Background
Rice breeding program needs to focus on development of nutrient dense rice for value addition and helping in reducing malnutrition. Mineral and vitamin deficiency related problems are common in the majority of the population and more specific to developing countries as their staple food is rice.

Results:
Genes and QTLs are recently known for the nutritional quality of rice. By comprehensive literature survey and public domain database, we provided a critical review on nutritional aspects like grain protein and amino acid content, vitamins and minerals, glycemic index value, phenolic and flavonoid compounds, phytic acid, zinc and iron content along with QTLs linked to these traits. In addition, achievements through transgenic and advanced genomic approaches have been discussed. The information available on genes and/or QTLs involved in enhancement of micro-nutrient element and amino acids are summarized with graphical representation.

Conclusion:
Compatible QTLs/genes may be combined together to design a desirable genotype with superior in multiple grain quality traits. The comprehensive review will be helpful to develop nutrient dense rice cultivars by integrating molecular markers and transgenic assisted breeding approaches with classical breeding.

Keywords:
Grain nutritional properties, Grain nutraceutical properties, Grain vitamins and minerals, Grain phytic acid, Grain protein, Grain amino acid, Grain phenolic and flavonoid compounds, Molecular markers

Background
Rice is the most well known cereal and staple food which serves as major carbohydrate for more than half of the world population. Half of the world’s population is suffering from one or more vitamin and/or mineral deficiency (World Food Program 2015). More than three billion people are affected by micronutrient malnutrition and 3.1 million children die each year out of malnutrition (Gearing 2015) and the numbers are gradually increasing (FAO 2009; Johnson et al. 2011). The developed countries are managing deficiency by adopting fortification programs, but same programs are not affordable to poor countries. Therefore, an alternative and less expensive strategy is to modify the nutritional quality of the major cereals consumed by the people. To improve the nutritional value of rice, research programs should be reoriented to develop high yielding cultivars with nutrient dense cultivars either by selective breeding or through genetic modification (Gearing 2015). Increase in literacy percentage and awareness of diet, people tend to be more health conscious and interested to have nutritionally enriched food. The quality of rice is an important character to determine the economic value in the export market and consumer acceptance (Pingali et al. 1997).

The genetic basis of the accumulation of micronutrients in the grain, mapping of the quantitative trait loci (QTL) and identification of genes will provide the basis for preparing the strategies and improving the grain micronutrient content in rice. Integrating marker assisted breeding with classical breeding makes, the possibility to track the introgression of nutritional quality associated QTLs and genes into a popular cultivar from various germplasm sources (Fig. 1). Till date classical breeding...
has a significant impact on improving biofortification of rice cultivars by making crosses, backcrosses and selection of the desired superior rice cultivars with high nutritional value. However, by availing technologies such as DNA markers, genetic engineering and allele mining offers an opportunity to use them as a tool to detect the allelic variation in genes underlying the traits and introgression of nutrition related QTLs/genes to improve the efficiency of classical plant breeding via marker-assisted selection (MAS).

Molecular markers such as SNPs (Ohstubo et al. 2002; Bao et al. 2006; Bert et al. 2008; Mammadov et al. 2012), SSRs (Anuradha et al. 2012; Nagesh et al. 2013; Gande et al. 2014), STS (Chandel et al. 2011; Gande et al. 2014), etc. have been developed. Integration of the markers into the breeding programs for effective selection of the plants at early stage of crop growth provides an opportunity to achieve the target earlier than the classical breeding program. Genomic approaches are particularly useful when working with complex traits having multigenic and influence of environment. In this new plant breeding era, genomics will be an essential aspect to develop more efficient nutritional rich rice cultivars (Perez-de-Castro et al. 2012), for reducing human health problems relating to mineral nutrition. Therefore, this is an effective approach for future rice breeding to reduce the malnutrition. By availing the different molecular approaches and advanced genomic technologies such as SNPs array, genome sequencing, genome-wide association mapping, transcriptome profiling, etc. could be strategically exploited to understand molecular mechanism and their relation between the genotypes and phenotypic traits leading to development of improved rice varieties (Chandel et al. 2011; Varshney et al. 2014; Malik et al. 2016; McCouch et al. 2016; Peng et al. 2016).

**Traits for improvement of the grain nutritive value**

In the present situation, attention on grain quality and nutritional value has become a primary thought for producers and consumers. Rice grain is relatively low in some essential micronutrients such as iron (Fe), zinc (Zn) and calcium (Ca) as compared to other staple crops like wheat, maize, legumes and tubers (Adeyeye et al. 2000). However, rice grain consists of ~80% starch and its quality is dependent on combination of several traits. Another component of nutritive value of rice is bran, an important source of protein, vitamins, minerals, antioxidants, and phytosterols (Iqbal et al. 2005; Liu 2005; Schramm et al. 2007; Renuka and Arumughan 2007). Rice bran protein has a great potential in the food industry, having unique

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**Fig. 1** Integration of phenotypic and molecular breeding approaches for improvement of nutraceutical properties in rice grain
nutraceutical properties (Saunders 1990) and reported as hypoallergenic food ingredient in infant formulations (Helm and Burks 1996) and having anti-cancer properties (Shoji et al. 2001). Improvement in these components in the grain can be useful to reduce malnutrition.

**Nutritional and nutraceutical properties of rice**

**Grain protein and amino acid content**

Protein energy malnutrition affects 25% of children where their dietary intake is mainly on rice and staple crops have low levels of essential amino acids (Gearing 2015). Therefore, attempts to improve the nutritional value of rice have been concentrated on protein content (PC) and other nutritional quality (Fig. 2). The amount of PC in rice is relatively low (8.5%) as compared to other cereals like wheat (12.3%), barley (12.8%) and Millet (13.4%) and an average of PC in milled rice is about 7 and 8% in brown rice. The total seed protein content of rice is composed of 60–80% glutelin and 20–30% prolamin, controlled by 15 and 34 genes respectively (Kawakatsu et al. 2008; Xu and Messing 2009). Rice supplies about 40% of the protein to human through diet in developing countries and quality of PC in rice is high, due to rich in lysine (3.8%) (Shobha Rani et al. 2006). Therefore, improvement of PC in rice grain is a major target for the plant breeders and biotechnologists. So far, by classical breeding effort, very limited success has been achieved because of the complex inheritance nature and the large effect of environment on protein content (Coffman and Juliano 1987). According to Iqbal et al. (2006), more than 170 million children and nourishing mothers suffered from Protein-calorie malnutrition (PCM) in developing Afro-Asian countries. In comparison with meat, plant proteins are much less expensive and nutritionally imbalanced because of their deficiency in certain essential amino acids (EAAs).

![Depicted diagram of molecular marker positions associated with grain nutritional quality of rice distributed on 12 chromosomes from comprehensive literature survey. Molecular marker on right and their position (cM) on left side of the chromosomes: MPGQ milling properties of grain quality, GA grain appearance (red), CP cooking properties (blue), NF nutrition factors (pink), FRG fragrance of rice grain (green) (colors indicate markers related to nutritional quality traits in rice)](image)
0.8–2.0 vs. 1.0%), and threonine (Thr, 2.7–3.9 vs. 4.0%). Pulses and most vegetable protein contain 1.0–2.0% of sulfur containing amino acid (methionine and cysteine), compared with the 3.5% of the WHO reference protein (Sun 1999). Therefore, these EAAs become the limiting amino acids in cereals and legumes. Recently, Han et al. (2015) compared the quality of rice bran protein (RBP) with animal and vegetable proteins. The digestibility of RBP (94.8%) was significantly higher than that of endosperm protein (90.8%), soy protein (91.7%) and whey protein (92.8%) which is same as that of casein. Among the total grain PC, rice bran protein appears to be a promising protein source with good biological value and digestibility.

