# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**
Open access books available

**122,000**
International authors and editors

**135M**
Downloads

Our authors are among the

**154**
Countries delivered to

**TOP 1%**
most cited scientists

**12.2%**
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

## Interested in publishing with us?
## Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

# Breeding for Biofortification Traits in Rice: Means to Eradicate Hidden Hunger

*Vinay Sharma, Dinesh Kumar Saini, Ashish Kumar, Hari Kesh and Prashant Kaushik*

## Abstract

Rice (*Oryza sativa* L.) supplies nourishment to about half of the population of the world's inhabitants. Of them, more than 2 billion people suffer from 'hidden hunger' in which they are unable to meet the recommended nutrients or micronutrients from their daily dietary intake. Biofortification refers to developing micronutrient-rich diet foods using traditional breeding methods and modern biotechnology, a promising approach to nutrition enrichment as part of an integrated strategy for food systems. To improve the profile of rice grain for the biofortification-related traits, understanding the genetics of important biofortification traits is required. Moreover, these attributes are quantitative in nature and are influenced by several genes and environmental variables. In the course of past decades, several endeavours such as finding the important quantitative trait loci (QTLs) for improving the nutrient profile of rice seeds were successfully undertaken. In this review, we have presented the information regarding the QTLs identified for the biofortification traits in the rice.

**Keywords:** QTLs, biofortification, malnutrition, hidden hunger, marker-assisted breeding

## 1. Introduction

Rice (*Oryza sativa* L.) provides energy and nutrition to almost half of the world's population [1]. In most developing countries, especially in Asia, rice is consumed in significant quantities and is the main component diet. In the present scenario, high-yielding rice varieties are low in mineral elements. Milled or polished rice is not a significant source of any major mineral elements, and therefore, it cannot meet up with the recommended daily dietary intake for mineral elements. Moreover, around 792.5 million people across the world are malnourished, out of which 780 million people live in developing countries [2]. Thus, most rice-eating, resource-poor people in Southeast Asia, Africa, and Latin America suffer from chronic micronutrient malnutrition, often referred to as hidden hunger [3]. Protein-energy malnutrition affects 25% of children those with the dietary intake of predominantly rice, and staple crops have low levels of an essential amino acid [4]. Further, rice has relatively low (8.5%) protein content as compared to other cereals such as wheat, barley, and millets. Moreover, the average protein content in milled rice is around 7%. However, the total seed protein content of rice consists of 60–80% glutelin and

20–30% prolamin [5]. Interestingly, rice supplies about 40% of the total protein requirement of humans in developing countries [6].

Phytate is a crucial mineral storage compound in seed, with a mixed cation salt of phytic acid accounting for approximately 75% of total seed phosphorus content [7]. The significant portion of the phosphorus taken from the soil by plants is ultimately translocated to the seed and further synthesised into phytic acid. Phytate is vital for the development of seeds and also as an antioxidant, anticancer agent, lowering chronic disease rates, and preventing coronary disease [8]. Phytic acid is known as an anti-nutritional factor because it forms complexes in seeds with proteins and essential minerals such as Fe, Zn, and Ca [9] and leads to the impairment of the bioavailability of the same.

Mineral elements are critical for several cellular and metabolic activities [10]. Biofortification of staple crops provides a sustainable methodology to triumph over the mineral deficiency. Attempts were made for the development, release, and distribution of biofortified crops with the help of agronomic practices and biotechnological techniques and also by using plant breeding methods. Various old rice varieties with high grain iron and zinc content were screened, and breeding methods with improved agronomic characteristics combined the higher mineral characteristics. In 2013, the Bangladesh Rice Research Institute released zinc-enriched rice varieties (BRRIdhan 62, BRRIdhan 72, and BRRIdhan 64), claiming to contain 20–22 ppm of zinc in brown rice. An improved line (IR68144-3B-2-2-3) has been identified in India and Philippines in a cross between a high-yielding variety (IR72) and a large, traditional variety (ZawaBonday) with a top grain iron concentration about 21 ppm in brown rice [11].

Similarly, Jalmagna, a traditional variety with almost double the iron and zinc concentration of common rice variety, has been identified for further breeding programs to improve iron and zinc concentration by nearly 40 percent more than that of conventional rice variety [11]. ICAR-Indian Institute of Rice Research, Hyderabad, Telangana, developed biofortified pure line variety, DRR Dhan 45. It possesses high zinc (22.6 ppm) in polished grain. It has been released and notified in 2016 for Karnataka, Tamil Nadu, Andhra Pradesh, and Telangana. Its average grain yield is 50.0 q/ha. It matures in 125–130 days [12, 13]. Another pure line variety DRR Dhan 49 with high zinc (25.2 ppm) in polished grain is released and notified in 2018 for Gujarat, Maharashtra, and Kerala. Its average grain yield is 50.0 q/ha and matures in 125–130 days [13].

