



Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (*Oryza sativa* L.)

Nitika Rana, Mohammed Saba Rahim, Gazaldeep Kaur, Ruchi Bansal, Surbhi Kumawat, Joy Roy, Rupesh Deshmukh, Humira Sonah & Tilak Raj Sharma

To cite this article: Nitika Rana, Mohammed Saba Rahim, Gazaldeep Kaur, Ruchi Bansal, Surbhi Kumawat, Joy Roy, Rupesh Deshmukh, Humira Sonah & Tilak Raj Sharma (2019): Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (*Oryza sativa* L.), Critical Reviews in Food Science and Nutrition, DOI: [10.1080/10408398.2019.1685454](https://doi.org/10.1080/10408398.2019.1685454)

To link to this article: <https://doi.org/10.1080/10408398.2019.1685454>



View supplementary material [↗](#)



Published online: 13 Nov 2019.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)

REVIEW



Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (*Oryza sativa* L.)

Nitika Rana, Mohammed Saba Rahim, Gazaldeep Kaur, Ruchi Bansal, Surbhi Kumawat, Joy Roy, Rupesh Deshmukh, Humira Sonah, and Tilak Raj Sharma

National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India

ABSTRACT

Rice nutritional quality is one of the major concerns along with productivity enhancement to feed the continuously growing population. To address wide-spread malnutrition influencing global health, novel high yielding rice cultivars with better nutritional quality need to be bred. No doubt, the conventional breeding approaches have helped to decrease the gap between demand and supply for yield and nutrition; however, to meet today's demands more advanced approaches need to be employed. This review discusses approaches for the improvement of nutritional quality of rice and gauges the availability of omics resources. Recent omics advances providing numerous tools and techniques for the efficient exploration of genetic resources as well as for the understanding of molecular mechanism involved in the trait development have been discussed. Understanding of genes or loci governing different traits has been found to be effective in accelerating the crop breeding programs. In this regard, approaches like QTL (quantitative trait loci) mapping, genome-wide association study and genomic selection are discussed in light of their utilization for rice nutritional quality improvements. Efficient integration of different omics approaches is recognized as a promising way to achieve the desired improvements in rice cultivars. Therefore, advances in omics branches like transcriptomics, proteomics, ionomics, and metabolomics being efficiently explored for rice improvement programs are also addressed. This article provides a catalog of genes, loci, mutants, online resources and computational approaches for rice improvement. The information provided here will be helpful for pursuing present progress and directing rice research program for better future.

KEYWORDS

Oryza sativa; nutritional quality; conventional rice breeding; omics assisted breeding; micronutrient deficiency; biofortification; genomic selection

Introduction

Rice, one of the most important staple foods across the world, is being cultivated on approximately 167 million hectares of area under varied climatic conditions in tropical and subtropical regions (FAOSTAT 2017). The annual global milled rice production in 2018 was 487.35 million tons (Ricestat 2019). Unpolished rice is a rich source of complex carbohydrates, minerals, vitamins, amino acids and dietary fiber. Since rice is a staple food for several developing countries, it provides 715 kcal/caput/day with around 27% energy supply and 20% protein supply from diet (FAOSTAT 2001). With a contribution of 71% to protein in diet, a person consumes an average of ~187/kg/year of rice in many countries where rice is a staple (Prolla et al. 2013). Increasing world population has made it imperative that crop production and quality need to be increased two-fold to meet the increasing demands. According to an estimate, around 100 million more tons of rice is required to feed an increase of one billion people to the world's population (Mohanty 2013). In addition to yield, grain quality

and nutrition are of paramount importance for nutritional security.

The parameters outlined by World Health Organization (WHO) specifying the essential composition and quality factors of rice are mainly based on protein, carbohydrate, vitamin, mineral, amino acid, phenolic and flavonoid content in rice grains. Rice without any impurities, inorganic and organic extraneous matter within permissible limits and free from heavy metal contaminants such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg); is deemed to be of consumable quality. Rice is high in nutritional content including excellent source of minerals like calcium (Ca), phosphorus (P), iron (Fe), sodium (Na), potassium (K), and vitamins. Brown rice, a good source of proteins, phosphorus and iron, is widely considered as the more nutritious type of rice (Verma and Shukla 2011). On one hand basmati rice contains around 364 kcal/100 g of energy, 19 g/100 g of proteins and 6 g/100 g of total lipids; jasmine rice consists of 356 kcal/100 g of energy, 6.67 g/100 g of proteins and minimal total lipids (fats) content (U.S. Department of Agriculture 2019a, 2019b). Similarly, total protein and lysine

CONTACT Tilak Raj Sharma ✉ trsharma@nabi.res.in; trsharma1965@gmail.com; Humira Sonah ✉ biohuma@gmail.com 📍 National Agri-Food Biotechnology Institute (NABI), Sector-81 (Knowledge City), PO Manauli, S.A.S. Nagar, Mohali-140306, Punjab, India.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

📎 Supplemental data for this article can be accessed at <https://doi.org/10.1080/10408398.2019.1685454>.

content are considered as one of the most important parameters which determine nutritional quality of rice (Yan and Bao 2014). For instance, brown rice contains 3.8 g/16 g N of lysine (FAO 2019).

Middle and low income populations with limited access to mineral, protein and vitamin-rich foods; and which consume high amounts of rice are primarily affected by micronutrient deficiencies due to lack of a heterogeneous source of nutrients. According to a report by Food and Agriculture Organization (FAO), hidden hunger affects more than two billion people world-wide. During the past few decades, developing and underdeveloped countries have focused on increasing yield and availability of staple crops; which are the main sources of calories. Similar research and availability is lacking in other crops which are a source of micronutrients, vitamins and minerals. Therefore, economically backward sections of society have although managed to meet their calorie demand from staple food crops but they are unable to meet requirements for micronutrients and suffer from various nutrient deficiency diseases like anemia (iron deficiency; Lucca, Hurrell, and Potrykus 2002), beriberi (thiamine deficiency; Carpenter 2000), scurvy (vitamin C deficiency; Ricaurte, Kewan, and Daw 2019), rickets (vitamin D deficiency; Sahay and Sahay 2012) and night-blindness (vitamin A deficiency; Dowling and Wald 1958). In this regard, bio-fortification of rice is one of the most promising approaches (Kok et al. 2018). Bio-fortification of rice can be performed via agronomic practices, conventional breeding and genetic engineering. Agronomic practices for fortification include application of fertilizers to soil and plant parts such as leaves (De Valença et al. 2017). Conventional breeding implies breeding of crops where desirable traits are selected and crossed over a few generations. The advanced techniques being employed for biofortification such as genetic engineering include methods for modifying gene sequences for desirable traits. According to WHO, fortification of rice is the most important task to eradicate malnutrition for a considerably large population all over the world affected with deficiency of iron, vitamin A and folic acid. Bio-fortification through genetic improvement is sustainable and affordable option to make fortified food available for the economically backward sections of society (Bouis and Saltzman 2017).

Although conventional breeding methods have contributed immensely for crop improvement, they are limited to the available genetic resources within the primary gene pool. In contrast, transgenic techniques which explore genetic resources across the living species provide an opportunity to engineer plants with desired traits (Sonah et al. 2011). One of the prominent contributions of transgenic approach particularly for the enhancement of nutritional quality is 'Golden Rice' (Paine et al. 2005). The transgenic Golden Rice is an intervention for vitamin A deficiency at almost the same cost as white rice. Golden Rice was bio-fortified with beta-carotene which is converted into vitamin A in the human body. Vitamin A is essential to fight night-blindness and for improved immunity. According to a UNICEF estimate, around 124 million children in the world suffer from

vitamin A deficiency. Since rice is consumed worldwide, it was envisioned to be used as a delivery system for beta carotene, a vitamin A precursor (<http://www.goldenrice.org/index.php>). Golden rice has faced challenges related to proper handling of Genetically Modified Organisms (GMO) and skepticism faced from opponents of GMO crops. The controversies resulted in slow down of many other transgenic efforts in rice and other crops and forced scientists to bypass the transgenic technology. Few such strategies termed as genome editing techniques have shown immense utility and lesser social stigma as compared to GMOs. Genome editing methods are derived from principles of living organisms such as DNA repair and defense posed by bacteria towards pathogens and does not necessitate introduction of foreign genes into the genome of interest (Maywa Montenegro 2016). Efficient exploration of the genome editing technology will be helpful to bypass the hurdles usually faced for the commercial release of transgenic crops (Hefferon and Herring 2017). As like golden rice, development of golden wheat also looks promising to eradicate hidden hunger world-wide. In addition, genetic improvement through omics assisted breeding is widely accepted as safe, sustainable, and efficient way for the crop improvement. Omics approaches include technologies from different platforms viz. genetics, genomics, proteomics, metabolomics, and ionomics which enable researchers to detect, identify and measure various biological molecules such as DNA, RNA, proteins, metabolites and ions present in the living system (Chaudhary et al. 2015; Deshmukh et al. 2014). Huge amounts of whole-genome and transcriptome sequence data has been generated for important crop plants in the past decade owing to developments in sequencing techniques (Pathak et al. 2018; Sharma et al. 2018). In this article, we have provided an overview of efforts made towards the nutritional quality enhancement, present goals and challenges for the rice breeders and available omics resources and approaches to accelerate the rice improvement programs.

Genomic resources in cultivated rice (*O. sativa*) and other *Oryza* species

Rice is an annual grass of gramineae family and can be classified into eleven genomic groups labeled AA to LL. Out of these eleven genome types, six are diploid and include AA, BB, CC, EE, FF, GG type genomes and five are polyploid with BBCC, CCDD, HHJJ, HHKK, KKLL type genomes (Stein et al. 2018). *Oryza* genus includes 25 other species in addition to two cultivated species: *O. sativa* (Asian rice) and *O. glaberrima* (African rice). Multiple species such as in genus *Oryza* include *O. australiensis*, *O. brachyantha*, *O. meyeriana*, *O. officinalis*, *O. longistaminata*, *O. glumaepatula*, *O. nivara* and *O. glaberrima* which has been domesticated from its wild ancestor *O. barthii* (Mondal et al. 2017; Song et al. 2019). *Oryza sativa* domesticated from the wild rice *O. rufipogon*; contains two major subspecies: *Japonica* (round and short-grained) cultivated in cooler zones of subtropics and temperate zones and *Indica* (long to short-grained and

Table 1. Details of significant efforts employed for whole genome re-sequencing, and for the development of sequence resource for rice research.

Genotypes re-sequenced	Details	References
Four upland rice varieties along with parental lines WAB56–104 and CG14 (<i>O. glaberrima</i>)	Approximately 3.1, 0.10, and 0.40 million single nucleotide polymorphisms, multi nucleotide polymorphisms, and short insertions/deletions were reported	(Yamamoto et al. 2018)
A set of 151 RILs derived from SN265 X R99	Re-sequencing data used for QTL mapping. Total 79 QTLs related to 15 agronomic traits in three ecological conditions were identified	(Li et al. 2018)
A set of 17 rice genotypes, including nine non-anthocyanin genotypes and eight high-anthocyanin genotypes	A total of 1176,226 SNPs identified. Candidate genes for anthocyanin have been selected.	(Kim et al. 2015)
A set of 126 japonica rice genotypes	Re-sequencing helped to discover lineage-specific nucleotide change in rice	(Arthur and Bennetzen 2018)
A set of 150 RILs derived from Indica cv. 93-11 and Japonica cv. Nipponbare	Total 1.7 M segregating SNPs have been detected and used for QTL mapping.	(Huang et al. 2009)
A collection of ~3,000 rice accessions from 89 countries	Discovery of about 18.9 million SNPs using <i>Oryza sativa japonica</i> Nipponbare cultivar as reference	(Li, Wang, and Zeigler 2014)
50 cultivated and wild rice (<i>Oryza rufipogon</i> and <i>Oryza nivara</i>) accessions re-sequenced	6.5 million high-quality SNPs were obtained	(Xu et al. 2012)
Re-sequencing of japonica rice variety 'Longdao24'	420,475 SNPs, 95,624 InDels, and 14,112 SVs were discovered in 'Longdao24' when reference genome was <i>japonica</i> cultivar Nipponbare.	(Jiang et al. 2017)
Re-sequencing of 132 'Liang-You-Pei-Jiu' recombinant inbred lines (RILs)	Enabled fine-mapping of yield-associated loci qSN8 and qSPB1	(Gao et al. 2013)

somewhat slender) grown extensively in tropics and subtropics. Rice was the first crop genome that was sequenced under 'The International Rice Genome Sequencing Project' (IRGSP) which started in 1997 as a collaborative efforts including eleven participating nations (IRGS Project 2005). The technique employed for rice genome sequencing was a classical clone-by-clone shotgun sequencing, where bacterial artificial clones containing large genomic fragments of rice were first mapped in order to represent the entire chromosome and then systematically sequenced by the Sanger's sequencing method (IRGS Project 2005). The first draft of rice genome revolutionized not only the rice research but also helped to develop genomic resources for other crop plants.

With the invention of next generation sequencing (NGS) technologies, the high quality rice reference genome sequence produced by IRGSP served as a basis for the re-sequencing of hundreds of genotypes and also for thousands of transcriptomic data (Huang, Lu, and Han 2013). Re-sequencing has numerous applications including identification of sequence variation leading to better understanding of genetic regulations of traits and it also provides basis for the evolutionary studies (Guo et al. 2014). Recently, re-sequencing of interspecific hybrids between Asian and African rice varieties was performed for exploring agronomic traits of value such as grain shattering, awn-ness, and salt tolerance (Yamamoto et al. 2018). One of the seminal work which demonstrated the efficacy of the re-sequencing started in 2006 by The International Rice Functional Genomics Consortium (IRFGC) which performed re-sequencing of 21 rice genotypes (McNally et al. 2006). High-throughput genotyping by whole-genome re-sequencing effort for 150 rice recombinant inbred lines (RILs) have been used to develop a dense linkage map highly saturated with the single nucleotide polymorphism (SNP) markers (Huang et al. 2009). Recently, another notable study where re-sequencing of 30 rice accessions varying for anthocyanin content was performed to identify candidate genes involved in anthocyanin

biosynthesis pathway based on the sequence variation and differential-expression (Oh et al. 2018).

The 3000 rice genomes project started by Chinese Academy of Agricultural Sciences (CAAS), BGI in collaboration with International Rice Research Institute (IRRI) have performed a massive re-sequencing of 3,024 rice accessions collected from 89 countries across the world. Similarly, comprehensive databases such as RiceVarMap provide annotations to sequence variations for about 4,726 accessions which includes data from 'The 3000 Rice Genomes Project' as well (Zhao et al. 2015). Apart from such mega projects, numerous studies performed with specific objectives have developed a valuable genomic resource for the rice research (Table 1). These efforts serve for better exploration of genetic resources to develop elite lines for better quality rice.

QTL mapping for nutritional quality related traits in rice

During the past few decades, immense work has been done to increase yield and availability of food for resource-poor people. Starch, a complex carbohydrate is the major constituent of milled rice. Protein content of rice, although much lower compared to the pulses and wheat, is the second most important constituent in cereals, for which limited efforts have been employed. Rice contains about 6% protein content (Chattopadhyay et al. 2019) and its enhancement can help alleviate malnutrition in countries which largely depend on rice for meeting their demand for calories and lack a heterogeneous source of nutrients. Research in rice over last decade has started considering enrichment of micronutrients, minerals and proteins along with yield. Similarly, fortification of rice for vitamins, antioxidants, dietary fibers and modified starch are some of the desired traits to make rice a more balanced staple food at affordable costs. Genetic improvement of such traits requires in-depth understanding of genetic regulation, genes and molecular

Table 2. Details of important quantitative trait loci (QTLs) mapping studies performed to identify loci governing nutritional quality related traits in rice.