Recently, Mohanty et al. (2011) reported 16.41 and 15.27% of crude protein in brown rice of ARC 10063 and ARC 10075 respectively on dry weight basis. They observed the total free amino acid content to be higher in these accessions and lysine content was positively correlated with the grain protein content in contrary to the view of Juliano et al. (1964) and Cagampang et al. (1966). Subsequently, by exploiting ARC 10075 as a donor, CR Dhan 310 (IET 24780) rice variety was developed with high protein content of 11% and rich in threonine and lysine (NRRI Annual Report 2014–2015). Several reports claim the varying levels of PC from 4.91 to 12.08%, lysine of 1.73–7.13 g/16 g N and tryptophan from 0.25 to 0.86 g/16 g N in rice accessions (Banerjee et al. 2010). Utilizing the efficiency of molecular marker technology, PC in brown and milled rice were mapped using various rice populations (Tan et al. 2001; Aluko et al. 2004; Weng et al. 2008; Zhang et al. 2008; Yu et al. 2009; Zhong et al. 2011; Yun et al. 2014).

Vitamins and minerals
Forty-nine nutrients are required for normal growth and development and the demand is fulfilled by nutrients supplied by cereals, particularly rice (Welch and Graham 2004). Among these nutrients, mineral elements play beneficial role directly or indirectly in human metabolism. The wide spread occurrence of anemia and osteoporosis due to deficiency of iron and calcium respectively was observed in most developing countries as well as developed countries (Welch and Graham 1999). In the scenario, plant breeders started to pay more attention to improve the nutrient qualities especially mineral elements of major food grain crops (Zhang et al. 2004). Several researchers have reported genetic differences of mineral elements in rice (Gregorio et al. 2000; Zhang et al. 2004; Anandan et al. 2011; Ravindra Babu 2013; Jagadeesh et al. 2013). However, limited number of reports was observed for molecular level study and QTLs for vitamin and mineral content in rice. Brown rice is an important source of vitamins and minerals and by polishing the brown rice, several nutritional components such as dietary fiber, vitamins and phenols are eliminated that are beneficial to human health.

Glycemic index value
Glycemic index (GI) is an indicator for the response of blood sugar levels based on the amount of carbohydrate consumption (after ingestion), which can be measured by rapidly available glucose (RAG). Rice, as a staple food contains 80% of starch and increased consumption leads to risk of type II diabetes (Courage 2010) and is predicted to affect almost 330 million people by 2030 (Misra et al. 2010). Brand-Miller et al. (2000) categorized glycemic index foods into low (GI value <55), medium (GI value 56–69) or high (GI value >70) GI foods. Recent studies have shown the ability of lower GI value will help to improve glycemic control in diabetics and cardiovascular diseases (Brand-Miller et al. 2003; Srinivasa et al. 2013). Low GI foods more slowly convert the food into energy by the body, thereby blood glucose levels become more stable than diets based on high GI foods. Therefore, identification of lower GI crops would play a major role in managing the disease. Thus, the diabetic sufferers in low-income countries such as Bangladesh, India, Indonesia, Malaysia and Sri Lanka may offer an inexpensive way for managing the disease (Fitzgerald et al. 2011). GI range may vary among the genotypes as well as the growing regions. GI varied from 54 to 121 among rice genotypes (Manay and Shadaksharaswamy 2001).

The degree of gelatinization is proportional to the amount of amylose; the less amylose there is, the greater the degree of gelatinization and vice versa. In other words, starches with lower amylose content will have higher Glycemic Indexes. Inversely, starches with a higher amylose content will be less susceptible to gelatinization, that is, to breaking down into glucose, that which makes for low Glycemic Indexes. The amount of amylose content (AC), Waxy haplotype and digestibility of rice are significantly correlated (Fitzgerald et al. 2011) and observed that AC plays a key role in rate of starch digestion and GI (Kharabian-Masouleh et al. 2012). Apparent amylose content is primarily controlled by the Waxy gene which codes for granule bound starch synthase (Chen et al. 2008a). The combination of two single-nucleotide-polymorphism (SNP) markers in the Waxy gene allows for the identification of three marker haplotypes in this gene. The first SNP is at the leader intron splice site (In1 SNP), and the second polymorphism is in exon 6. The haplotypes explained 86.7% of the variation in apparent amylose content and discriminated the three market classes of low, intermediate and high AC rice from each other.
Chen et al. (2008a, b), Larkin and Park (2003) and Kharabian-Masouleh et al. (2012) reported that Waxy gene showed four haplotypes viz., In1T-Ex6A, In1G-Ex6C, In1G-Ex6A and In1T-Ex6C used for the classification of AC in rice. Conversely, Cheng et al. (2012) identified intron1 is insufficient to explain the genetic variations of AC in rice. Therefore, the study based on the AC and molecular analysis would be helpful for the selection of appropriate nutritional quality rice for diabetic. Angwara et al. (2014) characterized 26 Thai rice varieties for RAG and Waxy haplotype (In1-Ex6) as GI indicators. The four haplotypes, classified 26 Thai rice varieties into groups consisting four varieties having G-A, nine varieties harboring G-C, 13 varieties carrying T-A or T-C allele associated with high, intermediate and low amyllose respectively and the varieties having G-A haplotype exhibited low RAG.

**Phenolic and flavonoid compounds of rice grain**

The phytochemicals such as phenolic compounds (toco-pherol, tocotrienols and γ-oryzanol) and flavonoids (anthocyanin) are responsible for good source of natural antioxidant and grain colour respectively. Kernel of red rice is characterized by the presence of proanthocyanidins whereas black rice is characterized by the accumulation of anthocyanins, mainly cyanidin-3-glucoside and peonidin 3-glucoside. These compounds help in decreasing the toxic compounds and reduce the risk of developing chronic diseases including cardiovascular disease, type-2 diabetes, reduction of oxidative stress and prevention of some cancers (Ling et al. 2001; Kong et al. 2003; Hu et al. 2003; Iqbal et al. 2005; Yawadio et al. 2007; Shao et al. 2011).

Red rice has phenolic compounds in the range of 165.8–731.8 mg gallic acid equivalent (GAE) 100 g−1 (Shen et al. 2009) and black/purple rice reported to have higher amount of Fe, Zn, Ca, Cu and Mg than red rice (Meng et al. 2005). On the other hand, pigmented rice reported to have higher amount of antioxidative activity (Zhang et al. 2006; Nam et al. 2006; Chung and Shin 2007; Hiemori et al. 2009). The concept of the total antioxidant capacity, which represents the ability of different food antioxidants to scavenge free radicals, has been suggested as a tool for evaluating the health effects of antioxidant rich foods. In non-pigmented rice varieties, the bran fraction has a total phenolic content (TPC) of 596.3 mg GAE 100 g−1, which is close to that of the husk (599.2 mg GAE 100 g−1) followed by the whole grain (263.9 mg GAE 100 g−1) and the rice endosperm (56.9 mg GAE 100 g−1) (Goufo and Trindade 2014). The phenolic compounds are mainly associated with the pericarp colour, darker the pericarp higher the amount of polyphenols (Tian et al. 2004; Zhou et al. 2004; Yawadio et al. 2007). Shen et al. (2009) characterized coloured parameters of rice grain (white, red and black rice) in wide collection of rice germplasm and found significantly associated with total phenolics, flavonoid and antioxidant capacity in three types of rice grain. Moreover, the correlations among the white rice accessions are rather weak. Goffman and Bergman (2004) evaluated different colour of rice genotypes and their total phenolic content ranged from 1.90 to 50.32 mg GAE g−1 of bran, and between 0.25 and 53.5 mg GAE g−1 of grain. Recent evidence of Goufo and Trindade (2014), showed 12 phenolic acids are generally identified in rice ranging from 177.6 to 319.8 mg 100 g−1 in the bran, 7.3 to 8.7 mg 100 g−1 in the endosperm, 20.8 to 78.3 mg 100 g−1 in the whole grain, and 477.6 mg 100 g−1 in the husk, depending on the rice color. This suggest that, rice bran has highest source of phenolic acids than others consumable part of rice. Numerous literatures have shown that consumption of colored rice reduces oxidative stress and simultaneously increases in antioxidant capacity. Consumption of colored rice varieties is very limited in Western countries, but in some growing areas of Asia, traditional varieties with colored pericarp are particularly valued in local markets (Finocchiaro et al. 2007).