Mineral element accumulation in the grain is a complex process and is highly influenced by environmental factors. This resulted in less effective early-generation phenotypic selections for mineral grain elements and slowed progress in the breeding of biofortified rice varieties [14]. In-depth understanding of the genetic basis of mineral elements at the molecular level and the identification of significant effects of QTLs can help to speed up the development of biofortified rice varieties through marker-assisted breeding [15]. Rice is a model for cereal crops. Vast genomic resources are available, including genome-wide single nucleotide polymorphic (SNP) molecular markers and various advanced genomic platforms, to enable complex traits to be dissected at the molecular level [16]. Several studies to chart QTLs for biofortified traits include the use of introgression lines (ILs) [17] and double haploids (DHs) to uncover QTLs [18]. However, the stability of released genotypes is an important consideration to hope for a meticulous performance of released genotypes for stable produce for the farmers [19, 20]. Hence, molecular breeding approach for biofortification of crop offers a sustainable and long-term solution. Also, biofortified crops with increased bioavailability of essential protein, vitamins, and micronutrients are deployed to consumers through traditional farming and food trading practices, thus providing a feasible way to reach undernourished and low-income families with limited access to various diets, supplements, and fortified foods [21]. The common processes involved in the development of the biofortified rice variety (**Figure 1**).


**Figure 1.**
*Summary of the process involved in the biofortification of the rice.*

## 2. Protein content in rice

Grain protein content (GPC) in rice is one of the major factors which decides the nutritional value of rice food and influences the palatability of cooked rice [22]. Rice's seed protein content consists of 60–80% glutelin and 20–30% prolamin, regulated by 15 and 34 genes, respectively [5]. It supplies about 40% of the protein to humans through diet in developing nations, and rice GPC quality is high, owing to lysine richness (3.8%) [6]. Improving GPC in rice grain is, therefore, a significant goal for plant breeders and biotechnologists. More than 20 QTL mapping studies have been conducted in the last two decades to explore the genetic base of the protein content in rice. Moreover, to our knowledge, more than 80 stable and consistent QTLs for GPC have been identified and mapped on all 12 chromosomes of rice, although most of them were mapped on chromosomes 1, 2, 6, 7, 10, and 11 (**Table 1**). For the first time, Tan et al. [28] mapped two QTLs, one in the interval of markers C952-Wx on chromosome 6, with the phenotypic variance explain (PVE) 13.0%, and the other one in the interval markers R1245-RM234 on chromosome 7 with PVE 6.0%. In another study, Aluko et al. [29] identified and mapped four QTLs among 312 DH lines derived from the $BC_3F_1$ of an interspecific cross of *O. sativa* × *O. glaberrima* explaining the phenotypic variance of 4.8–15.0%. Among the four QTLs, one QTL, pro6, was closely associated with Wx gene influencing rice quality. Thereafter, several studies have been conducted to map the QTLs regulating GPC in rice [26, 40–43].

Zheng et al. [39] employed unconditional and conditional QTL mapping methods to analyse the developmental behaviour of protein content and protein