Nutritional traits	QTLs/Genomic region	Varieties	References
Zn, Fe, Cu, Mg, Na	OsNRAMP, OsNAS, OsZIP, OsYSL, OsFER, and OsZIFL	PSBRc82 x Joryeongbyeol and PSBRc82 x IR69428	(Swamy et al. 2018)
Fe and Zn	OsYSL1, OsMTP1, OsARD2, OsIRT1, OsNAS1, OsNAS2, OsNAS3, OsNRAMP1	Madhukar x Swarna	(Anuradha et al. 2012)
Ecological specific Ca, Fe, K, Mg, Mn, P, Zn	24 novel QTLs	Chunjiang 06 X TN1	(Du et al. 2013)
Fe, Ca, Zn, Mn and Cu	qCA-5, qCA-9, qCA-4, qZN-5, qZN-7, qZN-11, qMN-1, qFE-1, qFE-9	Zhenshan 97 and Minghui 63	(Lu et al. 2008)
Prolamin and glutelin content	16 QTLs on 8 chromosomes	Asominori X IR24	(Zhang et al. 2008)
Phytate, Fe and Zn	QTL on chromosome 5 and 12	IR64 x Azucena	(Stangoulis et al. 2007)
Protein and fat content	qRPC-5, qRPC-7, qRFC-2, qRFC-5	Gui 630 X 02428	(Hu et al. 2004)
Protein content and grain color	Wx gene for protein content and flour color QTLs on chromosome 1,3,4,5,6,7,8	Zhenshan97 x Minghui 63	(Tan et al. 2001)

mechanisms involved in the trait regulation along with environmental effects. Most of these traits are complex and governed by many small effect genes (Amarawathi et al. 2007; Deshmukh et al. 2010; Singh et al. 2012). In rice, exploring the easy crossing as well as strictly self-pollination nature of reproduction allows development of various types of mapping populations including doubled haploid (DH), F2 population, recombinant inbred line (RIL), and backcross inbred lines (BILs). Recently, more advanced mapping populations like nested association mapping (NAM) which is extension of bi-parental population like RIL, and multi-parent advanced generation inter-cross (MAGIC) populations have been developed and are being used for the mapping of complex traits including quality related parameters (Huang et al. 2015).

Numerous QTL mapping studies have been conducted to identify genetic regions underlying nutritional quality traits in rice (Table 2). A few biofortified crops have also been released in recent decades with high iron and zinc contents for instance, DDR-Dhan rice has high zinc content (22.6 ppm) (ICAR 2017). Another study on rice mutants for high Zn and Fe has also reported the range of Fe content from 0.91 to 28.10 mg/kg and Zn content between 15.36 and 28.95 mg/kg (Jeng et al. 2012). Complexity of nutritional quality related traits varies drastically; for instance, some traits like folate content involve few major-effect QTLs compared to traits like protein content which involve many small-effect QTLs. Major efforts are employed towards the identification of QTLs for grain protein content; which are mostly mapped on rice chromosomes 3, 5, 7 and 8 (Hu et al. 2004; Ye, Liang, and Wan 2010). Many of the traits related to nutritional quality are correlated and QTLs for such traits usually co-locate. For example, QTLs of amylose content, gel consistency, peak viscosity, breakdown viscosity, trough viscosity, final viscosity, setback viscosity, and retrogradation was found to be collocated on chromosome 6 of rice (Ponce, Ye, and Zhao 2018). Similarly, some nutritional quality related traits are negatively regulated and simultaneous improvement of such traits is a challenging task viz. grain iron concentration is negatively correlated with grain yield per plant (Soman et al. 2013). Similarly, phytic acid content affects mineral bio-accessibility in rice (Lee et al. 2015).

The QTLs identified in several studies are located within premises and possibly governed by the same genetic factor. Longer QTL spans and ambiguities in precise localization make utilization of QTL mapping difficult for breeding applications. QTLs identified from several studies vary in terms of their confidence intervals and number of genes included in them. For meta-QTL analysis, consensus QTLs are identified and a number of previously conducted studies are utilized to refine the aforementioned QTL locations. In addition, metaQTLs are defined at 95% of confidence interval (Islam, Ontoy, and Subudhi 2019). In this regard, MetaQTL approach provides an excellent opportunity to compile published QTL mapping data from different studies to calculate more accurate statistical significance level, phenotypic variations and it also defines the QTL span precisely. One such example entails common QTL for phytate and phosphorus on chromosome five (Stangoulis et al. 2007). Another research effort identified three MetaQTLs related to increased content of Fe and two for Zn respectively in rice (Dixit et al. 2019). Similar MetaQTL analysis have been conducted to identify candidate genes for traits like grain size, morphology of rice roots, and salt tolerance (Avni et al. 2018; Courtois et al. 2009; Islam, Ontoy, and Subudhi 2019; Singh et al. 2017a). Exploration of QTLs through marker assisted breeding to improve nutritional quality of rice has been widely conducted; still further efforts are required to study combined or counteractive effects of multiple QTLs for improved nutritional traits.

GWAS for nutritional quality related traits in rice

Despite being a powerful method to identify chromosomal segments associated with a trait; QTL mapping suffers from two major drawbacks viz. (i) only segregating alleles from parental lines can be studied and (ii) limited precision of mapping resolution. Genome wide association study (GWAS) approach overcomes the limitations of QTL mapping method (Korte and Farlow 2013; Sonah et al. 2015). GWAS is an approach to rapidly scan markers across the complete set of DNA, or genomes, of multiple varieties to find genetic variations associated with a particular trait. Upon identification of novel genetic associations; effective breeding strategies can employ this information for crop

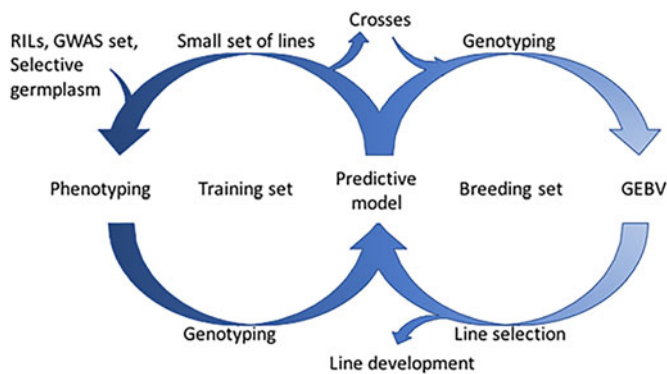


Figure 1. Schematic representation of genomic selection model in plants. A training dataset consisting of elite breeding lines, for which genotype and phenotype information is already available, is used to train the prediction model. Genomic Estimated Breeding Values (GEBVs) are calculated for the training dataset. For the development of improved lines, new candidate varieties are genotyped. GEBV values are calculated for these candidate varieties on the basis of GEBVs which were calculated from the training dataset. Lines with highest GEBV values are selected and crossed for development of improved varieties. Improved varieties are then genotyped and included in training dataset for increasing the efficiency of the prediction model.

improvement. GWAS approach also suffers from several limitations like requirement of high-throughput marker genotyping, large set of diverse germplasm resources and most importantly allelic information like representation of minor alleles at least in more than 5% of the germplasm set. For rare alleles which are present in very few genotypes, QTL mapping is always a preferred option. Considering the limitations of both the approaches it is advised to use them complementarily for the identification of QTLs. A study by Sonah et al. (2015) demonstrated the effectiveness of utilizing GWAS and QTL mapping approach simultaneously to dissect seed composition related traits in soybean. Genome-wide association mapping studies were conducted in approximately 300 rice varieties for concentrations of As, Cu, Mo and Zn in brown rice. Study across different environmental conditions revealed that variation in grain content of these elements was associated with several candidate genes and SNPs (Norton et al. 2014). In rice, GWAS and QTL mapping have been used together to study genetics of traits like aluminum accumulation in rice (Famoso et al. 2011) but combined studies for grain nutrient content are scarce. As compared to QTL mapping fewer efforts have been made using GWAS for the nutritional quality related traits.

Genomic selection efficiency and potentials to implement in breeding for the nutrient-rich rice

Marker assisted breeding, which is rather the most efficient method to incorporate precisely desired traits into the background of high yielding cultivars, requires prior information of loci governing the trait, linked markers, and screening of large segregating progenies to perform repetitive back-crossing. In addition, the newly made introgression may not result into expected trait improvement mostly due to different genetic backgrounds. Moreover, unwanted linkage drag is also a considerable problem with marker assisted breeding. Genomic selection (GS) introduced in 2001 by

Meuwissen, Hayes, and Goddard (2001) addresses these limitations by predicting the breeding values of lines in a population by analyzing their phenotypes and high-density marker scores (Figure 1). GS is a form of marker assisted selection in which genetic markers covering whole genomes are used so that all QTLs are in linkage equilibrium with at least one marker (Heslot et al. 2012). Besides being a decade old technology, GS is getting more attention after the availability of next generation based genotyping methods. The advent of cost effective genotyping techniques has increased GS efficiency several times. Even though many genotyping platforms and whole genome sequenced genotypes are publicly available very less efforts have been made in rice utilizing the GS approach. The efficacy of GS was first time evaluated in rice utilizing inbred lines to achieve genetic gain for grain quality traits such as grain yield, plant height and flowering time (Spindel et al. 2015). This study performed GWAS in conjunction with GS where the genomic prediction models were found to outperform to predict the phenotype as compared to prediction based on pedigree records alone. The study suggests that on one hand where cost-effectiveness of genotyping technologies has improved the utility of GS, on the other hand GWAS results of genetic architecture and population structure when combined with GS also increase efficiency of rice breeding.

As compared to yield related traits, inheritance of most of the quality related traits can be efficiently predicted. The relatively higher heritability of the quality traits also helps to efficiently implement the GS. The genomic estimated breeding values (GEBV) calculated through GS approach have shown wide range of accuracy for the traits including flowering time, plant height, grain yield and panicle weight (Grenier et al. 2015). Apart from the genetic architecture of traits, the accuracy of genomic prediction also varies greatly due to population structure and size, and marker density. A new strategy called genomic hybrid breeding which incorporates dominance and epistasis into the prediction model was proposed in 2014 (Xu, Zhu, and Zhang 2014). For an improved prediction of phenotypes of complex traits in diverse environments; a novel method for integrating genomic prediction with phenological modeling of Asian rice has also been proposed (Onogi et al. 2016). The proposed method showed greater accuracy in predicting heading dates of untested lines in untested environments. Although GS is being steadily used in quality traits for rice, exploration of utility of GS in studying nutritional traits still lags behind in rice (Table 3).

Mutagenesis approaches and mutation mapping for improving nutritional quality of rice: changing paradigm in NGS era

Mutations are a source of heritable genetic variability and are used to study the function of various genes. Where traditional hybridizations utilize existing genetic variations, novel mutations are also sometimes introduced for obtaining new traits (Tran and Ho 2017). Mutagenesis approaches can be spontaneous or induced (physical, chemical or

Table 3. Genomic selection efforts performed to improve the genetic potential for different traits in rice.

Trait	Training population/ breeding population	Details/significant out come	Prediction efficiency	Reference
Breeding strategy for hybrids in rice using GS	Double-haploid lines for maize, wheat, rye, barley, rice and triticale	Suggested key factors to consider in breeding strategies to maximize annual selection gain for grain yield	Recommends use of <i>GSrapid</i> strategy along with other key factors for hybrid breeding of cereals	(Marulanda et al. 2016)
Flowering time, plant height, grain yield, panicle weight	343 lines for 4 inter-related synthetic populations	Various factors affecting GEBV accuracy were investigated	Among a number of factors, phenotypic traits had the most significant effect on GEBV accuracy	(Grenier et al. 2015)
Grain yield, flowering time, and plant height	363 elite varieties	Effects of training population, statistical methods, markers and traits on accuracy of GS were studied	Genomic prediction models offered higher accuracy than results based only on pedigree data	(Spindel et al. 2015)
Yield	278 hybrids	The study proposes genomic best linear unbiased prediction method	Accuracy of prediction increases with inclusion of dominance and epistasis into the model	(Xu, Zhu, and Zhang 2014)

insertional) (Supplementary Table 1). Physical agents like fast neutron, gamma ray or ion beam irradiation using thermal neutrons, beta and alpha particles, X-rays, UV light; and chemical agents like diepoxybutane, alkylating agents like ethylmethanesulfonate and combination of sodium azide plus methyl-nitrosourea, intercalating agents like ethidium bromide and base analogs such as bromouracil; have been easy and inexpensive methods for mutagenesis and many of these have been adopted in rice (Mba et al. 2010; Till et al. 2007; Wu et al. 2005).

For instance, combined gamma radiation and hybridization approach have been utilized for development of new scented rice varieties with increased iron content suggesting utilization of hybrid approaches for generation of new cultivars with multiple improved traits (Tran and Ho 2017). Various mutant rice lines have been developed such as accumulation of anthocyanin content of Thai jasmine rice was increased to impart a deep blue color through bombardment with low-energy ion-beam (Semsang et al. 2012). The mutated variety BKOS extracts showed the highest total phenol content and improved antioxidant activities. Similarly, *lpa* (low phytic acid) mutant lines were developed from *Indica* and *Japonica* subspecies through physical and chemical mutagenesis with low phytic acid content which is commonly regarded as anti-nutritional component (Liu et al. 2007).