The antioxidant compounds in rice as γ-oryzanol, tocols and phenolic acids associated with reduced risk of developing chronic diseases (Liu 2007; Yawadio et al. 2007). Among the various phenolic compounds, ferulic acid (56–77% of total phenolic acids) found in the endosperm, bran, and whole grain, followed by p-coumaric acid (8–24%), sinapic acid (2–12%), gallic acid (1–6%), protocatechuic acid (1–4%), p-hydroxybenzoic acid (1–2%), vanillic acid (1%), and syringic acid (1%) (Goufo and Trindade 2014).

**Effect of phytic acid in rice grain**

An important mineral storage compound in seed is phytate, a mixed cation salt of phytic acid (InsP6) accounted approximately 75% of total phosphorus in seeds (Lott 1984; Suzuki et al. 2007; Raboy 2009). A considerable part of the phosphorus taken up by plants from soil is translocated ultimately to the seed and synthesized into phytic acid (PA). Therefore, this compound represents a major pool in the flux of phosphorus and recently estimated that, the amount of phosphorus synthesized into seed in the form of PA by crops each year represents a sum equivalent to >50% of phosphorus fertilizer used annually world-wide (Lott et al. 2000). Phytate being vital for seed development and higher seedling vigour, often considered as an anti-nutritional substance, but may have a positive nutritional role as an antioxidant, anti-cancer agent, lowering chronic disease rates, heart diseases in humans and prevents coronary diseases (Bohn et al.
for humans is 10–15 mg kg\(^{-1}\), whereas the recommended dietary intake of Zn for humans is 12–15 mg kg\(^{-1}\). The recommended dietary intake of Fe and Zn is equally important as Fe and vitamin A (Marschner 1995). Generally, the content of Zn in males within the age bracket of 15–74 years requires approximately 12–15 mg of Zn daily, while females within 15–74 years of age group need about 68 mg of Zn (Sandstead 1985). Generally, the content of Zn in polished rice has 2 mg kg\(^{-1}\), while the recommended dietary intake of Fe for humans is 10–15 mg kg\(^{-1}\). Therefore, globally more than 3 billion people were affected by Zn deficiency, particularly in developing countries (Graham et al. 1999; Welch and Graham 2004). Pregnancy maternal mortality by anemia leads to 1.15 lakh deaths per year, resulting in 3.4 million disability-adjusted life-years (DALYs), has been recognized to Fe deficiency (Stoltzfus et al. 2004). Hence, improvement of Fe content in rice grain is necessary, which is a major challenge to the plant breeders. In plants, Zn plays a significant role in the biosyntheses and turnovers of proteins, nucleic acids, carbohydrates and lipids, with functional aspects as integral cofactor for more than 300 enzymes, coordinating ion in the DNA-binding domains of transcription factors and equally important as Fe and vitamin A (Marschner 1995). Males within the age bracket of 15–74 years require approximately 12–15 mg of Zn daily, while females within 15–74 years of age group need about 68 mg of Zn (Sandstead 1985). Generally, the content of Zn in polished rice is an average of only 12 mg kg\(^{-1}\), whereas the recommended dietary intake of Zn for humans is 12–15 mg kg\(^{-1}\) (FAO 2001). About 17.3% of the global population is under risk of Zn deficiency and in some regions of the world, it is as high as 30% due to dietary inadequacy (Wessells and Brown 2013). Therefore, to enhance the concentration of these micronutrients in rice grain could be possible as signified the presence of vast genetic potential of various rice germplasm by adapting appropriate genetic approaches (Fig. 1). However, major attention to date has been paid on identification and development of genetically engineered rice grains with increased bioavailable contents of Fe and/or Zn. The list of rice cultivars that possess dense micronutrient are presented in Table 1. Recently, Indian Institute of Rice Research, Hyderabad has developed a genotype (IET 23832) that possesses high Zn (19.50 ppm). As the brown rice has higher amount of Fe and Zn, more than 70% of micronutrients are lost during polishing (Sellappan et al. 2009) as they are located on the outer layer of the kernel. Martinez et al. (2010) found 10–11 ppm Fe and 20–25 ppm Zn in brown rice, while 2–3 ppm Fe and 16–17 ppm Zn was observed in milled rice.

**Prerequisite for improvement of Fe and Zn content in rice grain**

Iron and zinc micronutrients are the most important elements, deficiency of which is a major cause for malnutrition. More than half of the world population is suffering from bioavailable nutrient deficiencies particularly in developing countries (Sheshadri 1997; Shahzad et al. 2014). The main reason of these deficiency occurred due to consumption of polished cereal based food crops as rice, wheat and maize (Pfeiffer and McClafferty 2007). Modern high yielding rice varieties are poor sources of essential micronutrients like Fe and Zn (Zimmerman and Hurrel 2002). On an average, polished rice has 2 mg kg\(^{-1}\), while the recommended dietary intake of Fe for humans is 10–15 mg kg\(^{-1}\). Therefore, globally more than 3 billion people were affected by Fe deficiency, particularly in developing countries (Graham et al. 1999; Welch and Graham 2004). Pregnancy maternal mortality by anemia leads to 1.15 lakh deaths per year, resulting in 3.4 million disability-adjusted life-years (DALYs), has been recognized to Fe deficiency (Stoltzfus et al. 2004). Hence, improvement of Fe content in rice grain is necessary, which is a major challenge to the plant breeders. In plants, Zn plays a significant role in the biosyntheses and turnovers of proteins, nucleic acids, carbohydrates and lipids, with functional aspects as integral cofactor for more than 300 enzymes, coordinating ion in the DNA-binding domains of transcription factors and equally important as Fe and vitamin A (Marschner 1995). Males within the age bracket of 15–74 years require approximately 12–15 mg of Zn daily, while females within 15–74 years of age group need about 68 mg of Zn (Sandstead 1985). Generally, the content of Zn in polished rice is an average of only 12 mg kg\(^{-1}\), whereas the recommended dietary intake of Zn for humans is 12–15 mg kg\(^{-1}\) (FAO 2001). About 17.3% of the global population is under risk of Zn deficiency and in some regions of the world, it is as high as 30% due to dietary inadequacy (Wessells and Brown 2013). Therefore, to enhance the concentration of these micronutrients in rice grain could be possible as signified the presence of vast genetic potential of various rice germplasm by adapting appropriate genetic approaches (Fig. 1). However, major attention to date has been paid on identification and development of genetically engineered rice grains with increased bioavailable contents of Fe and/or Zn. The list of rice cultivars that possess dense micronutrient are presented in Table 1. Recently, Indian Institute of Rice Research, Hyderabad has developed a genotype (IET 23832) that possesses high Zn (19.50 ppm). As the brown rice has higher amount of Fe and Zn, more than 70% of micronutrients are lost during polishing (Sellappan et al. 2009) as they are located on the outer layer of the kernel. Martinez et al. (2010) found 10–11 ppm Fe and 20–25 ppm Zn in brown rice, while 2–3 ppm Fe and 16–17 ppm Zn was observed in milled rice.