| Cross | Population type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/ chromosome arms | Marker intervals/nearest markers for major QTL (PVE) | References |
|---|---|---|---|---|---|---|
| **Amino acid content** | | | | | | |
| Indica rice (Zhenshan 97) × Indica rice (Nanyangzhan) | RILs (190) | 2 QTL clusters | 4.05–33.3 | 1, 7 | RM472-RM104 (Asp/Thr/Gly/Ala/Tyr/Pro/Lys/Ser/Glu/ Asp/Val/Met/Ile/Leu/Phe/His/Arg/Cys) (5.7–33.3) | [23] |
| Indica rice (Zhenshan 97) × Indica rice (Minghui 63) | RILs (241) | 10 (His) + 8 (Arg) | 12–35 (His); 16–33 (Arg) | 1, 2, 3, 6, 7, 10, 11, 12 (His); 2, 3, 5, 6, 7, 10, 11, 12 | R321–RM55 (12), RZ398–RM204 (12), RG101–G393 (15), C1003B–RG103 (15), RG118–C794 (20), RM53–RZ599 (22), RM258–RG561 (22), RG424–R2549 (23), RG528–RG128 (24), RM20b–C732 (35) [His]; C734b–RZ649 (16), R321–RM55 (18), RG424–R2549 (21),RM258–RG561 (21), R3203–RM20A (22), RM53–RZ599 (23), RG528–RG128 (23), RM20b–C732 (33) | [24] |
| Indica rice (Zhenshan 97) × Indica rice (Minghui 63) | RILs (241) | 12 | 3.4–48.8 | 1, 11 | R2632–C39 (Ser) (13.5), RG173–RM81A (Val) (14.5), RZ536–TEL3 (Met) (48.8) | [25] |
| Indica rice (Zhenshan 97B) × Indica rice (Delong 208) | RILs (188) | 3 QTL clusters | 4.2–31.7 | 1,7,9 | RM328–RM107 (Asp/Thr/Ser/Gly/Val/Ile/Phe/Lys/Taa) (13.2), MRG186–MRG4499(Asp/Thr/Ser/Glu/Gly/ Ala/ Cys/Val/Met/Ile/Phe/Arg/Pro/Taa) (14.4–27.5), RM493–RM562 (Asp/Thr/Glu/Gly/Ala/Val/Leu/Phe/ Arg/Pro/Taa) (24.2 31.7) | [26] |
| *O. sativa* (Dasanbyeo) × *O. sativa* (TR22183) | RILs (172) | 6 | 10.2–12.4 | 3 | id3015453-id3016090 (Ala-10.2, Phe-10.6, Iso-11.2, Val-12.4, Leu-12.4), id3001422 fd10 (Lys-10.8) | [27] |
| **Protein content** | | | | | | |
| Indica rice (Zhenshan 97) × Indica rice (Minghui 63) | RILs (238) | 2 | 6.0–13.0 | 6, 7 | C952-Wx (13) | [28] |
| Indica rice (Caiapo) × *Oryza glaberrima* (IRGC 103544) | DH lines (312) | 4 | 4.8–15.0 | 1, 2, 6, 11 | RM226–RM297 (15) | [29] |
| Indica rice (Gui630) × Japonica rice (02428) | DH lines (81) | 5 | 6.9–35.0 | 1, 4, 5, 6, 7 | C22-RG449d (16.5), ZG34B-G20 (22.5), RG435-RG172a (35.0) | [30] |

| Cross | Population type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/ chromosome arms | Marker intervals/nearest markers for major QTL (PVE) | References |
|---|---|---|---|---|---|---|
| *O. sativa* (V20A) × *O. glaberrima* (accession 103,544) | BC3(TC) F1 families (308) | 1 | 9.0–10.0 | 8 | | [31] |
| Japonica rice (Moritawase) × Japonica rice (Koshihikari) | RILs (92) | 3 | 2.3–16.3 | 2, 6, 9 | | [32] |
| Koshihikari/Indica rice (Kasalath)// Japonica rice (Koshihikari) | BILs (92) | 2 | 14.3–14.8 | 6, 10 | R1952 (14.3), R2447 (14.8) | [33] |
| Indica rice (Chuan) × Japonica rice (Nanyangzhan) | RILs (286) | 2 | 2.69–4.50 | 6, 7 | | [34] |
| Indica rice (Xieqingzao B) × Indica rice (Milyang 46) | RILs (209) | 5 | 3.9–19.3 | 3, 4, 5, 6, 10 | RM251–RM282 (10.5), RM190–RZ516 (19.3) | [35] |
| Indica rice (Zhenshan 97) × Indica rice (Minghui 63) | RILs (241) | 9 | 1.60–9.26 | 2, 3, 5, 6, 7, 10, 11, 12 | | [36] |
| Tongil variety (Samgang) × Japonica variety (Nagdong) | DH lines (120) | 3 | 6.92–22.98 | 1, 11 | RM287-RM26755 (21.21), 11,025-RM287 (22.98) | [37] |
| Japonica rice (Asominori) × Indica rice (IR24) | CSSLs (66) | 9 | 3.0–53.7 | 1, 2, 3, 6, 8, 11 | R1982 (10.4–14.2), XNpb113 (12.0–13.8), C1350 (23.6), G1149 (13.0–53.7) | [38] |
| Japonica rice (Asominori) × Indica rice (IR24) | RILs (71) | 10 | 8.53–23.70 | 1, 3, 4, 6, 7, 8, 9, 10, 12 | R265B-XNpb36 (10.50), C1003-C688 (12.67), XNpb212-G1318 (13.86), C606-XNpb238 (14.63), R1854-R2373 (15.65), XNpb24-C562 (17.60), XNpb338-C796 (19.59), R758-XNpb15 (19.74), XNpb268-R411 (23.70) | [39] |
| Indica rice (Zhenshan 97B) × Indica rice (Delong 208) | RILs (188) | 2 | 7.2–25.9 | 1, 7 | RM445–RM418 (25.9) | [26] |