Induction of mutation is much easier, but to map the causal mutation for further use in breeding programs needs substantial efforts. With the availability of NGS tools several methods for the mutation mapping have been developed. For instance, MutMap is a method being used for identification of mutant loci and mapping of causal mutation by screening sequence variation among segregating mutant lines (Abe et al. 2012). The method was first introduced in rice in 2012 (Abe et al. 2012). In MutMap, a mutant genotype is crossed to wild-type genotype followed by selfing which allows segregation of phenotypic differences in second filial (F_2) generation. MutMap+ is an extension of MutMap and it does not necessitate crossing between mutants and original wild-type parental lines, and identifies causal mutations by comparing SNP frequencies of DNA of mutant and

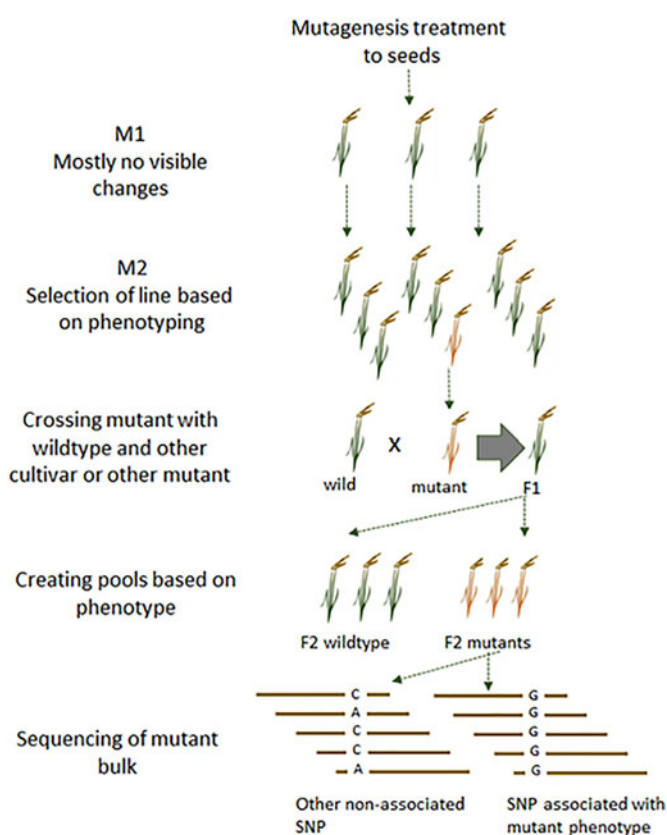


Figure 2. Basic steps involved in the MutMap approach. After mutagenesis treatment, the plants raised (M1 mutant generation) will be heterozygous for most of the mutations therefore no visible changes can occur. The subsequent generation (M2) will have lines (one line per are M1 plant) that will be segregating and plants homozygous for the mutation will show phenotypic changes. Based on the phenotyping (mutant and wildtype) selected plants with mutant phenotype need to be crossed with wild-type variety to obtain F1 and subsequent segregating F2 generation. Then the bulks formed based on wild-type and mutant phenotype sequenced using next-generation sequencing (NGS) will facilitate the identification of mutation associated with the mutant phenotype. MutMap technique was first demonstrated in rice (Abe et al. 2012).

wild-type progeny of M3 generation obtained after selfing of an M2 heterozygous line (Figure 2) (Fekih et al. 2013). A similar technique MutMap-GAP enables identification of genes from gaps in a reference genome; when combined with MutMap (Takagi et al. 2013). MutMap is used to

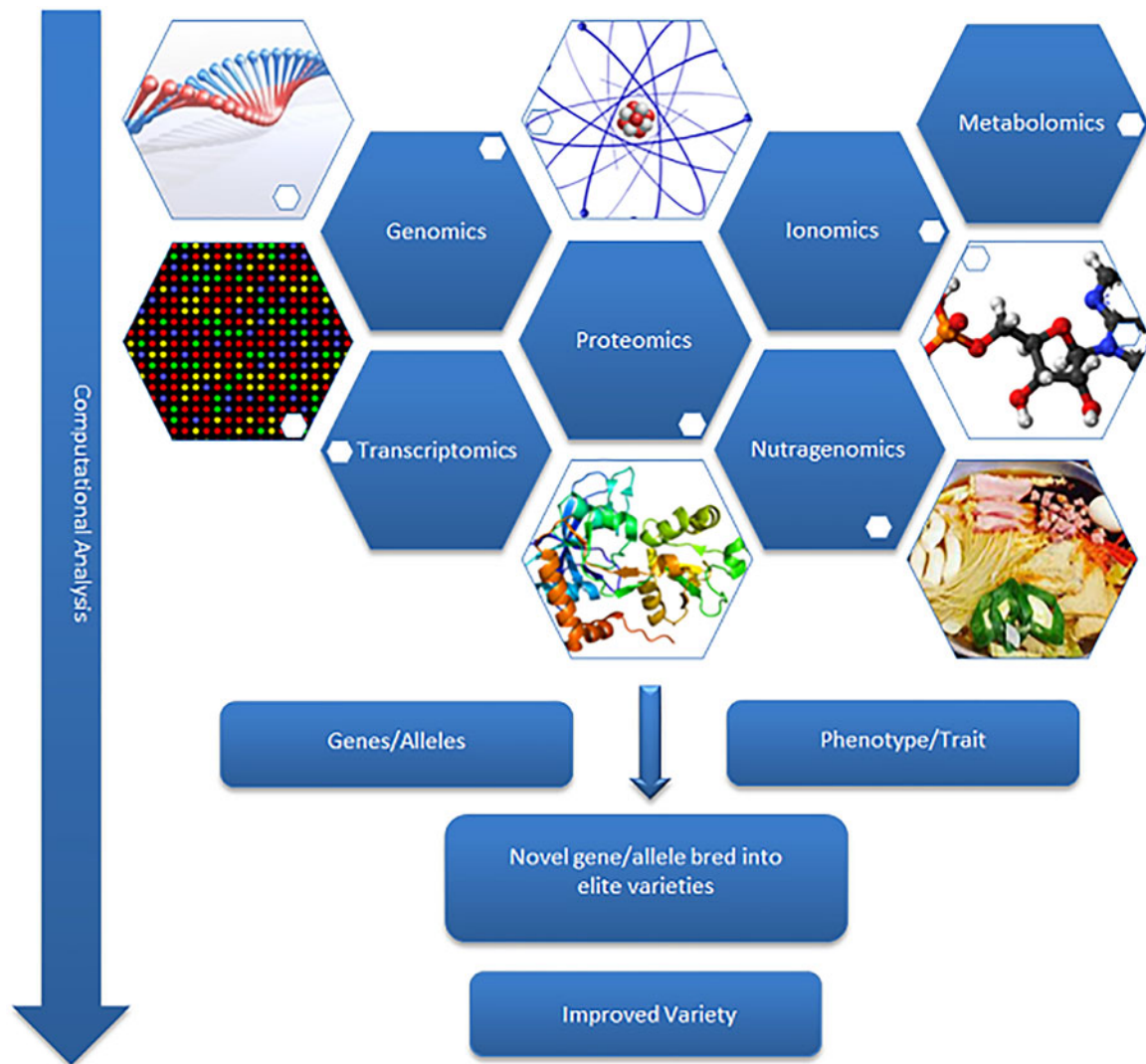


Figure 3. Major branches of 'omics' approaches being used for the understanding and genetic improvement of desired trait. The approaches are being used exclusively or integrated means data from all 'omics' related fields giving comprehensive overview of genetic architecture and phenotype is utilized to identify novel genes/alleles. These novel genes/alleles are then bred into elite lines to obtain improved varieties.

identify candidate region which contains targeted mutation. Once the targeted region is ascertained, de-novo assembly is performed followed by alignment and ultimately identification of mutations in the gapped regions (Takagi et al. 2013). Mutagenesis approaches have not only enabled us to identify genetic regions related to a trait of interest but have also helped to expand the diversity as well as develop mutant rice varieties with better agronomic traits.

Nutrition improvement in rice using genetic engineering technologies

Introduction of foreign genes into crop genomes termed genetic transformation enables stable integration of beneficial genetic regions or genes not originally found in plants under study. Various physical methods like biolistic gene gun, electroporation, microinjection, sonication; and biological methods such as *Agrobacterium*-mediated genetic engineering which is prominently used; have permitted manipulation of DNA. Various targets have been designed for the selection of appropriate transcription factors,

activators and promoters for tissue-specific or developmental-stage specific gene expression which are of paramount importance for effective genetic transformation (Mubeen et al. 2016).

The use of insertional mutagens includes T-DNA, maize transposable *Ac/Ds* system and *tyl*-copia retroelement *Tos17* (Guiderdoni et al. 2005). The use of DNA insertions is a preferred method for mutagenesis because the genomic positions are easily monitored to derive relationships between genotypes and phenotypes (Krishnan et al. 2009). Transgenic rice lines with increased Fe, Zn and beta-carotene in the rice endosperm were developed expressing *Arabidopsis thaliana*'s nicotianamine synthase 1 (*AtNAS1*), bean ferritin (*PvFerritin*), bacterial carotene desaturase (*CRTI*) and maize phytoene synthase (*ZmPSY*) in a single genetic locus (Singh, Gruijssem, and Bhullar 2017b). *Agrobacterium*-mediated transformation was employed to introduce beta-carotene biosynthetic pathway into rice endosperm in a single transformation (Ye et al. 2000). Similar studies have been performed by transfer of soybean ferritin gene into rice using *Agrobacterium*-mediated transformation

which was undertaken to increase iron content of rice (Goto et al. 1999). Similarly, iron fortification of rice was achieved through activation of nicotianamine synthase gene in 2009 (Lee et al. 2009). RNAi-mediated studies on Cd transporter OsLCT1 helped identify and describe its function in regulation of Cd transport into rice grains (Uraguchi et al. 2011). Similarly, another study elucidated the expression of a silicon transporter gene *low silicon rice 1* (lsil) using RNAi (Ma et al. 2006). Increase in Fe and Zn contents of rice grains has been observed by targeting rice *Nicotianamine Synthase* gene in various studies using genetic engineering approaches (Mahender et al. 2016). Similarly, rice grain lysine and methionine contents have also been increased through transgenesis. One such example entails transgenic rice expressing a polypeptide precursor of a seed storage protein leading to accumulation of sesame 2S albumin resulting in increased lysine content in seed (Galili and Amir 2013). Genetic transformation studies have enabled efficient introduction of desired genetic regions into rice for nutritionally quality important but the translation of the efforts into a product depends on worldwide acceptance of the transgenic technology (Figure 3).

Transcriptomics advances for the nutritional quality improvement in rice

Transcriptomics, a study of RNA expression profile addresses both coding RNA as well as regulatory non-coding RNA sequences inside the cells at a given time. Various techniques like microarray and RNA-sequencing (RNA-Seq) have been developed to study the gene expression profile of crop plants under varying conditions (Pandit, Shah, and Husaini 2018). In NCBI SRA database, RNA-seq data for over 35,241 rice samples have been deposited up to March 2019 and the frequency of RNA-seq data submission is expected to vigorously increase in near future. Similar to RNA-seq data, considerably high volume of transcriptome profiling data of rice generated through microarrays have also been publically available at gene expression omnibus (GEO: <https://www.ncbi.nlm.nih.gov/geo/>).

Initial attempts to study the whole transcriptome were started in 1990s (Lowe et al. 2017). For better elucidation of the complexity of rice transcriptome, whole-genome transcription profiles of *O. sativa japonica* and *indica* subspecies have been obtained using RNA-seq (Lu et al. 2010). In a study, large scale analysis of RNA-seq provided an improvement to the rice genome annotation with discovery of 1584 novel peptides and 101 new loci matched by novel peptides (Ren et al. 2019). Similarly, the role of alternative splicing in maintenance of mineral nutrient homeostasis in rice has also been studied (Dong et al. 2018). To understand the antagonistic interactions among the micronutrients, transcriptomic and microarray studies have been conducted. Zhang et al have performed the study to know the antagonistic interaction between Fe and P using microarray data in rice seedlings (Zheng et al. 2009). Characterization of molecular genetic basis for adaptation to macronutrient (N, P and K) deprivation was done using microarray analysis of

rice root where genes and important features of signaling pathways for these macronutrients were identified (Takehisa et al. 2013).

Transcriptomics enables analysis of gene expression changes, elucidation of un-annotated genes and regulation of genes (Lowe et al. 2017). Recently, expression profiling for N and P and the identification of three gene sets as indicators was conducted in rice under field conditions (Takehisa and Sato 2018). Large scale GWAS and transcriptome studies have helped to predict genes influencing glycaemic index (GI) in rice, as low GI is an important nutritional intervention for populations suffering from Type II diabetes (Anacleto et al. 2018) and several other metabolic diseases such as obesity and hypertension. Along with microarray profiling, laser microdissection was used to obtain a cell type transcriptome atlas for 40 cell types from rice to study cellular, functional and developmental hierarchies (Jiao et al. 2009). Most of the rice transcriptome profiling studies have been conducted in relation to stress response and relatively fewer efforts have been employed for the nutritional quality related traits.

Proteomics advances for nutritional quality improvement in rice

Proteomics entails the large-scale study of the dynamic proteomes or the complete set of proteins, their location, abundance and post-translational modifications in an organism. To study protein structure and function, proteomics complements genomics and transcriptomics to further explore molecular understanding. Several high-throughput techniques such as gel-free techniques like Tandem-MS, LC-MS-MS, MALDI-TOF/TOF, gel-based methods such as differential in-gel electrophoresis (DIGE) and combination of these are being used to generate enormous amounts of proteomics data (Supplementary Table 2). Proteomics aims at studying expression patterns of agronomic compounds in crop plants. Studies aimed at elucidating the differential expression patterns of bio-active compounds help to better explore nutritional quality of rice under varying conditions. A comparative proteomics and protein-profile of rice line KDML105 and Mali Daeng (MD) was performed to reveal differences in expression patterns of phenolic compounds, anthocyanin and antioxidant activity after germination (Maksup et al. 2018). The study revealed higher contents of phenolic compounds; anthocyanin and antioxidant activities in red rice MD as compared to white rice KDML105. A comparison of nutritional compositions and proteomics between transgenic and conventional rice was drawn in a study which showed no significant differences in the nutritional compositions between the two (Gayen et al. 2016). The proteomics approaches looks promising to evaluate possible impact of transgenic on food nutritional quality or any changes in gene expression followed by translation activities.

An investigation into the expression levels of seed storage proteins and their relation to nutrient quality of different commercial rice types have been performed to understand

the regulation at protein level (Ramli and Zin 2015). In a study, proteomic methods used to obtain the physiochemical function of seed proteins such as hydrophilic and catalytic activity and binding affinity involved in metabolic pathways have characterized 302 candidate proteins (Yang et al. 2013). An investigation into the proteomic and glycomic contents of rice chalky grains under high-temperature stress conditions suggested that starch degradation rather than starch synthesis is implicated in chalkiness of rice (Kaneko et al. 2016). Proteome analysis of expression of 25 metal-related genes from rice involved in Fe and/or Zinc concentrations in seeds has been undertaken, which is useful for bio-fortification purposes (Sperotto et al. 2010). The study provides insights of molecular mechanism involved in the metal transport from flag leaves to the seeds. A comparative study of genome and proteome of two high-yielding rice cultivars was conducted to understand cultivar specific variations in antioxidant and phenolic content in rice when exposed to different ozone concentrations (Sarkar et al. 2015). The study witnessed changes in antioxidant Defense pathways and proteome of leaf along with decrease in quality and yield of grains. Hence, proteome analysis combined with genetics of crops helps in gaining extensive insights into the dynamic protein content in agronomically important parts of the plant under varying conditions and an understanding of genes underlying the effective protein content in grains.

Ionomics advances for rice nutritional quality research

Study of mineral nutrient, low molecular weight molecules and trace element composition in different physiological conditions and developmental stages of a plant and the underlying molecular mechanisms using high-throughput elemental profiling approach is termed as ionomics. It represents the inorganic composition at cellular and organism levels. Ionomics emerging as an efficient omics approach has been found to be efficient for the identification of genes and gene networks particularly regulating the ionome (Huang and Salt 2016; Satismruti et al. 2013). Ionomics is an attractive technique owing to its comprehensiveness, cost-effectiveness and high-throughput attributes (Baxter 2010). Various techniques and tools are used in ionome analysis such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), X-Ray crystallography, Neutron Activation Analysis (NAA) (Supplementary Table 3). A comprehensive study of the underlying genetic architecture for varying ionome in rice was performed using GWAS for 17 mineral elements in grains of 529 rice accessions (Yang et al. 2018). The study gives details about genes and molecular mechanisms responsible for variations in micronutrients and mineral composition across rice accessions. Similarly, a study has been performed to evaluate the antioxidant activities, macro- (P, K, Ca, Mg) and micro-nutrient (Fe, Zn, Mn, Cu) composition of 6 local rice varieties (black glutinous rice, red rice, brown rice, white fragrant rice, white rice) from Malaysia (Shin et al. 2016). Such efforts at large scale

are required to explore the available genetic resources and for the better understanding of ionomic dynamics in rice tissues.