**QTLs linked to nutritional and nutraceutical properties of rice**

**QTLs for protein content in rice**

Protein content in rice grain is a key factor for the enhancement of nutritional values and influencing the palatability of cooked rice (Matsue et al. 1995). Tan et al. (2001) mapped two QTLs for PC in the interval of C952–Wx on chromosome 6 near to waxy gene with 13% PV and LOD score of 6.8 and another QTL was mapped within the interval of R1245–RM234 on chromosome 7, which accounted for 4.7% of the PV and LOD score of 3.2. On the other hand, Aluko et al. (2004) identified four QTLs located on chromosomes 1, 2, 6 and 11 in a DH population from an inter specific crosses between O. sativa and O. glaberrima. Among the four QTLs, one QTL was located on chromosome 6, which is closely associated with Wx gene influencing rice quality.

Three QTLs viz., qPC11.1, qPC11.1, and qPC111.2 were associated with PC of brown rice (Qin et al. 2009). Among them, qPC11.1, and qPC111.2 were identified on chromosome 11 exhibiting 22.10% and 6.92% PV with LOD score of 4.90 and 2.75, respectively. The QTL qPC111.2 was found to be consistent over two years of trial and linked with marker RM287. Yu et al. (2009) detected five QTLs for PC and four QTLs for fat content from 209 RILs. The five QTLs (qPC-3, qPC-4, qPC-5, qPC-6 and qPC-10) for PC were detected on chromosomes 3, 4, 5, 6 and 10 with LOD score of 6.25, 2.87, 2.28, 9.78 and 4.50 respectively. The QTL qPC111.2 was found to be consistent over two years of trial and linked with marker RM287. Yu et al. (2009) detected five QTLs for PC and four QTLs for fat content from 209 RILs. The five QTLs (qPC-3, qPC-4, qPC-5, qPC-6 and qPC-10) for PC were detected on chromosomes 3, 4, 5, 6 and 10 with LOD score of 6.25, 2.87, 2.28, 9.78 and 4.50 respectively.

Among these five loci, qPC-6 observed to be nearer to the Wx marker between RM190 and RZ516 on the short arm of rice chromosome 6, explaining 19.3% of the PV and other four QTLs explained 3.9–10.5% of the PV. Zhong et al. (2011) reported two consistent QTLs for PC in milled rice as qPrT and qPrT detected over two years and positioned in the marker interval of RM493–RM562 and RM445–RM418 on chromosome 1 and 7 respectively. Recently, three QTLs qPrO-8, qPrO-9 and qPrO-10 were detected on chromosome 8 flanked by RM506–RM1235 with a LOD score of 2.57, chromosome 9 in the interval of RM219–RM23914 with a LOD score of 2.66, and chromosome 10 separated by RM24934–RM25128 with a
Table 1 List of identified promising donors for Fe and Zn nutritional quality traits in rice

| S. no | Rice genotypes | Nutritional element | Reference |
|-------|----------------|---------------------|-----------|
| 1     | SL-32, Annada, ASD16, CH-45, Nagina 22, Swarna, IR-29, Pusa Sugandha-1, IRGC-106187, IR68144-3B-2-2-3, IRGC-105320, IRGC-105320, IRGC-86476, CH-45, Jyoti, HKR-126, Vansha, MSEA-9, Jalmagna, Zuchem, Kalabath, Pusa Basmati, Nootipattu, Pitchavari, Thanu, TKM-9, NDR-6279, and Aghonibora | Fe (>20 ppm) | Gregorio (2002), Anandan et al. (2011), Anuradha et al. (2012), Ravindra Babu (2013), Jagadeesh et al. (2013) |
| 2     | Nagina 22, Honduras, RG-187, SL-32, Aghoni bora, Annada, ASD-16, Jalmagna, CH-45, BPT-5204, Lalat, Sasyari, Swarna, IR-29, Pusa Sugandha-1, IRGC-106187, IRGC-105320, IRGC-86476, Benibhog, CH-45, Jyoti, HKR-126, Pant Sugandh-17, Ratna, Chitimutyalu, Ranbir basmati, IRRI-38, Jeerigesanna, Kalabath, Pusa Basmati, Nootipattu, Madhukar, Swarna, AM-141, Thanu, TKM-9, NDR-6279, Aghonibora and Pitchavari | Zn (>20 ppm) | Anandan et al. (2011), Anuradha et al. (2012), Ravindra Babu (2013), Berhanu et al. (2013), Vishnu et al. (2014), Gande et al. (2014) |

LOD score of 6.13 respectively for PC from 120 DH lines (Yun et al. 2014).

QTLs associated with amino acid in rice

Amino acid (AA) composition and mapping was reported in milled rice using 190 RILs and detected eighteen chromosomal regions for 17 out of 20 AA (except Tryptophan, Glutamine, and Asparagine), essential AA in total and total AA content in rice grain (Wang et al. 2008). Two major QTL clusters in RM472-RM104 (1–19) and RM125-RM542 (7–4, 5) were detected consistently in two years and explained about 30 and 40% of PV. Zhong et al. (2011) detected 48 and 64 QTLs related to AA in the year of 2004 and 2005, respectively. Most QTLs co-localized, forming 29 QTL clusters on the chromosomes with three major ones detected in both years, which were mapped on chromosomes 1, 7, and 9, respectively. The two QTL clusters for amino acid content, qAa1 and qAa7, influenced almost all the traits and the third QTL cluster for amino acid content, qAa9, increased the lysine content. Therefore, these identified QTLs and their association with particular grain quality nutrient trait results will be useful to find the candidate genes and favorable alleles to transfer into elite breeding rice cultivars through marker-assisted breeding program.

QTLs responsible for mineral contents in rice

Several QTLs related to nutritional quality traits have been reported in rice from different genetic backgrounds of intraspecific and interspecific crosses using molecular markers. The grain nutrient traits associated with various QTLs and linked/flanking markers are summarized in Table 2 and Fig. 2. Three loci explaining 19–30% variation for Fe content on chromosomes 7, 8, and 9 were observed by Gregorio et al. (2000). A major QTL explaining 16.5% of PV for Fe content on chromosome 2 was identified from a DH population derived from a cross between IR64 and Azucena (Stangouli et al. 2007). Besides, Garcia-Oliveira et al. (2008) reported a QTL for Fe content close to the marker RM6641 on chromosome 2 from an introgression line derived from a cross between Teqing and Oryza rufipogon. Wild rice (O. rufipogon) contributed favorable alleles for most of the QTLs (26 QTLS), and chromosomes 1, 9 and 12 exhibited 14 QTLS (45%) for these traits. One major effect of QTL for Zn content accounted for the largest proportion of phenotypic variation (11–19%) was detected near the simple sequence repeats marker RM152 on chromosome 8. James et al. (2007) used a DHs population for three Fe linked QTLS on chromosomes 2, 8, and 12, explaining 17, 18, and 14% of the total PV, respectively. They also reported two QTLS for Zn content on chromosomes 1 and 12, explaining PV of 15 and 13% respectively. Norton et al. (2010) reported ten QTLS for five mineral elements (Cu, Ca, Zn, Mn and Fe) and Fe (qFe-1) mineral trait explained the highest PV of 25.81% with LOD score of 7.66. Anuradha et al. (2012) identified 14 QTLS for Fe and Zn from unpolished rice of Madhukar/Swarna RILs. Seven QTLS each for grain Fe and Zn content were identified on chromosomes 1, 3, 5, 7 and 12 and the PV ranged from 29 to 71%. In addition, Gande et al. (2014) identified 24 candidate gene markers responsible for Zn content and four candidate genes namely OsNAC, OsZIP8a, OsZIP8c and OsZIP4b showed significant PV of 4.5, 19.0, 5.1 and 10.2%, respectively.