| Cross | Population type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/ chromosome arms | Marker intervals/nearest markers for major QTL (PVE) | References |
|---|---|---|---|---|---|---|
| Koshihikari/Indica rice (Kasalath)// Japonica rice (Koshihikari) | BILs (182) | 4 | 6.26–12.11 | 2, 3, 7, 10 | R250-C746 (10.04), C16-C809 (11.07), C847-C596 (12.11) | [40] |
| Indica rice (Cheongcheong) × Indica rice (Nagdong) | DH lines (133) | 1 | 39–41 | 2 | RM12532–RM555 (39–41) | [41] |
| Japonica cultivar (CJ06) × Indica rice cultivar (TN1) | DH lines (116) | 1 | 12.3–15.8 | 10 | RM216-RM467 (12.3–15.8) | [42] |
| Indica rice (Cheongcheong) × Indica rice (Nagdong) | DH lines (133) | 3 | 39–40 | 8,9,10 | RM506-RM1235 (39), RM24934-RM25128 (40), RM219-RM23914 (40) | [43] |
| *O. sativa* (M201) × *O. sativa* (JY293) | RILs (234) | 5 $ | 6.74–13.50 | 1, 2, 3, 4 | RM423-RM6375 (11.72), GS3-SLAF13430 (13.50) | [44] |
| Japonica variety (Sasanishiki) × Indica variety (Habataki) | CSSLs (39) | 1# | 10.38–15.43 | 1 | RM7124 (10.38–15.43) | [45] |
| Indica rice (Cheongcheong) × Indica rice (Nagdong) | DH lines (120) | 1 | 14 | 7 | RM8261 (14) | [46] |
| Naveen/O. sativa (ARC 10075)//O. sativa (Naveen) | BC3F5 lines (200) | 3 | 6.70–17.35 | 1, 2, 7 | CSCWR_Os01g02590__61041 (13.85), CSCWR_ Os02g10740_65058 (6.70–17.35) | [47] |
| **Iron and Zinc** | | | | | | |
| Indica variety (IR64) × Japonica variety (Azucena) | DH lines (129) | GZn-2; GFe-3 | q | 1, 12; 2, 8, 12 | RM235–RM17 (12.8), RM34–RM237 (15) [GZn]; RM270–RM17 (13.8), RM53–RM300 (16.5), RM137–RM325A (18.3) [GFe] | [18] |
| Indica cultivar (Zhengshan 97) × Indica cultivar (Minghui 63) | RILs (241) | GZn-3; GFe-2 | 5.3–18.61; 11.11–25.81 | 5, 7, 11; 1, 9 | R3166-RG360 (12.34), C794-RG118 (18.61) [GZn]; C472-R2638 (11.11), RG236-C112 (25.81) [GFe] | [48] |

| Cross | Population type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/ chromosome arms | Marker intervals/nearest markers for major QTL (PVE) | References |
|---|---|---|---|---|---|---|
| *O. sativa* ssp. Indica (Teqing) × *O. rufipogon* Griff. | ILs (85) | GZn-2; GFe- 1 | 5–11; 7 | 5, 8; 2 | RM152 (11) [Zn] | [17] |
| Indica rice (Bala) × Japonica rice (Azucena) | RILs (79) | GZn-4; GFe- 4 | 11.2–14.8; 9.7–21.4 | 6, 7, 10; 1, 3, 4, 7 | G1082 (11.2), G20 (11.4), AB0601 (14.7), C223 (14.8) [GZn]; R1440 (15.5), C949 (16.2), R1618 (21.4) [GFe] | [49] |
| Indica cultivar (ZYQ8) x Japonica cultivar (JX17) | DH lines (127) | GZn-2 | 10.83–12.38 | 4, 6 | CT206-G177 (10.83), RZ516-G30 (12.38) [GZn] | [34] |
| Indica rice (Madhukar) × Indica rice (Swarna) | RILs (168) | GZn-6; GFe-7 | 29–35; 69–71 | 3, 7, 12; 1, 5, 7, 12 | RM501–OsZip2 (29), RM7–RM517 (31), RM260–RM7102 (34), RM234–RM248 (35), RM248–RM8007 (35), RM17–RM260 (35) [GZn]; RM243–RM488 (69), RM488–RM490 (69.2), RM574–RM122 (69.2), RM234–RM248 (69), RM248–RM8007 (69), RM17–RM260 (71), RM 260–RM7102 (71) [GFe] | [50] |
| Indica rice (PAU201) x Indica rice (Palman 579) | F2 (247) | GZn-3; GFe- 8 | 4.7–19.1; 2.4–26.8 | 2, 10; 2, 3, 7, 10, 12 | 8RM474–RM184 (19.1) [Zn]; RM491–RM519 (16.9), RM228–RM496 (18.1), RM53–RM521 (21.4), RM221–RM208 (26.8) | [51] |
| Indica cultivar (Ce258) x Japonica breeding line (IR75862) and Indica cultivar (ZGX1) x Japonica breeding line (IR75862) | BILs (200 and 201) | GZn-4; GFe-1 | 2–24.4; 10.2–18.3 | 3, 6, 7, 8; 6, 11 | RM293–RM85 (11.1–14.4), RM407–RM152 (11.2–18.0) [GZn]; RM3–RM340 (10.2–18.3) [GFe] | [44] |
| Indica cultivar (Swarna) X Japonica rice (Moroberekan) | RILs (60) | GFe-1 | 39 | 1 | RM490-RM5 (39) | [52] |
| *O. sativa* (XB) × *O. rufipogon* (accession of DWR) | BILs (202) | GZn-6; GFe-3 | 5.3–11.8; 6.1–28.2 | 3, 4, 6, 7, 10, 12; 3, 6, 9 | RG172-RM340 (11.8) [GZn]; RG123-RG172 (16.7), RG510-RZ251 (28.2) | [53] |
| *O. sativa* (Nipponbare)/*O. meridionalis* (W1627)//Nipponbare | BRILs (151) | GZn-4 | 15.0–21.9 | 2, 9, 10 | RM171-RM590 (15.0), RM573 (15.2), RM6 (17.6), RM24085-RM566 (21.9) | [54] |