Identification of rice germplasms with enhanced grain nutritional quality, mineral content and trace element concentrations for whole grains of 1763 rice accessions was done (Pinson et al. 2015). Mineral content profiling in seed grains of diverse rice varieties were evaluated for phosphorus (P), magnesium (Mg), potassium (K), sulfur (S), calcium (Ca), arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), rubidium (Rb), strontium (Sr) and zinc (Zn) concentrations using ICP-MS. The study showed that accessions from *Indica* group mainly contained high Ca, Mg, or K; and low As was common among temperate *Japonica* varieties. A study conducted genetic mapping for ionome in leaves and grains of rice for identification of QTLs for 17 elements which included Fe, Se, Cd and As (Norton et al. 2010). The multi-element analysis resulted in detection of 36 QTLs which were responsible for element concentrations in leaves and 41 QTLs for grains. Moreover, epistatic interactions were detected for most elements in this study. Significant studies have been conducted which have established a relationship between rice Si and As levels. One such effort focused on effect of Si concentrations in soil on As, Fe and P accumulation in rice plant parts. As a result an inverse relationship between Si application and As accumulation in brown rice, polished rice, husk, straw plant parts was confirmed (Fleck, Mattusch, and Schenk 2013). Such ionomics studies when combined with genotyping techniques have enabled rapid identification of genes controlling mineral elements accumulation in plants.

Metabolomics advances in rice nutrient quality

Metabolomics, a term coined in 1998 is the comprehensive, systematic identification, qualitative and quantitative study of small molecules in a biological system (Barchet 2013). Various analytical techniques for analysis of plant metabolome include mass spectrometry (MS) like gas chromatography (GC-MS), liquid chromatography (LC-MS), capillary electrophoresis (CE), metabolic fingerprinting procedure using Fourier-transform ion cyclotron mass spectrometry (FT-MS), microscopy coupled with Raman spectroscopy and nuclear magnetic resonance (NMR) which is used for the identification of compounds otherwise unidentifiable by MS (Supplementary Table 4). Metabolomic analyses have revealed differences in bioactive compounds in different varieties of cooked as well as uncooked rice. A study detected around 3,097 compounds and SNPs for genes regulating metabolic pathways of nutritional importance (Heuberger et al. 2010). Variations in metabolome of cooked rice were explored and effect of SNPs in varying nutritional components of rice such as phenolics and Vitamin E content; was determined in this study. Metabolomics represents the molecular phenotype of a living system and signifies the biochemical activities undergoing in a living system. A method was developed and employed for metabolic

Table 4. Details of important online resources available for rice research.

Database	Features	Reference
SNP-Seek RiceXPro	SNP genotype data for the 3000 rice genomes project Rice Expression Profile Database has gene expression profiles from microarray analysis	https://doi.org/10.1093/nar/gkw1135 http://ricexpro.dna.affrc.go.jp/
RPAN RiceVarMap RGAP	Rice Pan-Genome Browser for rice pan-genome from 3K RGP Database for rice genomic variations Rice Genome Annotation Project database for rice annotation data	http://cgm.sjtu.edu.cn/3kricedb/ http://ricevarmap.ncpgr.cn/v2/ http://rice.plantbiology.msu.edu/
RAP-DB TENOR IC4R RED	The Rice Annotation Project Transcriptome Encyclopedia Of Rice Information Commons for Rice integrates multiple omics data Rice Expression Database for gene expression profiles from RNA-Seq data	https://rapdb.dna.affrc.go.jp/ https://tenor.dna.affrc.go.jp/ http://ic4r.org/ http://expression.ic4r.org/
Q-TARO RiceFOX	QTL Annotation Rice Online Database Rice Full-length c DNA over-expressed Arabidopsis mutant database	http://qtaro.abr.affrc.go.jp/ http://ricefox.psc.riken.jp/
GRAMENE QTL DB	QTL for agronomic traits in rice, maize, barley, oats, sorghum, pearl millet, foxtail millet and wild rice	http://archive.gramene.org/qtl/
RiceNET RicePPINet UnIVIO	A network prioritization server for <i>Oryza sativa</i> A computational Interactome for Rice Multiple Omics Database	https://www.inetbio.org/ricenet/ http://netbio.sjtu.edu.cn/riceppinet/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC358302/

phenotyping of natural variants in 68 rice accessions for 10 representative metabolites (Kusano et al. 2007). Such studies have enabled application of metabolite profiling in rice for deducing the genes, QTLs and variations responsible for nutritional quality of grains. A study conducted genetic analysis of metabolome of rice which resulted in identification of 2,800 QTLs for 900 metabolites. This led to identification of 24 candidate genes which are mainly responsible for phenolic compounds concentrations in rice (Gong et al. 2013). Numerous studies have demonstrated efficacy of metabolomics for the understanding of molecular mechanism involved in different quality related traits. For instance, a study was conducted which utilized a novel multi-platform metabolomics approach on multiple metabolomics data sets for identification of discriminatory compounds relevant for nutritional values in rice (Calingacion et al. 2012). Metabolomics has enabled simultaneous assessment of a large number of metabolites as well as identification of the genetic architecture underlying nutritionally important bioactive compounds in rice. Metabolomics data can aid selection of breeding material for development of elite rice varieties.

Metabolomics-assisted breeding is a crucial technique for crop genetic improvement (Gong et al. 2013). Rice bran metabolite profiling was done to reveal 209 amino acids, cofactors and vitamins, and secondary metabolites to improve upon the current available information of compounds which are provided during dietary supplementation (Zarei et al. 2017). Natural variations in the levels of 121 mature seed metabolites have been characterized in *Japonica* and *Indica* cultivars to reveal correlations between metabolic phenotype and geographical origins of rice (Hu et al. 2015). In addition to metabolomics analysis for cultivated rice, similar research on wild rice species has also explored identification and development of functional foods. For instance, wild rice species from North America (*Zizania palustris*) and China (*Zizania latifolia*) were compared for secondary metabolite contents and it was found that the two differed in 357 metabolites especially anthocyanins and catechins

(Yan et al. 2019). Similarly, metabolomics profiling of grains from giant embryo and normal embryo rice suggested that the former accumulates additional bioactive compounds suggesting better quality of rice grains which are derived from giant embryos (Zhao et al. 2019). Metabolomics has enabled a comprehensive study of small metabolites in crop plants which are closely linked to their phenotypes (Supplementary Table 4). The major challenge of metabolomics lies in extraction and interpretation of huge amount of information in biological context.

Computational resources for rice research

Past few decades have witnessed a surge in availability of information for crop plants, owing to improvements in sequencing technologies and techniques related to various omics fields (Sharma et al. 2018). Advancements in computational resources in the form of databases, software and tools have enabled efficient storage, easier retrieval and effortless data analysis. Data generated from various studies through different schemes of experimentation is available at successive stages viz. raw, processed and annotated data. By using computational platforms, researchers can easily design and perform extensive experiments like genome-wide profiling of numerous targets at genomic and proteomic level. Numerous repositories have been developed for data storage for extensive international sequencing efforts in rice like International Rice Genome Sequencing Project (IRGSP) and Rice Genome Annotation Project (RGAP). Similarly, various computational resources have been made available for easy retrieval, sharing and manipulation of crop data in rice (Table 4). One such online repository for rice re-sequencing data is Rice SNP-Seek Database (<http://snp-seek.irri.org/>) which is an extensive source of genetic variation data for 'The 3000 Rice Genomes Project'. The database provides extensive information of about 20 million rice SNPs and InDels in rice (Alexandrov et al. 2015). Similarly, genome sequencing of not only cultivated rice varieties but also of cultivars with specific characteristics such halophytic species

Table 5. List of major genes known to regulate different nutritional quality traits in rice.

Gene	Locus ID	Details	Reference
sd1,GA20ox2	LOC_Os01g66100	Grain protein content is controlled by semi-dwarf mutant allele	(Terao and Hirose 2015)
OsAAP6,qPC1	LOC_Os01g65670	Regulates grain protein content and nutrition quality	(Peng et al. 2014)
RBP-P	LOC_Os01g16090	Localizes glutelin and prolamine mRNA to endosperm cells	(Tian et al. 2018)
RISBZ1,OsZIP58,OsSMF1	LOC_Os07g08420	Participates in seed storage protein accumulation in grain	(Kawakatsu and Takaiwa 2010)
RAG2	LOC_Os07g11380	Functions in grain yield and quality	(Zhou et al. 2017)
glu4a	LOC_Os01g55690	Seed storage protein	(Qu et al. 2003)
OsMADS6	LOC_Os02g45770	Affects Endosperm nutrient accumulation	(Zhang et al. 2010)
RINO1	LOC_Os03g09250	Effect on phytic acid content	(Kuwano, Takaiwa, and Yoshida 2009)
XS-lpa2-1	LOC_Os03g04920	Effect on phytic acid content	(Xu et al. 2009)
lpaN15-186	LOC_Os03g52760	Effect on phytic acid content	(Kim et al. 2008b)
lpa1	LOC_Os02g57400	Involved in phytic acid metabolism	(Kim et al. 2008a)
OASA2	LOC_Os03g15780	Targeted for high-tryptophan content	(Saika et al. 2011)
CysR10	Os03g0766000, Os03g0766100	Role in protein body 1 formation	(Nagamine et al. 2011)
OsYSL2	LOC_Os02g43370	Long distance transport of Iron and Manganese	(Ishimaru et al. 2010)
OsNAS3	LOC_Os07g48980	Targeted for iron fortification	(Lee et al. 2009)
OsAAP6	Os01g0878700	Regulates grain protein content and nutritional content	(Peng et al. 2014)
OASA1D	LOC_Os03g61120	Tryptophan accumulation in seeds	(Wakasa et al. 2006)
OsVIT1	LOC_Os04g38940	Iron translocation	(Zhang et al. 2012)
OsVIT2	LOC_Os09g23300	Iron translocation	(Zhang et al. 2012)

has been conducted (Mondal et al. 2017). Such endeavors have fast-forwarded research in agronomically important crop plants throughout the world. The computational resources not only improve the access of large scale data but also improves analytical processing to solve more complex biological relevant questions which otherwise is difficult to answer.

Integrated omics approaches for nutritional quality related traits in rice

For a comprehensive knowledge of agronomically important traits in a crop, an integrated study encompassing high-throughput and large-scale experiments which include data from genomics, transcriptomics, proteomics, metabolomics, ionomics is of vital importance. Simultaneously, rapid development in high-throughput technologies, sequencing techniques, analysis tools and availability of complete genome sequence of rice has propelled efforts to improve agronomically and nutritionally important traits in crops. In addition to identification of genes responsible for agronomic traits, an integrated omics based research approach aims at deciphering the biological pathways and interactions between biomolecules and processes. Since the complete genome sequencing of rice became available over a decade ago, various individual transcriptome, proteome, metabolome and ionome studies based on the genomic data have been undertaken for improving nutritional quality of rice grain (Supplementary Tables 2–4). However, extensive integrated omics efforts have not been actively explored at its maximum potential.

Efficient exploration of the gene information can only be possible if in-depth understanding of genetic regulation, molecular pathways and complex gene x gene and gene x environment is available. In this regard, different omics tools provide opportunity to get holistic information required for the product designing. Presently, several genes regulating nutritional quality related traits have been extensively studied (Table 5). The information of these genes is expanding with the help of different omics platform and being

efficiently explored through molecular breeding, transgenic approach and even with relatively new technology like genome-editing.

Among the extensive integrated omics efforts generating a considerably large information include a study which discovered around 3,000 metabolites found in ten varieties of cooked rice using metabolomics (Heuberger et al. 2010). The genetic variations that caused this metabolite diversity were investigated by using functional genomics. As a result, the genetic diversity in genes for phenolic compounds was ascertained and SNPs were found in their un-translated regions (UTRs) which are equally important in regulation of gene expression. Similarly, efforts were undertaken to analyze comparative genomic and metabolomic variations in traditional and improved waxy rice varieties. The study resulted in determination of discriminatory compounds of nutritional importance as well as yield related differences among three rice cultivars (Calingacion et al. 2012). There is a dearth of similar integrated omics efforts to deduce relationships between and study thoroughly the agronomically and nutritionally important traits in rice crop.

Big data initiatives

Big data are datasets with dimensions beyond the analytical, storage and processing capabilities of traditional databases and tools. Agricultural data pertaining to crop genome sequences, weather forecast, biodiversity, fertilizers, pesticide interventions, nutrient availability in soil and harvest; is enormous and difficult to be managed on traditional data platforms (Kamilaris, Kartakoullis, and Prenafeta-Boldú 2017). Based on models developed from available big data analysis, more efficient solutions can be proposed to mitigate issues on the field (Lokers et al. 2016). Application of navigational satellite systems, remote sensing and cloud computing can help in efficient monitoring, crop management and decision-making processes. Big data is being used in weed control, crop protection, irrigation management (Sayad, Mousannif, and Le Page 2015) and for prediction of crop yield. One such example is European Space Agency

funded 'GeoRice' innovation platform for effective monitoring of rice crops. It uses Earth Observation data from satellite Sentinel-1 for crop monitoring. Such big data innovations are also helping in observing maturity, yield, growth, abnormalities in crop plants in real time. Similarly a case study on rice fields in Kanchipuram, India was undertaken using big data to predict crop quality based on cropping/farming systems (Senthilvadivu et al. 2016). Linking different big data efforts in an efficient way to gather and analyze such large scale multi-dimensional information is another challenge for rice community.

Nutrigenomics for rice improvement

Nutrigenomics aims at studying the relationship between nutrient intake and human health. It aims at understanding the effect of dietary regimes and nutrients on gene expression at the molecular level. The main cause of malnutrition is well known to be a chronic lack of vitamins and minerals. Nutrigenomics aims at improving the quality of food by enhancement of the bio-availability of macro and micro-nutrients in cereal vegetables and fruits or by incorporating bioactive compounds into crop plants (Tran and Kumar 2016). In addition to curbing impending diseases, nutrigenomics also concerns populations already suffering from various maladies. A study was undertaken to check the oxidative stress increase in type 2 diabetic rats when fed with white, brown or germinated brown rice and concluded the damaging effect of white rice as compared to other rice (Imam et al. 2012). Further the functional properties and nutrigenomic effects of bioactive compounds-rich germinated brown rice have been studied to understand its role in healthy diets (Imam and Ismail 2015). In spite of being a promising effort in personalized disease-intervention through diet, nutrigenomics suffers from a few limitations. Ethical dilemmas related to identity of patients, confidentiality of genetic information and targeted advertisement of supplements by companies aiming for higher profits highlights the need for strict management protocols in nutrigenomics. In addition, monitoring the relatively dynamic food intake habits of individuals, price and time-consuming protocols need long-term interventions (Castle and Ries 2009). Even though the importance of nutrigenomics is widely recognized very less efforts are being made towards its integration to crop improvement programs.