Garcia-Oliveira et al. (2008) identified 31 putative QTLS associated with microelements (Fe, Zn, Mn, Cu,) and macro elements (Ca, Mg, P and K) on all chromosomes except on chromosome 7. Among the total QTLS identified, chromosomes 1 and 9 had the highest number of QTLS having five QTLS each. Earlier reports showed several QTLS for the mineral content associated with different chromosomal regions of rice. QTLS for K on chromosomes 1 and 4 (Wu et al. 1998), P on chromosomes 1 and 12 (Ni et al. 1998; Wissuwa et al. 1998; Ming et al.
| S. no | Grain traits | Chr | QTLs | Markers | Type | Peak marker | Populations | References |
|-------|--------------|-----|------|---------|------|-------------|-------------|------------|
| 1     | PC           | 1   | qPr1 | RM493-RM562 | RILs | RM493-RM562 | Zhenshan97B/Delong 208 | Zhong et al. (2011) |
| 2     | PC           | 1   | qPC1.1 | 1008-RM575 | Dhs  | Samgang/Nagdong | Qin et al. (2009) |
| 3     | MAC-P        | 1   | qP1  | RM3411 | LT/TL-RILs | TeQing/Leont | Zhang et al. (2014) |
| 4     | MAC-K        | 1   | qK1  | RM5501 | LT/TL-RILs | Leont/TeQing | Zhang et al. (2014) |
| 5     | PC           | 1   | qPC1 | RM472-RM104 | RILs | Zhenshan97/ Nanyangzhan | Peng et al. (2014) |
| 6     | AAC          | 1   | qAa1 | RM493-RM562 | RILs | Zhenshan97B/Delong 208 | Zhong et al. (2011) |
| 7     | MAC-P        | 1   | qP1  | RM495 | LT-RILs | Madhukar/Swana | Anuradha et al. (2012) |
| 8     | Fe           | 1   | qFe1.1 | RM495 | RILs | Lemont/TeQing | Zhang et al. (2014) |
| 9     | MAC-Ca       | 1   | qCa1-1 | RM6480 | ILs | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 10    | MAC-P        | 1   | qP1-1 | RM212 | ILs | Lemont/TeQing | Zhang et al. (2014) |
| 11    | MAC-Ca       | 1   | qCa1 | RM490 | LT/TL-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 12    | MAC-Ca       | 1   | qCa1 | RM490 | LT/TL-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 13    | MAC-P        | 1   | qP1-1 | RM212 | ILs | Lemont/TeQing | Zhang et al. (2014) |
| 14    | Fe           | 1   | qFe1.1 | RM495 | RILs | Madhukar/Swana | Anuradha et al. (2012) |
| 15    | Fe           | 1   | qFe1.2 | RM490 | RILs | Madhukar/Swana | Anuradha et al. (2012) |
| 16    | AAC-Asp/Thr/Glu/Gly/Ala/Cys/5yr/Pro/Eaa/total | | qAA.1 | RM472-RM104 | RILs | RM472 | Zhenshan97/ Nanyangzhan | Wang et al. (2008) |
| 17    | Fe           | 1   | qFe1  | RM259-RM243 | RILs | RM259-RM243 | Zhenshan97/Minghui 63 | Kaiyang et al. (2008) |
| 18    | Mic-Fe       | 2   | qFe2-1 | RM641 | ILs | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 19    | PC           | 2   | qPC-2 | RM5897-RM6247 | RILs | Chuan7/Nanyanghan | Lou et al. (2009) |
| 20    | MIC-Cu       | 2   | qCu2  | RM6378 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 21    | MAC-Sr       | 2   | qSr2  | RM3688 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 22    | Fe           | 2   | qFe2  | RM324-RM301 | RILs | RM324-RM301 | Zhenshan97/ Nanyangzhan | Wang et al. (2008) |
| 23    | AAC-His      | 2   | qAA.2 | RM322-RM321 | RILs | RM322-RM321 | Zhenshan97/ Nanyangzhan | Wang et al. (2008) |
| 24    | AAC-Val/Ile/Leu/His/Phe | 2   | qAA.2 | RM5619-RM1211 | Dhs | RM5619-RM1211 | Cheongcheong/ Nagdong | Yun et al. (2014) |
| 25    | PC           | 2   | qLip-2 | RM5619-RM1211 | Dhs | RM5619-RM1211 | Cheongcheong/ Nagdong | Lee et al. (2014) |
| 26    | PC           | 2   | qPro-2 | RM5897-RM6247 | RILs | Chuan7/Nanyanghan | Lou et al. (2009) |
| 27    | MIC-Mn       | 2   | qMn2-1 | RM6378 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 28    | MIC-Mn       | 2   | qMn2-1 | RM6378 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 29    | MAC-Sr       | 2   | qSr2  | RM2666 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 30    | MAC-Ca       | 2   | qCa3  | RM5266-RM16 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 31    | MAC-Rb       | 3   | qRb3  | RM489 | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 32    | AAC-Tyr      | 3   | qAA.3 | RM520-RM468 | RILs | RM520 | Zhenshan97/ Nanyangzhan | Wang et al. (2008) |
| 33    | MAC-Mg       | 3   | qMg3-1 | RM5488 | ILs | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 34    | Ca           | 3   | qCa3  | RM200-RM227 | RILs | Zhenshan97/Minghui 63 | Kaiyang et al. (2008) |
| 35    | PC           | 3   | qPC-3 | RM251-RM282 | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| 36    | Zn           | 3   | qZn3.1 | RM7-RM517 | RILs | Madhukar × Swarna | Anuradha et al. (2012) |
| 37    | PC           | 3   | qPC-3 | RM251-RM282 | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| S. no | Grain traits | Chr | QTLs | Markers | Type | Peak marker | Populations | References |
|-------|--------------|-----|------|---------|------|-------------|-------------|------------|
| 38    | Mn           | 3   | qMn.3| RM227-R1925 | RILs | Zhenshan 97/Minghui | Kaiyang et al. (2008) |
| 39    | Cu           | 3   | qCu.1| R1925-RM148  | RILs | R1925-RM148  | Wang et al. (2008) |
| 40    | AAC-Thr/Gly/His/Arg | 4   | qAA.4| RM348-RM131   | RILs | Zhenshan/ Nanyangzhan | Kepiro et al. (2008) |
| 41    | CPB          | 4   | qcpb4| E12M61.256   | RILs | Cypress/Panda | Kepiro et al. (2008) |
| 42    | CPH          | 4   | qcpb4| E12M61.256   | RILs | Zhenshan 97/Minghui | Kaiyang et al. (2008) |
| 43    | Cu           | 5   | qCu.5| C1447-RM31   | RILs | Zhenshan 97/Minghui | Kaiyang et al. (2008) |
| 44    | PA           | 5   | qPA.5| RM305-RM178  | DHs  | IR64/Azucena | James et al. (2007) |
| 45    | FC           | 5   | qFC.5| RG480-RM274  | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| 46    | Fe           | 5   | qFe.5| RM574-RM122  | RILs | Madhukar/ Swarna | Anuradha et al. (2012) |
| 47    | CPB          | 4   | qcpb4| E12M61.256   | RILs | Zhenshan 97/Minghui | Kaiyang et al. (2008) |
| 48    | MAC-Ca       | 5   | qCa.6| RM598        | ILs  | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 49    | LC           | 6   | qLp.6| RM586-RM1163 | DHs  | Cheongcheong/ Nangdong | Yun et al. (2014) |
| 50    | PC           | 6   | qPC.6| RM190-RZ516  | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| 51    | FC           | 6   | qPC.6| RM190-RZ516  | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| 52    | MIC-Cu       | 6   | qCu.6| RM204        | ILs  | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 53    | Zn           | 6   | qZn.6| RZ398-RM204  | RILs | Zhenshan 97/Minghui | Kaiyang et al. (2008) |
| 54    | PC           | 6   | qPC.6| RM190-RZ516  | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| 55    | MAC-Mg       | 6   | qMg.6| OSR 21       | LT/TL-RILs | Xieqingzao B/Milyang | Zhang et al. (2014) |
| 56    | PC           | 7   | qPC.7| RM270-C751   | DHs  | Yuefu/IRAT109 | Yongmei et al. (2007) |
| 57    | MIC-Mn       | 7   | qMn.7| RM214        | LT/TL-RILs | Leont/TeQing | Zhang et al. (2014) |
| 58    | AAC-Pro/Gly/Met/Arg | 7   | qAA.7| RM125-RM214  | RILs | Zhenshan 97/Minghui | Wang et al. (2008) |
| 59    | Zn           | 7   | qZn.73| RM501-OsZip2 | RILs | Madhukar/ Swarna | Anuradha et al. (2012) |
| 60    | Fe           | 7   | qFe.71| RM234-RM248  | RILs | Zhenshan 97/Minghui | Zhong et al. (2011) |
| 61    | MAC-P        | 7   | qPC.1| R1245-RM234  | RILs | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 62    | PC           | 7   | qPr.7| RM445-RM418  | RILs | Zhenshan 97/B/Delong 208 | Zhong et al. (2011) |
| 63    | MIC-Zn       | 8   | qZn8-1| RM152        | ILs  | O. rufipogon/ Teqing | Garcia-Oliveira et al. (2008) |
| 64    | AAC-Tyr      | 8   | qAA.8| RM137-RM556  | RILs | Zhenshan 97/Minghui | Wang et al. (2008) |
| 65    | AAC-Cys      | 8   | qAA.8| RM447-RM458  | RILs | Zhenshan 97/Minghui | Wang et al. (2008) |
| 66    | MAC-K        | 8   | qK8.1| RM3572       | ILs  | Zhenshan 97/Nanyangzhan | Anuradha et al. (2012) |
| 67    | Zn           | 8   | qZn8 | RM25-R1629   | RILs | Zhenshan 97/Minghui | Kaiyang et al. (2008) |
| 68    | Cu           | 8   | qCu.8| RM201-C472   | RILs | Zhenshan 97/B/Delong 208 | Zhong et al. (2011) |
| 69    | Fe           | 8   | qFe.8| RM137-RM325A | DHs  | IR64/Azucena | James et al. (2007) |
| 70    | AAC          | 8   | qAA.9| RM328-RM1107 | RILs | Zhenshan 97/B/Delong 208 | Zhong et al. (2011) |
| 71    | MAC-P        | 9   | qP9.1| RM201        | ILs  | O. rufipogon/Teqing | Garcia-Oliveira et al. (2008) |
| 72    | MAC-Mg       | 9   | qMg.10| RM467        | LT-RILs | Lemont/TeQing | Zhang et al. (2014) |
| 73    | AAC-Cys/Leu/Ile/Phe | 10  | qAA.10| RM467-RM271  | RILs | Zhenshan 97/Nanyangzhan | Wang et al. (2008) |
Table 2 continued