| Cross | Population type and size | No. of total QTLs | PVE range (additive effect QTLs) | Chromosomes/ chromosome arms | Marker intervals/nearest markers for major QTL (PVE) | References |
|---|---|---|---|---|---|---|
| Indica cultivar (PSBRc82) x Korean rice (Joryeongbyeo) and PSBRc82 x Indica breeding line (IR69428) | DH lines (130 and 97) | GZn-8; GFe-1 | 7.5–22.8; 9.4 | 2, 3, 6, 8, 11, 12; 4 | 2,140,834–2,147,095 (10.3), 13,048,465–13,057,679 (12.3), 8,803,052–8,832,534 (14.3), 6,025,827–6,047,367 (15.3), 606,341- id6006214 (16.1), 2,110,566-id2009463 (17.3), 2,783,884–2,785,595 (20.3), 10,858,811-id11000778 (22.8) [GZn] | [55] |
| Indica cultivar (IR64) × Breeding line (IR69428) and Indica cultivar (BR29) × Breeding line (IR75862) | DH lines (111 and 146) | GZn-8 | 8.6–27.7 | 2, 3, 5, 7, 8, 9, 11 | wd9002310–9,831,169 (10.3), 5,645,339–5,648,872 (11.5), 2,048,774–2,054,640 (12.2), 3,538,410–3,548,096 (12.2), 7,062,019–7,089,136 (12.6), 5,027,770–5,077,125 (18.4), 10,907,196-id11001107 (27.7) [GZn] | [56] |
| Indica rice (PAU201) x Indica rice (Palman) | F4 Population (579) | GZn-1; GFe-5 | 25; 34.6–95.2 | 6; 5, 7, 9 | RM585-RM3 (25) [GZn]; RM2488-RM440 (64.1), RM440-RM31 (95.2), RM440-RM31 (95.2), RM432-RM429 (95.2), RM566-RM434 (36.6) [GFe] | [57] |

**Table 1.**
*List of QTLs identified for biofortification traits in rice.*

index in rice. At four stages of grain filling, viz. 7, 14, 21, and 28 DAF, they mapped 10 unconditional QTLs and 6 conditional QTLs, explaining 8.53–19.59% and 8.76–23.70% of PVE for GPC, respectively, and 11 unconditional QTLs and 9 conditional QTLs explaining 7.46–16.97% and 7.46–18.88% of PVE for protein index, respectively. A strategy to detect more QTLs for rice grain quality within subpopulations [44]. Xu et al. [58] detected a total of 29 QTLs in the whole population and 10 QTLs in the two subpopulations for 7 traits, 4 of which (1 qPRO3.1 for protein content) were detected in the entire population but the remaining 6 QTLs were not. These six QTLs with minor effects might have been covered by the Wx locus when mapped in the whole population. In addition to usual biparental populations such as recombinant inbred lines, backcross inbred lines, and doubled haploid lines, advanced population, i.e. chromosome segment substitution line (CSSL) populations, has also been employed [45]. Yang et al. [45] used a CSSL population derived from the cross of a Japonica variety (Sasanishiki) with Indica variety (Habataki) and identified a total of seven QTLs in three environments, although only one QTL (qPC-1) was detected across three environments explaining 10.38–15.43% of PVE. Furthermore, they developed $F_2$ and $F_3$ segregating populations from the cross between a CSSL with low PC, SL402, harbouring qPC-1 and Sasanishiki, and delimited the region of qPC-1 to a 41-kb on chromosome 1. These results may be helpful to introgress the QTL for GPC into rice cultivars using marker-assisted selection. In one study, Bruno et al. [46] observed compromised heritability percentage for protein while higher heritability percentage for the amylose content in a DH population derived from a cross between Cheongcheong and Nagdong. They mapped a QTL for GPC on chromosome 7 linked with the marker RM8261, explaining 14% of PVE.

