scale omics (metabolomics, proteomics, transcriptomics) data generation, statistics and modelling [54], with great potential to dissect the genetic architecture and unravel biologically meaningful regulatory networks from gene function through to complex phenotype [55–58].

Regarding the specific topic of rice grain quality, metabolomics has a particular role to play (see <http://www.meta-phor.eu>) where advances in analytical technologies, combined with dedicated data analysis tools, are already beginning to reap benefits [3,52,59]. Reverse genetics approaches, benefiting greatly from access to extensive genomic sequence information for rice, are already proving useful. For example, artificial micro-RNAs have been successfully used to induce gene silencing in rice with ‘unprecedented specificity’, resulting in the modulation of agronomically important traits such as plant height and tillering [60]. Extensive knowledge of biochemical pathways and their associated genetics has also facilitated successful food biofortification strategies, as demonstrated by a 100× enhancement of folate levels in (cooked) rice grains [61,62]. Transgenic success unlocks real opportunities for targeting a host of nutritional issues by designing rice plants that express novel (for rice) genes and pathways in their grains. Advances in metabolomic techniques can provide guarantees to consumers that compounds resulting from the novel pathways meet expectations of health improvement, taste and safety.

More such developments are anticipated in the future, and these must also be exploited to advance rice improvement, be it related to volatiles, starch, morphology or any other aspect of grain quality.

Future prospects

With the available rice genome sequence information, growing data on metabolic networks, genotype–phenotype associations and gene regulatory networks and the emergence of systems biology approaches [63], the rice community is poised to make major advances in our understanding of the molecular, genetic and biochemical bases of important grain quality traits. We live in a time of grave concern about world hunger, shortages in fuel and water, impact of climate change, population growth and future food security. Rice production is threatened by increasing urbanization leading to loss of farming land, urban migration leading to fewer farmers producing food [64], biofuel policies that negatively affect the availability of grain for consumption [65], decreased funding for rice research [66] and issues of climate change [22]. All of these factors, directly or indirectly, influence both the quantity and the quality of rice that is available for consumption.

Tackling the problems demands a united effort and utilization of all available tools. Combining expertise to work jointly on technology development (e.g within multi-partner EU projects such as META-PHOR: <http://www.meta-phor.eu>) and establishing international working groups such as the INQR (International Network for Quality Rice: <http://www.irri.org/inqr/>) are a start. Other joint international initiatives such as Harvest Plus and The Challenge Programmes are of major importance, but additional effort, for example in the development of new genotyping and sequencing platforms, accurate phenotyping tools and quantitative ex-ante analyses for setting priorities for investment, are all needed to constructively engage the scientific community in solving current and future problems associated with food production, security, distribution and quality.

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