| S. no | Grain traits | Chr | QTLs | Markers | Type | Peak marker | Populations | References |
|-------|--------------|-----|------|---------|------|-------------|-------------|------------|
| 75    | PC           | 10  | qPC-10 | RM184-RM3229B | RILs | Xieqingzao B/Milyang | Yu et al. (2009) |
| 76    | PC           | 10  | qPro-10 | RM24934-RM25128 | DHs | RM24934 | Cheongcheong-Nagdong | Yun et al. (2014) |
| 77    | MAC-Mg       | 11  | qMg.11 | RM332 | LT/TL-RILs | Lembert/TeQing | Zhang et al. (2014) |
| 78    | MIC-Cu       | 11  | qCu.11 | RM167 | LT-RILs | Samgang and Nagdong | Qin et al. (2009) |
| 79    | PC           | 11  | qPCI.11 | 1027-RM287 | DHs | RM287 | Samgang and Nagdong | Kaiyang et al. (2008) |
| 80    | Fe           | 11  | qFe.11 | RZ536-TEL3 | RILs | Zhen 97/Minghui 63 | Bres-Patry et al. (2001) |
| 81    | PC           | 11  | qPCI.11 | RM287-RM26755 | DHs | RM287 | IR64/Azucena | James et al. (2007) |
| 82    | PA           | 12  | qPA12 | RM247-RM179 | DHs | | |
| 83    | Fe           | 11  | qFe.12 | RM270-RM17 | DHs | | |
| 84    | Zn           | 12  | qZn.12 | RM235-RM17 | DHs | | |
| 85    | Fe           | 12  | qFe.12 | RM260-RM7102 | RILs | Madhukar/Swarna | Anuradha et al. (2012) |
| 86    | Fe           | 12  | qFe.12 | RM17-RM260 | RILs | | |
| 87    | Zn           | 12  | qZn.12 | RM260-RM7102 | RILs | | |

## 2001; Wissuwa and Ae 2001a, b) and Mn on chromosome 10 (Wang et al. 2002) were reported. Lu et al. (2008) observed 10 QTLs for Ca, Fe, Mn, and Zn accumulation in rice grains on seven chromosomes. Zhang et al. (2014) reported 134 QTLs for 16 elements in unmilled rice grain and among them, six were considered strongly associated and validated.

### QTLs for phenolic compounds in rice grain

The Rc locus regulates pigmentation of the rice bran layer, and selection for the rc allele (white pericarp) occurred during domestication of the crop. Two loci, Rc and Rd were found to be responsible for the formation of pericarp colour (Sweeney et al. 2006; Furukawa et al. 2007). Rc produces brown pericarp and seed coat, with Rd it develops red pericarp and seed coat, while Rc alone has no phenotype. Rc encodes a regulatory protein (Basic Helix-Loop-Helix Protein) that allows the accumulation of proanthocyanidins (Sweeney et al. 2006), while Rd encodes the enzyme DFR (dehydroflavonolreductase), which is involved in anthocyanin and proanthocyanidins pathway (Furukawa et al. 2007). Consequently, wild-type allele (Rc), the domestication allele (rc) and a mutant allele (Rc-s) were cloned and sequenced. The allele rc was found to be null with 14-bp deletion, responsible for frame shift mutation and a premature stop codon (Brooks et al. 2008). Through classical genetic approaches, Yoshimura et al. (1997) identified two loci, Pb (Prp-b) and Pp (Prp-a), located on chromosomes 4 and 1, respectively for the pericarp pigmentation with anthocyanin of black rice. Further, Wang and Shu (2007) mapped Pb gene responsible for purple pericarp on chromosome 4 and suggested that, the gene Pb may be a mutant of gene Ra caused by a two bases deletion (GT) within exon 7 of the Ra.

## Associated QTLs for phytic acid

In rice, phytic acid (PA) is a major source of P for support of seedling growth on P-deficient soil and important role of anti-nutritional factor. Liu et al. (2005) reported the amount of PA and protein content (PC) in 24 cultivars of rice and found to be no significant correlation between them. Among the cultivars, PA content ranged from 0.68% for Xiu217 to 1.03% for Huai9746, with a mean of 0.873%, and PC ranged between 6.45% for Xiu52 and 11.10% for K45, with a mean of 8.26%. The molecular mechanism and genetic trait of phytate accumulation in rice grain is necessary to understand for designing a breeding program. James et al. (2007) identified two QTLs for phytate concentration on chromosomes 5 and 12 with LOD score of 5.6 and 3.5 explaining 24.3 and 15.4% of PV, respectively. In addition, they reported significant positive correlation of phytate with inorganic P and total P (R = 0.99), indicating that the majority of P in grain was stored in the form of phytate.
Achievements through transgenic approaches to enhance nutritional values