As has been shown by previous studies, identification of robust QTLs for GPC in rice grains has been restricted because of lack of appropriate donors, non-utilisation of high-throughput phenotyping and genotyping platforms, and high genotype × environment (G × E) interaction. To overcome these restrictions, recently Chattopadhyay et al. [47] genotyped a $BC_3F_4$ mapping population derived from the cross between grain protein donor, ARC10075 and high-yielding cultivar Naveen, using 40 K Affymetrix custom SNP array, and identified three stable QTLs (viz. qGPC1.1, qSGPC2.1, and qSGPC7.1) for GPC explaining 13, 14, and 7.8% of PVE, respectively. QTLs identified in this study can be useful to improve the nutritional quality of rice grain. The closely linked markers that flanked the identified QTLs can be used to aid quality selection in breeding programs. And the results of the coincidence between the QTL detected, and the loci involved in protein biosynthesis pathways, might be helpful for gene cloning by the candidate gene method.

## 3. Amino acid content

In addition to GPC, improvement in the amino acid composition is important to meet the food demands of a growing global population. A major function of proteins in nutrition is to supply adequate amounts of required amino acids [59, 60]. Depending on requirement and availability in animal metabolic processes, essential amino acids cannot be synthesised by animals, but play a crucial role in metabolism [61]. Therefore, improving amino acid content in rice grain is an important objective. Several studies using the linkage mapping approach with various mapping populations have provided useful genetic information for improving the amino acid composition (AAC) in rice grains. Wang et al. [23] identified 18 chromosomal

regions for 19 components of AAC in 2 years, viz. 2002 and 2004. They found a total of 10 QTL clusters in 2002 and 6 in 2004.

Interestingly, they also detected a wide coincidence between the QTLs and the loci involved in amino acid metabolism pathways, including N assimilation, transfer and protein biosynthesis. In a similar study, Zhong et al. [26] reported 48 and 64 QTLs, each contributing 4.0–43.7% to the total phenotypic variance, in 2004 and 2005, respectively. They also reported good coincidence between the detected QTL and the loci involved in amino acid metabolism pathways in nitrogen assimilation and transport, or protein biosynthesis. In another study, Zheng et al. [24] mapped a total of 10 QTLs explaining 12–35% of PVE for histidine on chromosomes 1, 2, 3, 6, 7, 10, 11, and 12 and 8 QTLs explaining 16–33% of PVE for arginine on chromosomes 2, 3, 5, 6, 7, 10, 11, and 12. All QTLs showed significant additive effects from the triploid endosperm and diploid maternal plant, while two QTLs for histidine and two for arginine content also showed significant dominant main effects from the triploid endosperm. Various interactions between QTLs and the environment were detected for five QTLs associated with histidine content and two QTLs associated with arginine contents. QTLs associated with amino acid contents and linked/flanking markers are summarised in **Table 1**. Recently, Yoo [27] mapped a total of six main-effect QTLs located on chromosome 3, contributing 10.2–12.4% PVE for the content of six amino acids. The QTL cluster (qAla3, qVal3, qPhe3, qIle3, and qLeu3) in the interval of markers id3015453 and id3016090 was found to be associated with the contents of five amino acids and accounted for PVE from 10.2 to 12.8%. Although they also detected 26 digenic interactions for the content of 7 amino acids, viz. Asp, Ser, His, Gly, Arg, Ala, and Tyr, involving 25 loci distributed on the 9 chromosomes, but they did not find any interaction for the other 9 amino acids. Therefore, these identified QTL results will be useful to find the candidate genes and favourable alleles for the enrichment of nutritional value in rice grain.