General conclusions

Rice research has focused primarily on yield-related traits with limited emphasis on the nutritional quality improvements. With the current changing nutritional requirements due to increasing population and dwindling resources, utilization of modern tools and techniques on a large scale becomes necessary for crop improvement. Compared to other omics branches, significant progress has been achieved in genomics and transcriptomics and subsequent integration of genomics and transcriptomics has become more frequent. Up to now several QTL mappings and GWAS for

marketable quality-related traits like grain size, elongation ratio, and aroma have been performed but such efforts to address nutritional quality are limited. Resources developed for the QTL and GWAS can be efficiently employed to develop a prediction model for genomic selection. Simultaneous utilization of different approaches sharing similar resources will be an effective strategy for crop improvement programs. High-throughput genotyping techniques coupled with QTLs mapping, GWAS and GS seems feasible and cost-effective. Similarly, the omics scale resources publically available for rice need to be explored efficiently. The gap between the development of omics resources and its utilization is widening over the time which warrants more efforts demonstrating efficient utilization of such resources. Mutagenesis is one such area which became more effective with the advent of NGS technologies. New mutation mapping approaches are more precise, quick and cost-effective. Similar approaches exploring the integration of different tools and techniques are expected. Besides achieving tremendous advances in different omics branches, interdisciplinary studies and integrated approaches have not been explored at its full potential to succeed desired quality rice grains. Complementing conventional breeding, an integrated omics approach has proven to be more efficient for trait improvement. Integration of omics tools like genomics, transcriptomics, proteomics, metabolomics, and ionomics are paramount to gain a comprehensive overview of the nutritional quality-related traits in rice. The critical review of the progress made towards rice improvement highlighted the lack of affordable and feasible high-throughput phenotyping platforms to cope up with other omics branches for its efficient exploration. Omics interventions for the enhancement of nutritional quality in rice will be helpful to eradicate hidden hunger and to archive sustainable food security for growing world population.

Acknowledgments

Authors are thankful to the Department of Science and Technology, Govt of India for the award of JC Bose National Fellowship to TRS, and Department of Biotechnology, Govt of India for the award of Ramalingaswami Fellow to RD and HS.

References

- Abe, A., S. Kosugi, K. Yoshida, S. Natsume, H. Takagi, H. Kanzaki, H. Matsumura, K. Yoshida, C. Mitsuoka, M. Tamiru, et al. 2012. Genome sequencing reveals agronomically important loci in rice using MutMap. *Nature Biotechnology* 30 (2):174. doi: [10.1038/nbt.2095](https://doi.org/10.1038/nbt.2095).
- Alexandrov, N., S. Tai, W. Wang, L. Mansueto, K. Palis, R. R. Fuentes, V. J. Ulat, D. Chebotarov, G. Zhang, Z. Li, et al. 2015. SNP-Seek database of SNPs derived from 3000 rice genomes. *Nucleic Acids Research* 43 (D1):D1023–D1027. doi: [10.1093/nar/gku1039](https://doi.org/10.1093/nar/gku1039).
- Amarawathi, Y., R. Singh, A. K. Singh, V. P. Singh, T. Mohapatra, T. R. Sharma, and N. K. Singh. 2007. Mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). *Molecular Breeding* 21 (1):49–65. doi: [10.1007/s11032-007-9108-8](https://doi.org/10.1007/s11032-007-9108-8).
- Anacleto, R., S. Badoni, S. Parween, V. M. Butardo, Jr., G. Misra, R. P. Cuevas, M. Kuhlmann, T. P. Trinidad, A. C. Mallillin, and C. Acuin. 2018. Integrating a genome-wide association study with a

- large-scale transcriptome analysis to predict genetic regions influencing the glycaemic index and texture in rice. *Plant Biotechnology Journal* doi: [10.1111/pbi.13051](https://doi.org/10.1111/pbi.13051).
- Anuradha, K., S. Agarwal, Y. V. Rao, K. Rao, B. Viraktamath, and N. Sarla. 2012. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar \times Swarna RILs. *Gene* 508 (2):233–40. doi: [10.1016/j.gene.2012.07.054](https://doi.org/10.1016/j.gene.2012.07.054).
- Arthur, R. A., and J. L. Bennetzen. 2018. Discovery of lineage-specific genome change in rice through analysis of resequencing data. *Genetics* 209 (2):617–26. doi: [10.1534/genetics.118.300848](https://doi.org/10.1534/genetics.118.300848).
- Avni, R., L. Oren, G. Shabtay, S. Assili, C. Pozniak, I. Hale, R. Ben-David, Z. Peleg, and A. Distelfeld. 2018. Genome based Meta-QTL analysis of grain weight in tetraploid wheat identifies rare alleles of GRF4 associated with larger grains. *Genes* 9 (12):636. doi: [10.3390/genes9120636](https://doi.org/10.3390/genes9120636).
- Awais, A., C. Nualsri, and W. Soonswon. 2019. Induced mutagenesis for creating variability in Thailand's upland rice (Cv. Dawk Payawm and Dawk Kha 50) using ethyl methane sulphonate (EMS). *Sarhad Journal of Agriculture* 35 (1) doi: [10.17582/journal.sja/2019/35.1.293.301](https://doi.org/10.17582/journal.sja/2019/35.1.293.301).
- Barchet, G. 2013. A brief overview of metabolomics: What it means, how it is measured, and its utilization. *The Science Creative Quarterly* 8.
- Baxter, I. 2010. Ionomics: The functional genomics of elements. *Briefings in Functional Genomics* 9 (2):149–56. doi: [10.1093/bfpg/elp055](https://doi.org/10.1093/bfpg/elp055).
- Bouis, H. E., and A. Saltzman. 2017. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security* 12:49–58. doi: [10.1016/j.gfs.2017.01.009](https://doi.org/10.1016/j.gfs.2017.01.009).
- Bruce, M., A. Hess, J. Bai, R. Mauleon, M. G. Diaz, N. Sugiyama, A. Bordeos, G.-L. Wang, H. Leung, and J. E. Leach. 2009. Detection of genomic deletions in rice using oligonucleotide microarrays. *BMC Genomics* 10 (1):129. doi: [10.1186/1471-2164-10-129](https://doi.org/10.1186/1471-2164-10-129).
- Calingacion, M. N., C. Boulaphanh, V. D. Daygon, R. Anacleto, R. Sackville Hamilton, B. Biais, C. Deborde, M. Maucourt, A. Moing, R. Mumm, et al. 2012. A genomics and multi-platform metabolomics approach to identify new traits of rice quality in traditional and improved varieties. *Metabolomics* 8 (5):771–83. doi: [10.1007/s11306-011-0374-4](https://doi.org/10.1007/s11306-011-0374-4).
- Castle, D., and N. Ries. 2009. *Nutrition and genomics: issues of ethics, law, regulation and communication*. USA: Academic Press.
- Carpenter, K. J. 2000. *Beriberi, white rice, and vitamin B: A disease, a cause, and a cure*. Berkeley: University of California Press.
- Chattopadhyay, K., L. Behera, T. B. Bagchi, S. S. Sardar, N. Moharana, N. R. Patra, M. Chakraborti, A. Das, B. C. Marndi, A. Sarkar, et al. 2019. Detection of stable QTLs for grain protein content in rice (*Oryza sativa* L.) employing high throughput phenotyping and genotyping platforms. *Scientific Reports* 9 (1):3196. doi: [10.1038/s41598-019-39863-2](https://doi.org/10.1038/s41598-019-39863-2).
- Chaudhary, J., G. B. Patil, H. Sonah, R. K. Deshmukh, T. D. Vuong, B. Valliyodan, and H. T. Nguyen. 2015. Expanding omics resources for improvement of soybean seed composition traits. *Frontiers in Plant Science* 6:1021. doi: [10.3389/fpls.2015.01021](https://doi.org/10.3389/fpls.2015.01021).
- Chen, L., Y. Huang, M. Xu, Z. Cheng, D. Zhang, and J. Zheng. 2016. iTRAQ-based quantitative proteomics analysis of black rice grain development reveals metabolic pathways associated with anthocyanin biosynthesis. *PLoS One* 11 (7):e0159238. doi: [10.1371/journal.pone.0159238](https://doi.org/10.1371/journal.pone.0159238).
- Courtois, B., N. Ahmadi, F. Khowaja, A. H. Price, J.-F. Rami, J. Frouin, C. Hamelin, and M. Ruiz. 2009. Rice root genetic architecture: Meta-analysis from a drought QTL database. *Rice* 2 (2–3):115. doi: [10.1007/s12284-009-9028-9](https://doi.org/10.1007/s12284-009-9028-9).
- De Valença, A., A. Bake, I. Brouwer, and K. Giller. 2017. Agronomic biofortification of crops to fight hidden hunger in Sub-Saharan Africa. *Global Food Security* 12:8–14. doi: [10.1016/j.gfs.2016.12.001](https://doi.org/10.1016/j.gfs.2016.12.001).
- Deshmukh, R., A. Singh, N. Jain, S. Anand, R. Gacche, A. Singh, K. Gaikwad, T. Sharma, T. Mohapatra, and N. Singh. 2010. Identification of candidate genes for grain number in rice (*Oryza sativa* L.). *Functional & Integrative Genomics* 10:339–47. doi: [10.1007/s10142-010-0167-2](https://doi.org/10.1007/s10142-010-0167-2).
- Deshmukh, R., H. Sonah, G. Patil, W. Chen, S. Prince, R. Mutava, T. Vuong, B. Valliyodan, and H. T. Nguyen. 2014. Integrating omic approaches for abiotic stress tolerance in soybean. *Frontiers in Plant Science* 5:244. doi: [10.3389/fpls.2014.00244](https://doi.org/10.3389/fpls.2014.00244).
- Dixit, S., U. M. Singh, R. Abbai, T. Ram, V. K. Singh, A. Paul, P. Virk, and A. Kumar. 2019. Identification of genomic region (s) responsible for high iron and zinc content in rice. *Scientific Reports* 9 (1): 8136. doi: [10.1038/s41598-019-43888-y](https://doi.org/10.1038/s41598-019-43888-y).
- Dong, C., F. He, O. Berkowitz, J. Liu, P. Cao, M. Tang, H. Shi, W. Wang, Q. Li, Z. Shen, et al. 2018. Alternative splicing plays a critical role in maintaining mineral nutrient homeostasis in rice (*Oryza sativa*). *The Plant Cell* 30 (10):2267–85. doi: [10.1105/tpc.18.00051](https://doi.org/10.1105/tpc.18.00051).
- Dowling, J. E., and G. Wald. 1958. Vitamin A deficiency and night blindness. *Proceedings of the National Academy of Sciences of Sciences* 44 (7):648. doi: [10.1073/pnas.44.7.648](https://doi.org/10.1073/pnas.44.7.648).
- Du, J., D. Zeng, B. Wang, Q. Qian, S. Zheng, and H.-Q. Ling. 2013. Environmental effects on mineral accumulation in rice grains and identification of ecological specific QTLs. *Environmental Geochemistry and Health* 35 (2):161–70. doi: [10.1007/s10653-012-9473-z](https://doi.org/10.1007/s10653-012-9473-z).
- Famoso, A. N., K. Zhao, R. T. Clark, C.-W. Tung, M. H. Wright, C. Bustamante, L. V. Kochian, and S. R. McCouch. 2011. Genetic architecture of aluminum tolerance in rice (*Oryza sativa*) determined through genome-wide association analysis and QTL mapping. *PLoS Genetics* 7 (8):e1002221. doi: [10.1371/journal.pgen.1002221](https://doi.org/10.1371/journal.pgen.1002221).
- FAO. 2019. Nutrient composition and protein quality of rice relative to other cereals.
- FAOSTAT. 2001. Nutritional contribution of rice and impact of biotechnology and biodiversity in rice-consuming countries.
- FAOSTAT. 2017. Crops Statistics Rice. Fekih, R., H. Takagi, M. Tamiru, A. Abe, S. Natsume, H. Yaegashi, S. Sharma, S. Sharma, H. Kanzaki, H. Matsumura, et al. 2013. MutMap+: Genetic mapping and mutant identification without crossing in rice. *PLoS One* 8 (7): e68529. doi: [10.1371/journal.pone.0068529](https://doi.org/10.1371/journal.pone.0068529).
- Fleck, A. T., J. Mattusch, and M. K. Schenk. 2013. Silicon decreases the arsenic level in rice grain by limiting arsenite transport. *Journal of Plant Nutrition and Soil Science* 176:785–94. doi: [10.1002/jpln.201200440](https://doi.org/10.1002/jpln.201200440).
- Galili, G., and R. Amir. 2013. Fortifying plants with the essential amino acids lysine and methionine to improve nutritional quality. *Plant Biotechnology Journal* 11 (2):211–22. doi: [10.1111/pbi.12025](https://doi.org/10.1111/pbi.12025).
- Gao, Z.-Y., S.-C. Zhao, W.-M. He, L.-B. Guo, Y.-L. Peng, J.-J. Wang, X.-S. Guo, X.-M. Zhang, Y.-C. Rao, C. Zhang, et al. 2013. Dissecting yield-associated loci in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. *Proceedings of the National Academy of Sciences* 110 (35):14492–7. doi: [10.1073/pnas.1306579110](https://doi.org/10.1073/pnas.1306579110).
- Gayen, D., S. Paul, S. N. Sarkar, S. K. Datta, and K. Datta. 2016. Comparative nutritional compositions and proteomics analysis of transgenic Xa21 rice seeds compared to conventional rice. *Food Chemistry* 203:301–7. doi: [10.1016/j.foodchem.2016.02.058](https://doi.org/10.1016/j.foodchem.2016.02.058).
- Gong, L., W. Chen, Y. Gao, X. Liu, H. Zhang, C. Xu, S. Yu, Q. Zhang, and J. Luo. 2013. Genetic analysis of the metabolome exemplified using a rice population. *Proceedings of the National Academy of Sciences* 110 (50):20320–5. doi: [10.1073/pnas.1319681110](https://doi.org/10.1073/pnas.1319681110).
- Goto, F., T. Yoshihara, N. Shigemoto, S. Toki, and F. Takaiwa. 1999. Iron fortification of rice seed by the soybean ferritin gene. *Nature Biotechnology* 17 (3):282. doi: [10.1038/7029](https://doi.org/10.1038/7029).
- Grenier, C., T.-V. Cao, Y. Ospina, C. Quintero, M. H. Châtel, J. Tohme, B. Courtois, and N. Ahmadi. 2015. Accuracy of genomic selection in a rice synthetic population developed for recurrent selection breeding. *PLoS One* 10 (8):e0136594. doi: [10.1371/journal.pone.0136594](https://doi.org/10.1371/journal.pone.0136594).
- Guiderdoni, E., P. Piffanelli, C. Sallaud, E. Bourgeois, and A. T. Johnson. 2005. *Insertional mutagenesis: Lessons from Arabidopsis, gaining experience in rice*. Italy: Avenue Media.
- Guo, L., Z. Gao, and Q. Qian. 2014. Application of resequencing to rice genomics, functional genomics and evolutionary analysis. *Rice* 7 (1):4. doi: [10.1186/s12284-014-0004-7](https://doi.org/10.1186/s12284-014-0004-7).