Genetic engineering, an alternative approach to enhance nutritional values, has been considered to be the potential tool for the sustainable and efficient strategy for increasing the nutritional quality traits in target area of plants (Uzogara 2000; Lucca et al. 2001; Zimmerman and Hurrel 2002; Dias and Ortiz 2012). The world population would likely to reach 8 billion by 2030. Therefore, the problem of malnutrition would further exaggerated to 93% (Khush 2005, 2008). Numerous evidences are piling up showing significant increase of bioavailable content in rice grains by transfer of biofortification genes through biolistic and Agrobacterium-mediated transformation method (Table 3). Through the transgenic approaches, Goto et al. (1999) first observed 3-fold enhancement of Fe in the starchy endosperm of rice by transferring the ferritin gene of soybean. Similarly, in 2001 Lucca et al. introduced ferritin gene from common bean into rice showed 2-fold concentration of Fe in seeds as compared to controls. Vasconcelos et al. (2003) transferred soybean ferritin gene into rice and observed 3-fold increase of Fe in milled rice and 2-fold in rough rice. Similarly, Khalekuzzaman et al. (2006) observed increase in Fe T1 brown rice. Similarly, Lucca et al. (2001) introduced ferritin gene of soybean. Khalekuzzaman et al. (2006) observed increase in Fe T1 brown rice. Similarly, in 2001 Lucca et al. introduced ferritin gene from common bean into rice showed 2-fold concentration of Fe in seeds as compared to controls. Vasconcelos et al. (2003) transferred soybean ferritin gene into rice and observed 3-fold increase of Fe in milled rice and 2-fold in rough rice. Similarly, Khalekuzzaman et al. (2006) observed increase in Fe T1 brown seeds and T2 Polish rice seeds compared to control. Thus, the Fe content increased more than 2-fold in transgenic lines. Subsequently many researchers have attempted to increase Fe content in rice endosperm by over expressing genes involved in Fe uptake from the soil and translocation from root, shoot, flag leaf to grains, and by increasing the efficiency of Fe storage proteins (Kobayashi and Nishizawa 2012; Lee et al. 2012; Bashir et al. 2013; Masuda et al. 2013; Slamet-Loedin et al. 2015). Several studies exhibited the associated increase in Fe and Zn content in rice grain obtained by over expression or activation of the Nicotianamine Synthase (NAS) genes or influenced with other transporters genes (Table 3). Masuda et al. (2009) transferred NAS gene of Hordeum vulgare to rice observed significant enhancement of the target trait, which accumulated 2- to 3-fold higher iron and zinc in polished rice grain. Zheng et al. (2010) observed 5-fold iron accumulation in polished rice grain through the over expression of endosperm specific endogenous NAS gene. Through the higher expression of three rice NAS homologous proteins, (OsNAS1, OsNAS2, and OsNAS3), Johnson et al. (2011) observed 2-fold increase in Fe and Zn concentration in polished rice (Table 4). Similarly, Lee et al. (2009) observed transfer of NAS gene (OsNAS3-D1) increases the expression of Fe (2.9-fold), Zn (2.2-fold), and Cu (1.7-fold) compared to wild type grain at seedling stage. Soumitra et al. (2012) observed 7.8-fold increase of Fe content in a line 276-1-2 and six lines showed a 4.1 to 4.5-fold increment over control by over expression of ferritin gene. Masuda et al. (2013) introduced multiple genes viz., OsSLT1 promoter-driven OsYSL2, ferritin gene under the control of endosperm-specific promoter, barley IDS3 genome fragment and NAS over expression and observed significant increase in 1.4-fold, 2-fold, 6-fold, 3-fold of Fe concentration respectively as compared to polished rice seeds. These results suggest that, targeting multiple genes would be more successful in enhancing nutritional values of rice.

Rice lacks the ability to produce β-carotene, the precursor of Vitamin A. Ye et al. (2000) developed golden rice that yields 1.6–2.0 μg g⁻¹ of β-carotene of dry rice which is very beneficial for retina (Vitamin A) to create visual pigment and ultimately leads decreasing of night blindness and particularly useful for people in developing countries. It was possible by introgression of major four genes phytoene synthase, phytoene desaturase, β-carotene desaturase, and lycopene β-cyclase into rice.

Advanced genomic technologies

The ever-increasing demand for rice production with higher quality drives to the identification of superior and novel rice cultivars. To meet these challenges, plant breeders and biotechnologist together has to explore efficient breeding strategies that integrate genomic technologies by using available germplasm resources to a new revolution in the field of plant breeding for better understanding of genotype and its relationship with the phenotype, in particular for complex traits. Genomic approaches are particularly useful when working with complex traits having multigenic and environmental effects. In this new plant breeding era, genomics will be an essential aspect to develop more efficient nutritional rich rice cultivars for reducing human health problems relating to mineral nutrition (Perez-de-Castro et al. 2012).

Sequenced rice genome has provided new technologies and tools in functional genomics, transcriptomics and proteomics of important agronomic traits in rice. At present, trends in molecular biology are fully updated. Therefore, by availing the different molecular approaches as, whole genome sequencing of 3000 rice accessions (Li et al. 2014), Genome-wide association mapping (Huang et al. 2010; Zhao et al. 2011; Varshney et al. 2014; McCouch et al. 2016; Yano et al. 2016; Wang et al. 2016; Edzesi et al. 2016; Biscarini et al. 2016; Si et al. 2016); Whole Genome SNP Array (Hu et al. 2013; Yu et al. 2014; Singh et al. 2015; Malik et al. 2016), Genomic-based genotyping platforms and re-sequencing (Gao et al. 2013; Han and Huang 2013; Chen et al. 2013; Barabaschi et al. 2016; Guo et al. 2014; Xu and Bai 2015), Genome-guided RNA-seq (Loraine et al. 2013; Szczesniak et al. 2013; Biselli et al. 2015; Peng et al. 2016; Badoni et al. 2016),
Map-based cloning approach (Salvi and Tuberosa 2005; Price 2006; Shomura et al. 2008; Zhang et al. 2013), Transcriptome profiling (Mochida and Shinozaki 2010; Chandel et al. 2011; Venu et al. 2011), Genomics approaches (Mochida and Shinozaki 2010; Swamy and Kumar 2013; Varshney et al. 2014; Spindel et al. 2015; Okazaki and Saito 2016) Sequencing-By-Synthesis (SBS) (Venu et al. 2011; Sun et al. 2015), Next generation sequencing (NGS) technologies (Uchida et al. 2011; Miyao et al. 2012; James et al. 2013; Guo et al. 2014; Wang et al. 2016; Matsunoto et al. 2016) and etc. could be strategically exploited to understand molecular mechanism and their relation between the genotypes and phenotypic traits.

In 2011, Zhao et al. genotyped 413 diverse accessions of *O. sativa* with 44,100 SNP and phenotyped them for 34 traits including grain quality parameters. Deep transcriptional analysis by MPSS and SBS brought out several differentially expressed genes that affect milling yield and eating quality trait in rice (Venu et al. 2011). The genes that expressed were identified to be involved in biosynthesis of starch, aspartate amino acid metabolism, seed maturation and storage proteins.