## 4. Zn and Fe contents in rice

Zn deficiency in grown-up children and adolescent males causes retarded growth and dwarfism, retarded sexual development, impaired sense of taste and poor appetite, and mental lethargy [62]. Several roles of zinc are found to be involved in an abundant number of proteins in biological systems to maintain their structural stability function. It has been found that Zn is essential for gene regulation and expression under stress conditions and is therefore required for protection against infections and disease [63]. Likewise, iron has so many vital functions in the body like as a carrier of oxygen to the tissues from the lungs [64].

In last two decades, more than 80 QTLs have been identified and mapped on all 12 chromosomes of rice for zinc and iron contents using various mapping populations derived from different intraspecific and interspecific crosses. QTLs associated with zinc and iron contents and linked/flanking markers are summarised in **Table 1**. As per our knowledge, for the first time, Stangoulis et al. [18] mapped two QTLs for Zn and three QTLs for Fe on chromosomes 1, 2, 8, and 12 explaining 12.8–15% and 13.8–18.3% of PVE, respectively. Besides, Garcia- Oliveira et al. [17] detected one major effect QTL explaining the most significant proportion of PVE (11–19%) for zinc, flanking SSR marker RM152 on chromosome 8. In other various studies, several QTLs have been reported which explained a large amount of PVE either for zinc or for both iron and zinc contents [34, 48–52].

Ishikawa et al. [53] mapped four QTLs on chromosomes 2, 9, and 10 explaining 15.0–21.9% of PVE for grain zinc content using backcross recombinant inbred lines (BRILs) derived from *O. sativa* 'Nipponbare' and *O. meridionalis* W1627. Further, they fine-mapped QTL (named qGZn9) present on chromosome 9 and identified two tightly linked loci, qGZn9a (candidate region-190 kb) and qGZn9b (950 kb). They also showed the association of wild chromosomal segment covering qGZn9a with fertility reduction, and hence they recommended the use of qGZn9b as a valuable allele for breeding rice with high Zn in the grains. In another study, Swamy et al. [55] identified 20 QTLs for agronomic traits and total 59 QTLs for several biofortification traits including 8 QTLs for grain zinc and one QTL for grain iron, mapped on chromosomes 2, 3, 4, 6, 8, 11, and 12. They also detected eight epistatic interactions for Zn, Cu, Mg, and Na in a double haploid population.

Furthermore, they identified several candidate genes near grain zinc QTL (OsNRAMP, OsNAS, OsZIP, OsYSL, OsFER, and OsZIFL family), which may be useful for marker-assisted breeding for this important trait. Recently in 2019, two critical studies were conducted; in the first study, Descalsota-Empleo et al. [55] phenotyped two DH populations at two seasons and genotyped with a 6 K SNP chip and identified a total of 15 QTLs for agronomic traits and 50 QTLs for grain element concentration including 8 QTLs explaining 8.6–27.7% PVE for grain zinc. They also analysed the combined effect of QTL in both populations. Among the single-QTL lines, those with qZn9.1 showed highest mean grain Zn of 18.1 and 19.1 mg $kg^{-1}$ in two consecutive seasons, respectively. They reported an increase in the content of zinc with the increase in number of QTLs and observed highest grain Zn of 28.2 and 24.3 mg $kg^{-1}$ in two seasons, respectively, in four QTL lines (qZn2.1 + qZn5.1 + qZn5.1 + qZn11.1). Their results showed the possibility of QTL pyramiding for improving the zinc content in rice. In another study, Kumar et al. [57] detected one QTL for Zn and five QTLs for Fe having PVE 25% and 34.6–95.2%, respectively, using $F_4$ population (579 individuals) derived from a cross between PAU 201 and Palman. These identified QTLs can significantly enhance the efficacy of breeding programs to improve the Zn and Fe density in rice. The Zinc fortified rice varieties are released globally (**Figure 2**).