- Hefferon, K. L., and R. J. Herring. 2017. The end of the GMO? Genome editing, gene drives and new frontiers of plant technology. *Review of Agrarian Studies* 7 (1):1–32.
- Heslot, N., H.-P. Yang, M. E. Sorrells, and J.-L. Jannink. 2012. Genomic selection in plant breeding: A comparison of models. *Crop Science* 52 (1):146–60. doi: [10.2135/cropsci2011.09.0297](https://doi.org/10.2135/cropsci2011.09.0297).
- Heuberger, A. L., M. R. Lewis, M.-H. Chen, M. A. Brick, J. E. Leach, and E. P. Ryan. 2010. Metabolomic and functional genomic analyses reveal varietal differences in bioactive compounds of cooked rice. *PLoS One* 5 (9):e12915. doi: [10.1371/journal.pone.0012915](https://doi.org/10.1371/journal.pone.0012915).
- Hu, C., J. Shi, S. Quan, B. Cui, S. Kleessen, Z. Nikoloski, T. Tohge, D. Alexander, L. Guo, H. Lin, et al. 2015. Metabolic variation between japonica and indica rice cultivars as revealed by non-targeted metabolomics. *Scientific Reports* 4 (1):5067. doi: [10.1038/srep05067](https://doi.org/10.1038/srep05067).
- Hu, Z.-L., P. Li, M.-Q. Zhou, Z.-H. Zhang, L.-X. Wang, L.-H. Zhu, and Y.-G. Zhu. 2004. Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. *Euphytica* 135 (1):47–54. doi: [10.1023/B:EUPH.0000009539.38916.32](https://doi.org/10.1023/B:EUPH.0000009539.38916.32).
- Huang, B. E., K. L. Verbyla, A. P. Verbyla, C. Raghavan, V. K. Singh, P. Gaur, H. Leung, R. K. Varshney, and C. R. Cavanagh. 2015. MAGIC populations in crops: Current status and future prospects. *Theoretical and Applied Genetics* 128 (6):999–1017. doi: [10.1007/s00122-015-2506-0](https://doi.org/10.1007/s00122-015-2506-0).
- Huang, X.-Y., and D. E. Salt. 2016. Plant ionomics: From elemental profiling to environmental adaptation. *Molecular Plant* 9 (6):787–97. doi: [10.1016/j.molp.2016.05.003](https://doi.org/10.1016/j.molp.2016.05.003).
- Huang, X., Q. Feng, Q. Qian, Q. Zhao, L. Wang, A. Wang, J. Guan, D. Fan, Q. Weng, T. Huang, et al. 2009. High-throughput genotyping by whole-genome resequencing. *Genome Research* 19 (6):1068–76. doi: [10.1101/gr.089516.108](https://doi.org/10.1101/gr.089516.108).
- Huang, X., T. Lu, and B. Han. 2013. Resequencing rice genomes: An emerging new era of rice genomics. *Trends in Genetics* 29 (4):225–32. doi: [10.1016/j.tig.2012.12.001](https://doi.org/10.1016/j.tig.2012.12.001).
- ICAR. 2017. High zinc rice variety – DRR Dhan 45 (IET 23832).
- Imam, M. U., and M. Ismail. 2015. An overview on germinated brown rice and its nutrigenomic implications. In *Genomics, proteomics and metabolomics in nutraceuticals and functional foods*. 2nd ed., eds. D. Bagchi, A. Swaroop, and M. Bagchi, 504–17. Hoboken: Wiley.
- Imam, M. U., S. N. A. Musa, N. H. Azmi, and M. Ismail. 2012. Effects of white rice, brown rice and germinated brown rice on antioxidant status of type 2 diabetic rats. *International Journal of Molecular Sciences* 13 (12):12952–69. doi: [10.3390/ijms131012952](https://doi.org/10.3390/ijms131012952).
- IRGS Project. 2005. The map-based sequence of the rice genome. *Nature* 436:793.
- Ishimaru, Y., H. Masuda, K. Bashir, H. Inoue, T. Tsukamoto, M. Takahashi, H. Nakanishi, N. Aoki, T. Hirose, R. Ohsugi, and N. K. Nishizawa. 2010. Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. *The Plant Journal* 62 (3):379–90. doi: [10.1111/j.1365-3113.2010.04158.x](https://doi.org/10.1111/j.1365-3113.2010.04158.x).
- Islam, M., J. Ontoy, and P. K. Subudhi. 2019. Meta-analysis of quantitative trait loci associated with seedling-stage salt tolerance in rice (*Oryza sativa* L.). *Plants* 8 (2):33. doi: [10.3390/plants8020033](https://doi.org/10.3390/plants8020033).
- Jeng, T. L., Y. W. Lin, C. S. Wang, and J. M. Sung. 2012. Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the indica type cultivar IR64. *Journal of Food Composition and Analysis* 28 (2):149–54. doi: [10.1016/j.jfca.2012.08.008](https://doi.org/10.1016/j.jfca.2012.08.008).
- Jiang, C., Z. Cheng, C. Zhang, T. Yu, Q. Zhong, J. Q. Shen, and X. Huang. 2014. Proteomic analysis of seed storage proteins in wild rice species of the *Oryza* genus. *Proteome Science* 12 (1):51. doi: [10.1186/s12953-014-0051-4](https://doi.org/10.1186/s12953-014-0051-4).
- Jiang, S., S. Sun, L. Bai, G. Ding, T. Wang, T. Xia, H. Jiang, X. Zhang, and F. Zhang. 2017. Resequencing and variation identification of whole genome of the japonica rice variety “Longdao24” with high yield. *PLoS One* 12 (7):e0181037. doi: [10.1371/journal.pone.0181037](https://doi.org/10.1371/journal.pone.0181037).
- Jiao, Y., S. Lori Tausta, N. Gandotra, N. Sun, T. Liu, N. K. Clay, T. Ceserani, M. Chen, L. Ma, M. Holford, et al. 2009. A transcriptome atlas of rice cell types uncovers cellular, functional and developmental hierarchies. *Nature Genetics* 41 (2):258. doi: [10.1038/ng.282](https://doi.org/10.1038/ng.282).
- Kamilaris, A., A. Kartakoullis, and F. X. Prenafeta-Boldú. 2017. A review on the practice of big data analysis in agriculture. *Computers and Electronics in Agriculture* 143:23–37. doi: [10.1016/j.compag.2017.09.037](https://doi.org/10.1016/j.compag.2017.09.037).
- Kaneko, K., M. Sasaki, N. Kuribayashi, H. Suzuki, Y. Sasuga, T. Shiraya, T. Inomata, K. Itoh, M. Baslam, and T. Mitsui. 2016. Proteomic and glycomic characterization of rice chalky grains produced under moderate and high-temperature conditions in field system. *Rice* 9 (1):26. doi: [10.1186/s12284-016-0100-y](https://doi.org/10.1186/s12284-016-0100-y).
- Kawakatsu, T., and F. Takaiwa. 2010. Differences in transcriptional regulatory mechanisms functioning for free lysine content and seed storage protein accumulation in rice grain. *Plant and Cell Physiology* 51 (12):1964–74. doi: [10.1093/pcp/pcq164](https://doi.org/10.1093/pcp/pcq164).
- Kim, C.-K., Y.-J. Seol, Y. Shin, H.-M. Lim, G.-S. Lee, A.-R. Kim, T.-H. Lee, J.-H. Lee, D.-S. Park, S. Yoo, et al. 2015. Whole-genome resequencing and transcriptomic analysis to identify genes involved in leaf-color diversity in ornamental rice plants. *PLoS One* 10 (4):e0124071. doi: [10.1371/journal.pone.0124071](https://doi.org/10.1371/journal.pone.0124071).
- Kim, J. K., S.-Y. Park, S.-H. Lim, Y. Yeo, H. S. Cho, and S.-H. Ha. 2013. Comparative metabolic profiling of pigmented rice (*Oryza sativa* L.) cultivars reveals primary metabolites are correlated with secondary metabolites. *Journal of Cereal Science* 57 (1):14–20. doi: [10.1016/j.jcs.2012.09.012](https://doi.org/10.1016/j.jcs.2012.09.012).
- Kim, S. I., C. Andaya, S. Goyal, and T. Tai. 2008a. The rice OsLpa1 gene encodes a novel protein involved in phytic acid metabolism. *Theoretical and Applied Genetics* 117 (5):769–79. doi: [10.1007/s00122-008-0818-z](https://doi.org/10.1007/s00122-008-0818-z).
- Kim, S. I., C. Andaya, J. Newman, S. Goyal, and T. Tai. 2008b. Isolation and characterization of a low phytic acid rice mutant reveals a mutation in the rice orthologue of maize MIK. *Theoretical and Applied Genetics* 117 (8):1291. doi: [10.1007/s00122-008-0863-7](https://doi.org/10.1007/s00122-008-0863-7).
- Koide, Y., A. Ogino, T. Yoshikawa, Y. Kitashima, N. Saito, Y. Kanaoka, K. Onishi, Y. Yoshitake, T. Tsukiyama, H. Saito, et al. 2018. Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice. *Proceedings of the National Academy of Sciences* 115 (9):E1955–E1962. doi: [10.1073/pnas.1711656115](https://doi.org/10.1073/pnas.1711656115).
- Kok, A. D.-X., L. L. Yoon, R. Sekeli, W. C. Yeong, Z. N. B. Yusof, and L. K. Song. 2018. Iron biofortification of rice: Progress and prospects. In *Rice crop: Current developments*, eds. F. Shah, Z. Khan, and A. Iqbal, 25. London: InTech.
- Koller, A., M. P. Washburn, B. M. Lange, N. L. Andon, C. Deciu, P. A. Haynes, L. Hays, D. Schieltz, R. Ulaszek, J. Wei, et al. 2002. Proteomic survey of metabolic pathways in rice. *Proceedings of the National Academy of Sciences* 99 (18):11969–74. doi: [10.1073/pnas.172183199](https://doi.org/10.1073/pnas.172183199).
- Korte, A., and A. Farlow. 2013. The advantages and limitations of trait analysis with GWAS: A review. *Plant Methods* 9 (1):29. doi: [10.1186/1746-4811-9-29](https://doi.org/10.1186/1746-4811-9-29).
- Krishnan, A., E. Guiderdoni, G. An, Y. C. Hsing, C. Han, M. C. Lee, S.-M. Yu, N. Upadhyaya, S. Ramachandran, Q. Zhang, et al. 2009. Mutant resources in rice for functional genomics of the grasses. *Plant Physiology* 149 (1):165–70. doi: [10.1104/pp.108.128918](https://doi.org/10.1104/pp.108.128918).
- Kusano, M., A. Fukushima, M. Kobayashi, N. Hayashi, P. Jonsson, T. Moritz, K. Ebana, and K. Saito. 2007. Application of a metabolomic method combining one-dimensional and two-dimensional gas chromatography-time-of-flight/mass spectrometry to metabolic phenotyping of natural variants in rice. *Journal of Chromatography B* 855 (1):71–9. doi: [10.1016/j.jchromb.2007.05.002](https://doi.org/10.1016/j.jchromb.2007.05.002).
- Kuwano, M., F. Takaiwa, and K. T. Yoshida. 2009. Differential effects of a transgene to confer low phytic acid in caryopses located at different positions in rice panicles. *Plant and Cell Physiology* 50 (7):1387–92. doi: [10.1093/pcp/pcp071](https://doi.org/10.1093/pcp/pcp071).
- Lee, H., S. Loh, C. Bong, S. Sarbini, and P. Yiu. 2015. Impact of phytic acid on nutrient bioaccessibility and antioxidant properties of dehusked rice. *Journal of Food Science and Technology* 52 (12):7806–16. doi: [10.1007/s13197-015-1918-9](https://doi.org/10.1007/s13197-015-1918-9).
- Lee, J., and H.-J. Koh. 2011. A label-free quantitative shotgun proteomics analysis of rice grain development. *Proteome Science* 9 (1):61. doi: [10.1186/1477-5956-9-61](https://doi.org/10.1186/1477-5956-9-61).