Peng et al. (2016) developed a stable variant line (YVB) having greatly improved grain quality traits using restriction-site associated DNA sequencing technology (RADseq) from a BC1F5 backcross population derived

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### Table 3 Incorporation of various nutritional genes into rice cultivars through genetic engineering approaches

| S. no | Nutrient | Gene | Increases to fold expression (compare to wild type/ non-transformed) | References |
|-------|----------|------|------------------------------------------------------------------|------------|
| 1     | Vit A    | Npy51, Euq1l | 1.6-fold | Ye et al. (2000) |
| 2     | Fe       | Osnas2 | 4.2-fold | Johnson et al. (2011) |
| 3     | Zn       | Activation tagging of Osnas1 | 4 to 6.3-fold | Wirth et al. (2009) |
| 4     | Fe       | Activation tagging of Osnas2 | 2.9-fold | Lee et al. (2009) |
| 5     | Fe and Zn| Activation tagging of Osnas2 | 2.9-fold | Johnson et al. (2011) |
| 6     | Fe and Zn| Gm ferritin, Afpypholate, and Osnas2 | 1.6-fold | Wirth et al. (2009) |
| 7     | Fe and Zn| Gm ferritin, Afpypholate, and Osnas1 | 4.4-fold Fe | Vasconcelos et al. (2003) |
| 8     | Fe       | Ferritin gene | 2.0-fold Fe and 3.0-fold Zn | Masuda et al. (2009) |
| 9     | Fe       | SoxferH1 | 4.2-fold | Johnson et al. (2011) |
| 10    | Fe and Zn| SoxferH1 | 4.2-fold Fe and 1.1-fold Zn | Lee et al. (2009) |
| 11    | Fe       | Pyferritin, rgyMT | 2.0-fold | Lucca et al. (2002) |
| 12    | Fe and Zn| OsFRO2 | 2.8-fold Fe and 1.4-fold Zn | Ogo et al. (2011) |
| 13    | Fe and Zn| OsYSL15 | 1.1-fold Fe and 1.0-fold Zn | Lee et al. (2009) |
| 14    | Fe and Zn| HvNAS1, HvNAS1, HvNAAT, and IDS3 | 1.2-fold Fe and 1.4-fold Zn | Suzuki et al. (2008) |
| 15    | Fe and Zn| OsNAS1 | 1.0-fold Fe and 1.3-fold Zn | Zheng et al. (2010) |
| 16    | Fe and Zn| SoxferH1 | 2.5-fold Fe and 1.5-fold Zn | Paul et al. (2014) |
| 17    | Fe and Zn| OsNAS2 | 3.0-fold Fe and 2.7-fold Zn | Lee et al. (2012) |
| 18    | Fe and Zn| HvNAS1 | 2.5-fold Fe and 1.5-fold Zn | Higuchi et al. (2001) |
| 19    | Fe       | OysL2 | 4.4-fold Fe | Ishimaru et al. (2010) |
| 20    | Fe and Zn| AtNAS1, Pyferritin, and Apfylpatase | 6.3-fold Fe and 1.6-fold Zn | Wirth et al. (2009) |
| 21    | Fe and Zn| SoxferH2, HvNAS1, and OsYSL2 | 3.4-fold Fe and 1.3-fold Zn | Aung et al. (2013) |
| 22    | Fe and Zn| SoxferH2, HvNAS1, HvNAAT-A, -B and IDS3 genome fragments | 2.5-fold Fe and 1.4-fold Zn | Masuda et al. (2013) |
| 23    | Zn, Cu, and Ni| OsNAS3 | 2.1, 1.5, and 1.3-fold | Lee et al. (2009) |
| 24    | Fe and Zn| OsNAS3-D1 | 1.7-fold Fe in shoots, 1.6-fold in Fe roots and 2.0-fold Zn in shoots, 1.6-fold Zn in roots | Lee et al. (2009) |
| 25    | Fe       | Ferritin gene | 2.0-fold Fe | Khalekuzzaman et al. (2006) |
| 26    | Fe and Zn| Osfer2 | 2.09-fold Fe and 1.37-fold zinc | Soumitra et al. (2012) |
from an *indica* hybrid rice maintainer line V20B and YVB line. The YVB is a stable variant line derived from V20B by transferring the genomic DNA of *O. minuta* into V20B using SIM method (Zhao et al. 2005). The deep re-sequencing of genomes of both the parents V20B and YVB showed read coverage of 89.04 and 93.13% and depth of sequencing 41.26-fold and 87.54-fold respectively. A total of 322,656 homologous SNPs were identified between V20B and YVB. A total of 17 QTLs for rice grain quality were detected on chromosomes 3, 5, 6, 8, and 9 through genetic map analysis with PV ranging from 5.67 to 35.07%. Invention of SIM technology enabling introduction of exogenous DNA helped in creating a large number of new rice germplasm accessions and the variants were analyzed using molecular markers (Pena et al. 1987; Zhao et al. 2005).

**Conclusion**

The nutritional value enrichment of rice grain is very much essential to reduce malnutrition of developing countries in the post green revolution era. The current gain in knowledge on the nutritional value related genes and QTLs will help into develop desired genotypes for the humankind. The availability of gene based markers and advanced tool will assist breeders to accumulate specific alleles of genes known to play a role in nutritional grain quality traits in rice. In recent years, significant achievement has been made in genetic studies on grain protein and amino acid content, vitamins and minerals, glycemic index value, phenolic and flavinoid compounds, phytic acid, zinc and iron content along with QTLs linked to these traits but needs more research for processing and curative properties. Recent release of high protein and zinc rich rice varieties in India gives the positive note on progressive move in crop improvement program in rice. The transgenic approach will further strengthen to enrich grain nutrition to desired level rapidly. The recent development of genomic technologies may augment for improving the nutritional quality in rice when it goes hand in hand with breeding program.

**Abbreviations**

GI: glycemic index; PA: phytic acid; MIC: micro-elements; MAC: macro-elements; PC: protein content; EAA: essential amino acids; RBP: rice bran protein; RAG: rapidly available glucose; AC: amylose content; GAE: galleic acid equivalent; TPC: total phenolic content; AA: amino acid; DH: double haploid; PV: phenotypic variance; RIL: recombinant inbred lines; SNP: single nucleotide polymorphism; SSR: simple sequence repeats; STS: sequence tagged site.

**Authors’ contributions**

AM and AA conceived the idea and carried out the literature survey. SKP and EP helped in interpretation and drafting of manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

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**Table 4 Utilization of micronutrient traits related genes in rice for the improvement**

| S. no | Gene            | Functions                                      | References                        |
|------|-----------------|------------------------------------------------|-----------------------------------|
| 1    | OsZIP1          | Vascular bundles, Epidermis and mesophyll cells | Lee et al. (2010), Ishimaru et al. (2011) |
| 2    | OsZIP3          | Vascular bundles, Epidermal cells in stem      | Ishimaru et al. (2011)           |
| 3    | OsZIP4          | Meristem, Vascular bundles, Epidermis and mesophyll cells | Lee et al. (2010), Ishimaru et al. (2011) |
| 4    | OsNAS3          | Vascular bundles, Epidermis                    | Lee et al. (2010), Ishimaru et al. (2011) |
| 5    | OsYSL15         | Fe transporters                                | Masuda et al. (2013)             |
| 6    | OsYSL2, OsNAAT1 and OsNAC | High grain Zn content                           | Chandel et al. (2011)           |
| 7    | OsNAC, OsZIP8a, OsZIP8c and OsZIP4 | grain zinc content | Gande et al. (2014) |
| 8    | OsZIP8          | Leaf blade, root, stem, anther, ovary and embryo | Bashir et al. (2012)            |
| 9    | OsNRAMP7        | High grain Zn content                          | Chandel et al. (2011)           |
| 10   | OsNRAMP75       | Mid grain filling stage                         |                                   |
| 11   | OsNAAT1         | High grain zn content                           | Chandel et al. (2011)           |
| 12   | OsVIT1          | High grain zn content                           | Chandel et al. (2011)           |
| 13   | OsAAP6          | grain protein content and nutritional quality   | Peng et al. (2014)              |
| 14   | Osfer2          | Increases of iron content in grain              | Paul et al. (2012)              |
| 15   | MOT1 (molybdenum transporter 1) | grain molybdenum concentration                   | Norton et al. (2014)            |
| 16   | COPT1 and COPT2 (copper transport) | grain copper concentration                       | Norton et al. (2014)            |
| 17   | Lsi1 (arsenic transport) | inter and extra cellular transporters of arsenic | Ma et al. (2008), Norton et al. (2014) |

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