- Lee, S., U. S. Jeon, S. J. Lee, Y.-K. Kim, D. P. Persson, S. Husted, J. K. Schjorring, Y. Kakei, H. Masuda, N. K. Nishizawa, and G. An. 2009. Iron fortification of rice seeds through activation of the nicotianamine synthase gene. *Proceedings of the National Academy of Sciences* 106 (51):22014–9. doi: [10.1073/pnas.0910950106](https://doi.org/10.1073/pnas.0910950106).
- Li, G., L. Nunes, Y. Wang, P. N. Williams, M. Zheng, Q. Zhang, and Y. Zhu. 2013. Profiling the ionome of rice and its use in discriminating geographical origins at the regional scale, China. *Journal of Environmental Sciences* 25 (1):144–54. doi: [10.1016/S1001-0742\(12\)60007-2](https://doi.org/10.1016/S1001-0742(12)60007-2).
- Li, J.-Y., J. Wang, and R. S. Zeigler. 2014. The 3,000 rice genomes project: New opportunities and challenges for future rice research. *GigaScience* 3 (1):8. doi: [10.1186/2047-217X-3-8](https://doi.org/10.1186/2047-217X-3-8).
- Li, X., L. Wu, J. Wang, J. Sun, X. Xia, X. Geng, X. Wang, Z. Xu, and Q. Xu. 2018. Genome sequencing of rice subspecies and genetic analysis of recombinant lines reveals regional yield-and quality-associated loci. *BMC Biology* 16 (1):102. doi: [10.1186/s12915-018-0572-x](https://doi.org/10.1186/s12915-018-0572-x).
- Liu, Q.-L., X.-H. Xu, X.-L. Ren, H.-W. Fu, D.-X. Wu, and Q.-Y. Shu. 2007. Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 114 (5): 803–14. doi: [10.1007/s00122-006-0478-9](https://doi.org/10.1007/s00122-006-0478-9).
- Lokers, R., R. Knapen, S. Janssen, Y. van Randen, and J. Jansen. 2016. Analysis of big data technologies for use in agro-environmental science. *Environmental Modelling & Software* 84:494–504. doi: [10.1016/j.envsoft.2016.07.017](https://doi.org/10.1016/j.envsoft.2016.07.017).
- Lowe, R., N. Shirley, M. Bleackley, S. Dolan, and T. Shafee. 2017. Transcriptomics technologies. *PLoS Computational Biology* 13 (5): e1005457. doi: [10.1371/journal.pcbi.1005457](https://doi.org/10.1371/journal.pcbi.1005457).
- Lu, K., L. Li, X. Zheng, Z. Zhang, T. Mou, and Z. Hu. 2008. Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. *Journal of Genetics* 87 (3):305–10. doi: [10.1007/s12041-008-0049-8](https://doi.org/10.1007/s12041-008-0049-8).
- Lu, T., G. Lu, D. Fan, C. Zhu, W. Li, Q. Zhao, Q. Feng, Y. Zhao, Y. Guo, W. Li, et al. 2010. Function annotation of the rice transcriptome at single-nucleotide resolution by RNA-seq. *Genome Research* 20 (9):1238–49. doi: [10.1101/gr.106120.110](https://doi.org/10.1101/gr.106120.110).
- Lucca, P., R. Hurrell, and I. Potrykus. 2002. Fighting iron deficiency anemia with iron-rich rice. *Journal of the American College of Nutrition* 21 (sup3):184S–90S. doi: [10.1080/07315724.2002.10719264](https://doi.org/10.1080/07315724.2002.10719264).
- Luz, V. K. d., S. F. d. S. Silveira, G. M. d. Fonseca, E. L. Groli, R. G. Figueiredo, D. Baretta, M. M. Kopp, A. M. d. Magalhães Junior, L. C. d. Maia, and A. C. d. Oliveira. 2016. Identification of variability for agronomically important traits in rice mutant families. *Bragantia* 75 (1):41–50. doi: [10.1590/1678-4499.283](https://doi.org/10.1590/1678-4499.283).
- Ma, J. F., K. Tamai, N. Yamaji, N. Mitani, S. Konishi, M. Katsuhara, M. Ishiguro, Y. Murata, and M. Yano. 2006. A silicon transporter in rice. *Nature* 440 (7084):688. doi: [10.1038/nature04590](https://doi.org/10.1038/nature04590).
- Mahender, A., A. Anandan, S. K. Pradhan, and E. Pandit. 2016. Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *Springerplus* 5 (1):2086. doi: [10.1186/s40064-016-3744-6](https://doi.org/10.1186/s40064-016-3744-6).
- Maksup, S., S. Pongpakpian, S. Roytrakul, S. Cha-Um, and K. Supaibulwatana. 2018. Comparative proteomics and protein profile related to phenolic compounds and antioxidant activity in germinated *Oryza sativa* 'KDML105' and Thai brown rice 'Mali Daeng' for better nutritional value. *Journal of the Science of Food and Agriculture* 98 (2):566–73. doi: [10.1002/jsfa.8498](https://doi.org/10.1002/jsfa.8498).
- Marulanda, J. J., X. Mi, A. E. Melchinger, J.-L. Xu, T. Würschum, and C. F. H. Longin. 2016. Optimum breeding strategies using genomic selection for hybrid breeding in wheat, maize, rye, barley, rice and triticale. *Theoretical and Applied Genetics* 129 (10):1901–13. doi: [10.1007/s00122-016-2748-5](https://doi.org/10.1007/s00122-016-2748-5).
- Matsuda, F., Y. Okazaki, A. Oikawa, M. Kusano, R. Nakabayashi, J. Kikuchi, J. I. Yonemaru, K. Ebana, M. Yano, and K. Saito. 2012. Dissection of genotype–phenotype associations in rice grains using metabolome quantitative trait loci analysis. *The Plant Journal* 70 (4): 624–36. doi: [10.1111/j.1365-3113.2012.04903.x](https://doi.org/10.1111/j.1365-3113.2012.04903.x).
- Maywa Montenegro. 2016. OPINION: CRISPR is coming to agriculture—With big implications for food, farmers, consumers and nature.
- Mba, C., R. Afza, S. Bado, and S. M. Jain. 2010. Induced mutagenesis in plants using physical and chemical agents. *Plant Cell Culture: Essential Methods* 20:111–30.
- McNally, K. L., R. Bruskiewich, D. Mackill, C. R. Buell, J. E. Leach, and H. Leung. 2006. Sequencing multiple and diverse rice varieties. Connecting whole-genome variation with phenotypes. *Plant Physiology* 141 (1):26–31. doi: [10.1104/pp.106.077313](https://doi.org/10.1104/pp.106.077313).
- Meuwissen, T., B. Hayes, and M. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157 (4):1819–29.
- Mohanty, S. 2013. Trends in global rice consumption. *Rice Today* 12: 44–5.
- Mohapatra, T., S. Robin, N. Sarla, M. Sheshasayee, A. K. Singh, K. Singh, N. K. Singh, S. V. Amitha Mithra, and R. P. Sharma. 2014. EMS induced mutants of upland rice variety Nagina22: Generation and characterization. *Proceedings of the Indian National Science Academy* 80 (1):163–72. doi: [10.16943/ptinsa/2014/v80i1/55094](https://doi.org/10.16943/ptinsa/2014/v80i1/55094).
- Mondal, T. K., H. C. Rawal, K. Gaikwad, T. R. Sharma, and N. K. Singh. 2017. First de novo draft genome sequence of *oryza coarctata*, the only halophytic species in the genus *oryza*. *F1000Research* 4: XXX. doi: [10.12688/f1000research.12414.2](https://doi.org/10.12688/f1000research.12414.2).
- Mubeen, H. N., R. Z. Naqvi, A. Masood, M. Waseem Shoaib, and S. Raza. 2016. Gene transformation: Methods, uses and applications. *Journal of Pharmaceutical and Biological Sciences* 6:1750.
- Nagamine, A., H. Matsusaka, T. Ushijima, Y. Kawagoe, M. Ogawa, T. W. Okita, and T. Kumamaru. 2011. A role for the cysteine-rich 10 kDa prolamin in protein body I formation in rice. *Plant and Cell Physiology* 52 (6):1003–16. doi: [10.1093/pcp/pcr053](https://doi.org/10.1093/pcp/pcr053).
- Norton, G. J., C. M. Deacon, L. Xiong, S. Huang, A. A. Meharg, and A. H. Price. 2010. Genetic mapping of the rice ionome in leaves and grain: Identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant and Soil* 329 (1–2):139–53. doi: [10.1007/s11104-009-0141-8](https://doi.org/10.1007/s11104-009-0141-8).
- Norton, G. J., A. Douglas, B. Lahner, E. Yakubova, M. L. Guerinet, S. R. M. Pinson, L. Tarpley, G. C. Eizenga, S. P. McGrath, F.-J. Zhao, et al. 2014. Genome wide association mapping of grain arsenic, copper, molybdenum and zinc in rice (*Oryza sativa* L.) grown at four international field sites. *PLoS One* 9 (2):e89685. doi: [10.1371/journal.pone.0089685](https://doi.org/10.1371/journal.pone.0089685).
- Oh, J.-H., Y.-J. Lee, E.-J. Byeon, B.-C. Kang, D.-S. Kyeoung, and C.-K. Kim. 2018. Whole-genome resequencing and transcriptomic analysis of genes regulating anthocyanin biosynthesis in black rice plants. *3 Biotech* 8 (2):115. doi: [10.1007/s13205-018-1140-3](https://doi.org/10.1007/s13205-018-1140-3).
- Onogi, A., M. Watanabe, T. Mochizuki, T. Hayashi, H. Nakagawa, T. Hasegawa, and H. Iwata. 2016. Toward integration of genomic selection with crop modelling: The development of an integrated approach to predicting rice heading dates. *Theoretical and Applied Genetics* 129 (4):805–17. doi: [10.1007/s00122-016-2667-5](https://doi.org/10.1007/s00122-016-2667-5).
- Paine, J. A., C. A. Shipton, S. Chaggar, R. M. Howells, M. J. Kennedy, G. Vernon, S. Y. Wright, E. Hinchliffe, J. L. Adams, A. L. Silverstone, and R. Drake. 2005. Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nature Biotechnology* 23 (4):482. doi: [10.1038/nbt1082](https://doi.org/10.1038/nbt1082).
- Pandit, A. A., R. A. Shah, and A. M. Husaini. 2018. Transcriptomics: A time-efficient tool with wide applications in crop and animal biotechnology. *Journal of Pharmacognosy and Phytochemistry* 7:1701–4.
- Pathak, R. K., M. Baunthiyal, D. Pandey, and A. Kumar. 2018. Augmentation of crop productivity through interventions of omics technologies in India: Challenges and opportunities. *3 Biotech* 8 (11):454. doi: [10.1007/s13205-018-1473-y](https://doi.org/10.1007/s13205-018-1473-y).
- Peng, B., H. Kong, Y. Li, L. Wang, M. Zhong, L. Sun, G. Gao, Q. Zhang, L. Luo, G. Wang, et al. 2014. OsAAP6 functions as an important regulator of grain protein content and nutritional quality in rice. *Nature Communications* 5 (1):4847. doi: [10.1038/ncomms5847](https://doi.org/10.1038/ncomms5847).
- Pinson, S. R., L. Tarpley, W. Yan, K. Yeater, B. Lahner, E. Yakubova, X.-Y. Huang, M. Zhang, M. L. Guerinet, and D. E. Salt. 2015. Worldwide genetic diversity for mineral element concentrations in rice grain. *Crop Science* 55 (1):294–311. doi: [10.2135/cropsci2013.10.0656](https://doi.org/10.2135/cropsci2013.10.0656).

- Ponce, K. S., G. Ye, and X. Zhao. 2018. QTL identification for cooking and eating quality in indica rice using multi-parent advanced generation intercross (MAGIC) population. *Frontiers in Plant Science* 9: 868. doi: [10.3389/fpls.2018.00868](https://doi.org/10.3389/fpls.2018.00868).
- Prolla, I. R., M. Rafii, G. Courtney-Martin, R. Elango, L. P. da Silva, R. O. Ball, and P. B. Pencharz. 2013. Lysine from cooked white rice consumed by healthy young men is highly metabolically available when assessed using the indicator amino acid oxidation technique. *The Journal of Nutrition* 143 (3):302–6. doi: [10.3945/jn.112.166728](https://doi.org/10.3945/jn.112.166728).
- Qu, L., X. Wei, H. Satoh, T. Kumamaru, M. Ogawa, and F. Takaiwa. 2003. Biochemical and molecular characterization of a rice glutelin allele for the GluA-1 gene. *Theoretical and Applied Genetics* 107 (1): 20–5. doi: [10.1007/s00122-003-1228-x](https://doi.org/10.1007/s00122-003-1228-x).
- Ramli, N. S., and N. H. M. Zin. 2015. Proteomic analysis of rice seed storage proteins in relation to nutrient quality of three different commercial rice types. *Jurnal Teknologi* 77 (24):7–11. doi: [10.11113/jt.v77.6689](https://doi.org/10.11113/jt.v77.6689).
- Ren, Z., D. Qi, N. Pugh, K. Li, B. Wen, R. Zhou, S. Xu, S. Liu, and A. R. Jones. 2019. Improvements to the rice genome annotation through Large-Scale analysis of RNA-Seq and proteomics data sets. *Molecular & Cellular Proteomics* 18:86–98. doi: [10.1074/mcp.RA118.000832](https://doi.org/10.1074/mcp.RA118.000832).
- Ricaurte, F. R., T. Kewan, and H. Daw. 2019. Scurvy: A rare cause of anemia. *Cureus* 11 (9):e5694.
- Ricestat, I.R.R.I. 2019. World rice statistics.
- Sahay, M., and R. Sahay. 2012. Rickets-vitamin D deficiency and dependency. *Indian Journal of Endocrinology and Metabolism* 16 (2): 164. doi: [10.4103/2230-8210.93732](https://doi.org/10.4103/2230-8210.93732).
- Saika, H., A. Oikawa, F. Matsuda, H. Onodera, K. Saito, and S. Toki. 2011. Application of gene targeting to designed mutation breeding of high-tryptophan rice. *Plant Physiology* 156 (3):1269–77. doi: [10.1104/pp.111.175778](https://doi.org/10.1104/pp.111.175778).
- Sarkar, A., A. A. Singh, S. B. Agrawal, A. Ahmad, and S. P. Rai. 2015. Cultivar specific variations in antioxidative defense system, genome and proteome of two tropical rice cultivars against ambient and elevated ozone. *Ecotoxicology and Environmental Safety* 115:101–11. doi: [10.1016/j.ecoenv.2015.02.010](https://doi.org/10.1016/j.ecoenv.2015.02.010).
- Satishruti, K., N. Senthil, S. Vellaikumar, R. V. Ranjani, and M. Raveendran. 2013. Plant ionomics: A platform for identifying novel gene regulating plant mineral nutrition. *American Journal of Plant Sciences* 04 (07):1309. doi: [10.4236/ajps.2013.47162](https://doi.org/10.4236/ajps.2013.47162).
- Sayad, Y. O., H. Mousannif, and M. Le Page. 2015. Crop Management Using BIG DATA, 2015 International Conference on Cloud Technologies and Applications (CloudTech). IEEE, pp. 1–6.
- Selamassakul, O., N. Laohakunjit, O. Kerdchoechuen, L. Yang, and C. S. Maier. 2018. Isolation and characterisation of antioxidative peptides from bromelain-hydrolysed brown rice protein by proteomic technique. *Process Biochemistry* 70:179–87. doi: [10.1016/j.procbio.2018.03.024](https://doi.org/10.1016/j.procbio.2018.03.024).
- Semsang, N., R. Kawaree, R. Cutler, R. Chundet, L. Yu, and S. Anuntalabhochai. 2012. Improved antioxidant activity of BKOS Thai jasmine rice. *Natural Product Research* 26 (12):1145–51. doi: [10.1080/14786419.2011.561207](https://doi.org/10.1080/14786419.2011.561207).
- Senthilvadivu, S., S. V. Kiran, S. P. Devi, and S. Manivannan. 2016. Big data analysis on geographical segmentations and resource constrained scheduling of production of agricultural commodities for better yield. *Procedia Computer Science* 87:80–5. doi: [10.1016/j.procs.2016.05.130](https://doi.org/10.1016/j.procs.2016.05.130).
- Serrat, X., R. Esteban, N. Guibourt, L. Moysset, S. Nogués, and E. Lalanne. 2014. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. *Plant Methods* 10 (1):5. doi: [10.1186/1746-4811-10-5](https://doi.org/10.1186/1746-4811-10-5).
- Sharma, T. R., B. N. Devanna, K. Kiran, P. K. Singh, K. Arora, P. Jain, I. M. Tiwari, H. Dubey, B. Saklani, M. Kumari, et al. 2018. Status and prospects of next generation sequencing technologies in crop plants. *Current Issues in Molecular Biology* 27:1–36.
- Shin, S. Y., M. A. Ahmad, M. R. A. Rashid, N. T. A. Bakar, C. Machap, R. A. Z. Abidin, A. L. C. Kuang, R. Kamaruzaman, M. N. M. Yusof, and S. Simoh. 2016. Antioxidant activities, macro- and micro-element composition of selected Malaysian local rice varieties. *Transactions of the Genetics Society of Malaysia* 3:205–212.
- Singh, A. K., A. K. Tyagi, R. Srivastava, S. K. Parida, and A. V. Daware. 2017a. Regional association analysis of metaQTLs delineates candidate grain size genes in rice. *Frontiers in Plant Science* 8:807.
- Singh, R., A. K. Singh, T. R. Sharma, A. Singh, and N. K. Singh. 2012. Fine mapping of grain length QTLs on chromosomes 1 and 7 in basmati rice (*Oryza sativa* L.). *Journal of Plant Biochemistry and Biotechnology* 21 (2):157–66. doi: [10.1007/s13562-011-0080-3](https://doi.org/10.1007/s13562-011-0080-3).
- Singh, S. P., W. Gruissem, and N. K. Bhullar. 2017b. Single genetic locus improvement of iron, zinc and β -carotene content in rice grains. *Scientific Reports* 7 (1):6883. doi: [10.1038/s41598-017-07198-5](https://doi.org/10.1038/s41598-017-07198-5).
- Soman, R., N. K. Gande, R. Ambati, P. J. Kundur, A. R., B. D. Bekele, and S. H. E. 2013. Genetic variability and correlation studies for grain iron concentration and yield related traits in recombinant inbred lines of rice (*Oryza sativa* L.) grown under aerobic condition. *International Journal of Current Research* 6 (3):5869–74.
- Sonah, H., R. K. Deshmukh, V. P. Singh, D. K. Gupta, N. K. Singh, and T. R. Sharma. 2011. Genomic resources in horticultural crops: Status, utility and challenges. *Biotechnology Advances* 29 (2): 199–209. doi: [10.1016/j.biotechadv.2010.11.002](https://doi.org/10.1016/j.biotechadv.2010.11.002).
- Sonah, H., L. O'Donoghue, E. Cober, I. Rajcan, and F. Belzile. 2015. Identification of loci governing eight agronomic traits using a GBS-GWAS approach and validation by QTL mapping in soya bean. *Plant Biotechnology Journal* 13 (2):211–21. doi: [10.1111/pbi.12249](https://doi.org/10.1111/pbi.12249).
- Song, E.-H., H.-J. Kim, J. Jeong, H.-J. Chung, H.-Y. Kim, E. Bang, and Y.-S. Hong. 2016. A ¹H HR-MAS NMR-based metabolomic study for metabolic characterization of rice grain from various *Oryza sativa* L. cultivars. *Journal of Agricultural and Food Chemistry* 64 (15): 3009–16. doi: [10.1021/acs.jafc.5b05667](https://doi.org/10.1021/acs.jafc.5b05667).
- Song, S., D. Tian, Z. Zhang, S. Hu, and J. Yu. 2019. Rice genomics: Over the past two decades and into the future. *Genomics. Proteomics & Bioinformatics* doi: [10.1016/j.gpb.2019.01.001](https://doi.org/10.1016/j.gpb.2019.01.001).
- Sperotto, R. A., T. Boff, G. L. Duarte, L. S. Santos, M. A. Grusak, and J. P. Fett. 2010. Identification of putative target genes to manipulate Fe and Zn concentrations in rice grains. *Journal of Plant Physiology* 167 (17):1500–6. doi: [10.1016/j.jplph.2010.05.003](https://doi.org/10.1016/j.jplph.2010.05.003).
- Spindel, J., H. Begum, D. Akdemir, P. Virk, B. Collard, E. Redona, G. Atlin, J.-L. Jannink, and S. R. McCouch. 2015. Genomic selection and association mapping in rice (*Oryza sativa*): Effect of trait genetic architecture, training population composition, marker number and statistical model on accuracy of rice genomic selection in elite, tropical rice breeding lines. *PLoS Genetics* 11 (2):e1004982. doi: [10.1371/journal.pgen.1004982](https://doi.org/10.1371/journal.pgen.1004982).
- Stangoulis, J. C., B.-L. Huynh, R. M. Welch, E.-Y. Choi, and R. D. Graham. 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* 154 (3):289–94. doi: [10.1007/s10681-006-9211-7](https://doi.org/10.1007/s10681-006-9211-7).
- Stein, J. C., Y. Yu, D. Copetti, D. J. Zwickl, L. Zhang, C. Zhang, K. Chougule, D. Gao, A. Iwata, J. L. Goicoechea, et al. 2018. Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus *oryza*. *Nature Genetics* 50 (2):285. doi: [10.1038/s41588-018-0040-0](https://doi.org/10.1038/s41588-018-0040-0).
- Swamy, B. M., G. I. L. Descalsota, C. T. Nha, A. Amparado, M. A. Inabangan-Asilo, C. Manito, F. Tesoro, and R. Reinke. 2018. Identification of genomic regions associated with agronomic and biofortification traits in DH populations of rice. *PLoS One* 13 (8): e0201756. doi: [10.1371/journal.pone.0201756](https://doi.org/10.1371/journal.pone.0201756).
- Takagi, H., A. Uemura, H. Yaegashi, M. Tamiru, A. Abe, C. Mitsuoka, H. Utsushi, S. Natsume, H. Kanzaki, H. Matsumura, et al. 2013. MutMap-gap: Whole-genome resequencing of mutant F2 progeny bulk combined with de novo assembly of gap regions identifies the rice blast resistance gene Pii. *New Phytologist* 200 (1):276–83. doi: [10.1111/nph.12369](https://doi.org/10.1111/nph.12369).
- Takehisa, H., and Y. Sato. 2018. Transcriptome monitoring visualizes growth stage-dependent nutrient status dynamics in rice under field conditions. *The Plant Journal* doi: [10.1111/tpj.14176](https://doi.org/10.1111/tpj.14176).
- Takehisa, H., Y. Sato, B. A. Antonio, and Y. Nagamura. 2013. Global transcriptome profile of rice root in response to essential

- macronutrient deficiency. *Plant Signaling & Behavior* 8:e24409. doi: [10.4161/psb.24409](https://doi.org/10.4161/psb.24409).
- Talebi, A. B., A. B. Talebi, and M. Jafarpour. 2012a. Identify the lethal dose of EMS and gamma radiation mutagenesis in rice MR219. *International Proceedings of Chemical, Biological & Environmental Engineering* 48.
- Talebi, A. B., A. B. Talebi, and B. Shahrokhifar. 2012b. Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Sciences* 03 (12):1661. doi: [10.4236/ajps.2012.312202](https://doi.org/10.4236/ajps.2012.312202).
- Tan, Y., M. Sun, Y. Xing, J. Hua, X. Sun, Q. Zhang, and H. Corke. 2001. Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. *Theoretical and Applied Genetics* 103 (6–7):1037–45. doi: [10.1007/s001220100665](https://doi.org/10.1007/s001220100665).
- Terao, T., and T. Hirose. 2015. Control of grain protein contents through SEMIDWARF1 mutant alleles: Sd1 increases the grain protein content in dee-geo-woo-gen but not in reimei. *Molecular Genetics and Genomics* 290 (3):939–54. doi: [10.1007/s00438-014-0965-7](https://doi.org/10.1007/s00438-014-0965-7).
- Tian, L., H.-L. Chou, L. Zhang, S.-K. Hwang, S. R. Starkenburg, K. A. Doroshenko, T. Kumamaru, and T. W. Okita. 2018. RNA-binding protein RBP-P is required for glutelin and prolamine mRNA localization in rice endosperm cells. *The Plant Cell* 30 (10):2529–52. doi: [10.1105/tpc.18.00321](https://doi.org/10.1105/tpc.18.00321).
- Till, B. J., J. Cooper, T. H. Tai, P. Colowit, E. A. Greene, S. Henikoff, and L. Comai. 2007. Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biology* 7 (1):19. doi: [10.1186/1471-2229-7-19](https://doi.org/10.1186/1471-2229-7-19).
- Tran, L.-S P., and R. Kumar. 2016. Plant quality improvement and nutrigenomics. *Current Genomics* 17:153. doi: [10.2174/138920291703160329114532](https://doi.org/10.2174/138920291703160329114532).
- Tran, P. T., and C. Q. Ho. 2017. Breeding new aromatic rice with high iron using gamma radiation and hybridization. In *Biotechnologies for plant mutation breeding*, eds. J. Jankowicz-Cieslak, T. Tai, J. Kümlehn, and B. Till, 173–91. Cham: Springer.
- U.S. Department of Agriculture. 2019a. Basmati rice.
- U.S. Department of Agriculture. 2019b. Jasmine rice.
- Uraguchi, S., T. Kamiya, T. Sakamoto, K. Kasai, Y. Sato, Y. Nagamura, A. Yoshida, J. Kyoizuka, S. Ishikawa, and T. Fujiwara. 2011. Low-affinity cation transporter (OsLCT1) regulates cadmium transport into rice grains. *Proceedings of the National Academy of Sciences* 108 (52):20959–64. doi: [10.1073/pnas.1116531109](https://doi.org/10.1073/pnas.1116531109).
- Verma, D. K., and K. Shukla. 2011. Nutritional value of rice and their importance. *Indian Farmers Digest* 44 (1):21–35.
- Wakasa, K., H. Hasegawa, H. Nemoto, F. Matsuda, H. Miyazawa, Y. Tozawa, K. Morino, A. Komatsu, T. Yamada, and T. Terakawa. 2006. High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. *Journal of Experimental Botany* 57 (12):3069–78. doi: [10.1093/jxb/erl068](https://doi.org/10.1093/jxb/erl068).
- Wang, Y., W. Xu, W. Zhao, J. Hao, Y. Luo, X. Tang, Y. Zhang, and K. Huang. 2012. Comparative analysis of the proteomic and nutritional composition of transgenic rice seeds with Cry1ab/ac genes and their non-transgenic counterparts. *Journal of Cereal Science* 55 (2):226–33. doi: [10.1016/j.jcs.2011.12.004](https://doi.org/10.1016/j.jcs.2011.12.004).
- Wu, J.-L., C. Wu, C. Lei, M. Baraoidan, A. Bordeos, M. R. S. Madamba, M. Ramos-Pamplona, R. Mauleon, A. Portugal, V. J. Ulat, et al. 2005. Chemical-and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. *Plant Molecular Biology* 59 (1):85–97. doi: [10.1007/s11103-004-5112-0](https://doi.org/10.1007/s11103-004-5112-0).
- Xu, S., D. Zhu, and Q. Zhang. 2014. Predicting hybrid performance in rice using genomic best linear unbiased prediction. *Proceedings of the National Academy of Sciences* 111 (34):12456–61. doi: [10.1073/pnas.1413750111](https://doi.org/10.1073/pnas.1413750111).
- Xu, X.-H., H.-J. Zhao, Q.-L. Liu, T. Frank, K.-H. Engel, G. An, and Q.-Y. Shu. 2009. Mutations of the multi-drug resistance-associated protein ABC transporter gene 5 result in reduction of phytic acid in rice seeds. *Theoretical and Applied Genetics* 119 (1):75–83. doi: [10.1007/s00122-009-1018-1](https://doi.org/10.1007/s00122-009-1018-1).
- Xu, X., X. Liu, S. Ge, J. D. Jensen, F. Hu, X. Li, Y. Dong, R. N. Gutenkunst, L. Fang, L. Huang, et al. 2012. Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nature Biotechnology* 30 (1):105. doi: [10.1038/nbt.2050](https://doi.org/10.1038/nbt.2050).
- Yamamoto, N., R. Garcia, T. Suzuki, C. A. Solis, Y. Tada, R. Venuprasad, and A. Kohli. 2018. Comparative whole genome resequencing analysis in upland new rice for Africa: Insights into the breeding history and respective genome compositions. *Rice* 11 (1): 33. doi: [10.1186/s12284-018-0224-3](https://doi.org/10.1186/s12284-018-0224-3).
- Yan, N., Y. Du, X. Liu, M. Chu, J. Shi, H. Zhang, Y. Liu, and Z. Zhang. 2019. A comparative UHPLC-QqQ-MS-based metabolomics approach for evaluating Chinese and North American wild rice. *Food Chemistry* 275:618–27. doi: [10.1016/j.foodchem.2018.09.153](https://doi.org/10.1016/j.foodchem.2018.09.153).
- Yan, W., and J. Bao. 2014. *Rice: Germplasm, genetics and improvement*. Norderstedt: BoD – Books on Demand.
- Yang, M., K. Lu, F.-J. Zhao, W. Xie, P. Ramakrishna, G. Wang, Q. Du, L. Liang, C. Sun, and H. Zhao. 2018. Genetic basis of rice ionomic variation revealed by genome-wide association studies. *The Plant Cell*, TPC 00375:02018.
- Yang, Y., L. Dai, H. Xia, K. Zhu, H. Liu, and K. Chen. 2013. Protein profile of rice (*Oryza sativa*) seeds. *Genetics and Molecular Biology* 36 (1):87–92. doi: [10.1590/S1415-47572013000100012](https://doi.org/10.1590/S1415-47572013000100012).
- Ye, G., S. Liang, and J. Wan. 2010. QTL mapping of protein content in rice using single chromosome segment substitution lines. *Theoretical and Applied Genetics* 121 (4):741–50. doi: [10.1007/s00122-010-1345-2](https://doi.org/10.1007/s00122-010-1345-2).
- Ye, X., S. Al-Babili, A. Klöti, J. Zhang, P. Lucca, P. Beyer, and I. Potrykus. 2000. Engineering the provitamin A (β -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287 (5451):303–5. doi: [10.1126/science.287.5451.303](https://doi.org/10.1126/science.287.5451.303).
- Zarei, I., D. G. Brown, N. J. Nealon, and E. P. Ryan. 2017. Rice bran metabolome contains amino acids, vitamins & cofactors, and phytochemicals with medicinal and nutritional properties. *Rice* 10:24. doi: [10.1186/s12284-017-0157-2](https://doi.org/10.1186/s12284-017-0157-2).
- Zhang, J., B. R. Nallamilli, H. Mujahid, and Z. Peng. 2010. OsMADS6 plays an essential role in endosperm nutrient accumulation and is subject to epigenetic regulation in rice (*Oryza sativa*). *The Plant Journal* 64 (4):604–17. doi: [10.1111/j.1365-313X.2010.04354.x](https://doi.org/10.1111/j.1365-313X.2010.04354.x).
- Zheng, L., F. Huang, R. Narsai, J. Wu, E. Giraud, F. He, L. Cheng, F. Wang, P. Wu, and J. Whelan. 2009. Physiological and transcriptome analysis of iron and phosphorus interaction in rice seedlings. *Plant Physiology* 151:262–274.
- Zhang, M., S. R. Pinson, L. Tarpley, X.-Y. Huang, B. Lahner, E. Yakubova, I. Baxter, M. L. Guerinot, and D. E. Salt. 2014. Mapping and validation of quantitative trait loci associated with concentrations of 16 elements in unmilled rice grain. *Theoretical and Applied Genetics* 127 (1):137–65. doi: [10.1007/s00122-013-2207-5](https://doi.org/10.1007/s00122-013-2207-5).
- Zhang, W., J. Bi, L. Chen, L. Zheng, S. Ji, Y. Xia, K. Xie, Z. Zhao, Y. Wang, L. Liu, et al. 2008. QTL mapping for crude protein and protein fraction contents in rice (*Oryza sativa* L.). *Journal of Cereal Science* 48 (2):539–47. doi: [10.1016/j.jcs.2007.11.010](https://doi.org/10.1016/j.jcs.2007.11.010).
- Zhang, Y., Y. H. Xu, H. Y. Yi, and J. M. Gong. 2012. Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *The Plant Journal* 72 (3): 400–10. doi: [10.1111/j.1365-313X.2012.05088.x](https://doi.org/10.1111/j.1365-313X.2012.05088.x).
- Zhao, G.-C., Y.-X. Zhang, S.-Y. Sun, M.-X. Xie, C.-Y. Hu, Y.-Q. Shi, J.-X. Shi, and J.-Y. Li. 2019. Identification of the biochemical characteristics of developing giant embryo rice grains using non-targeted metabolomics. *Journal of Cereal Science* 85:70–6. doi: [10.1016/j.jcs.2018.10.011](https://doi.org/10.1016/j.jcs.2018.10.011).
- Zhao, H., W. Yao, Y. Ouyang, W. Yang, G. Wang, X. Lian, Y. Xing, L. Chen, and W. Xie. 2015. RiceVarMap: A comprehensive database of rice genomic variations. *Nucleic Acids Research* 43 (D1): D1018–D1022. doi: [10.1093/nar/gku894](https://doi.org/10.1093/nar/gku894).
- Zhou, W., X. Wang, D. Zhou, Y. Ouyang, and J. Yao. 2017. Overexpression of the 16-kD α -amylase/trypsin inhibitor RAG 2 improves grain yield and quality of rice. *Plant Biotechnology Journal* 15 (5):568–80. doi: [10.1111/pbi.12654](https://doi.org/10.1111/pbi.12654).