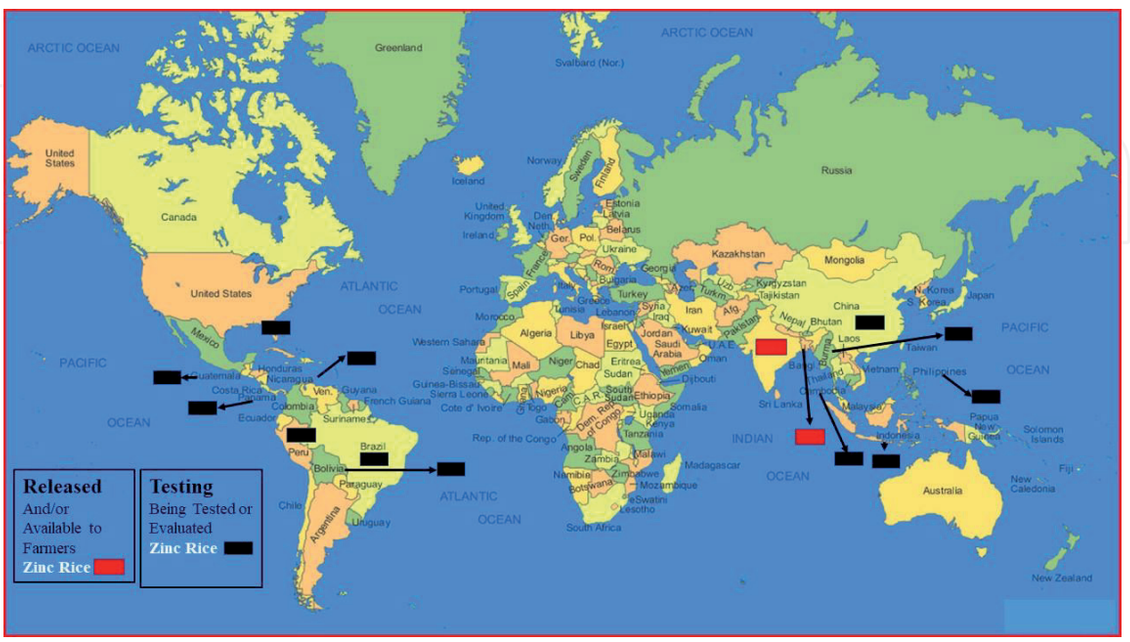


**Figure 2.**
*Map showing countries where zinc-biofortified rice varieties are released and being tested (information taken from HarvestPlus).*

## 5. Phytic acid

Phytic acid is an essential constituent in staple foods like legume and cereals, which has been of much concern [57]. In addition to its beneficial effect on human health, it has some anticancer and antioxidant functions and prevents coronary disease, and it is well known that phytic acid acts as strong chelating agent of mineral nutrients such as Ca, Zn, and Fe [65]. It has been seen that due to the presence of complex of phytic acid, in the form of phytate, there is a significant reduction found in bioavailability of nutrient elements [66]. It seems reasonable to control phytic acid contents in edible parts of crops to a level in which the medical and health functions of the food may be maintained and bioavailability of minerals is not much altered [67].

Liu et al. [68] assayed 72 cultivars for protein content and phytic acid and reported a wide range for phytic acid ranging from 0.685 to 1.03%, with an average of 0.873%. Interestingly, grain phytic acid and protein content were not correlated, which suggests the possibility of breeding rice for phytic acid and high protein content. Furthermore, they also reported a significant effect of varieties, locations, and their interactions on phytic acid content, with the location having the most considerable impact which suggests the necessity of multi-environment trials for the accurate evaluation of rice germplasm for phytic acid content.

Although sufficient genetic variation for phytic acid has been reported in various studies [68, 69], unfortunately, only one study has been conducted to map the QTLs for phytic acid in rice [18]. Stangoulis et al. [18] identified two QTLs explaining 15.4–24.3% of PVE for grain phytates from an IR64 × Azucena double haploid population. One common QTL for phytate and total P concentrations on chromosome 5 with the (high concentration) allele contributed from Azucena was identified. Furthermore, it was reported that Fe, Zn, and Mn contents in grains have different genetic regulation because the QTLs of phytate were not located on the same chromosomal regions as those found for Fe, Zn, and Mn [18]. So, there is a great possibility to find segregants having a low level of phytic acid and high level of Fe, Zn, and Mn content. Use of molecular marker in the breeding and selection to reduce grain phytic acid and improving the nutritional value of cereal grains.

## 6. Conclusions and future prospects

Biofortification is a promising, cost-effective, agricultural strategy to improve the nutritional status of the world's undernourished populations. Strategies for biofortification based on crop breeding, targeted genetic manipulation, and/or mineral fertiliser application have great potential to address human mineral malnutrition [70–72]. Developing biofortified food crops with improved nutrient content such as increased content of iron, zinc, Se, and provitamin A provides adequate levels of these and other such micronutrients that are often lacking in developed and developing diets. International initiatives, such as the CGIAR centres in collaboration with HarvestPlus and national initiatives, serve as pillars for achieving these objectives. These efforts have resulted in crops with the potential to increase both quantities and bioavailability of essential mineral elements in human diets, particularly in elementary cereal crops such as rice, wheat, maize, cassava, beans, and sweet potatoes. However, crop biofortification is a challenging task. Collaboration between plant breeders, nutritionists, genetic engineers, and molecular biologists is essential to achieving this. Breeding approaches are generalised and easy to accept and have been used to improve food nutritional qualities sustainably. Although greater emphasis is placed on molecular breeding-based approaches of which success rates are much higher as transgenically fortified crop plants, it faces

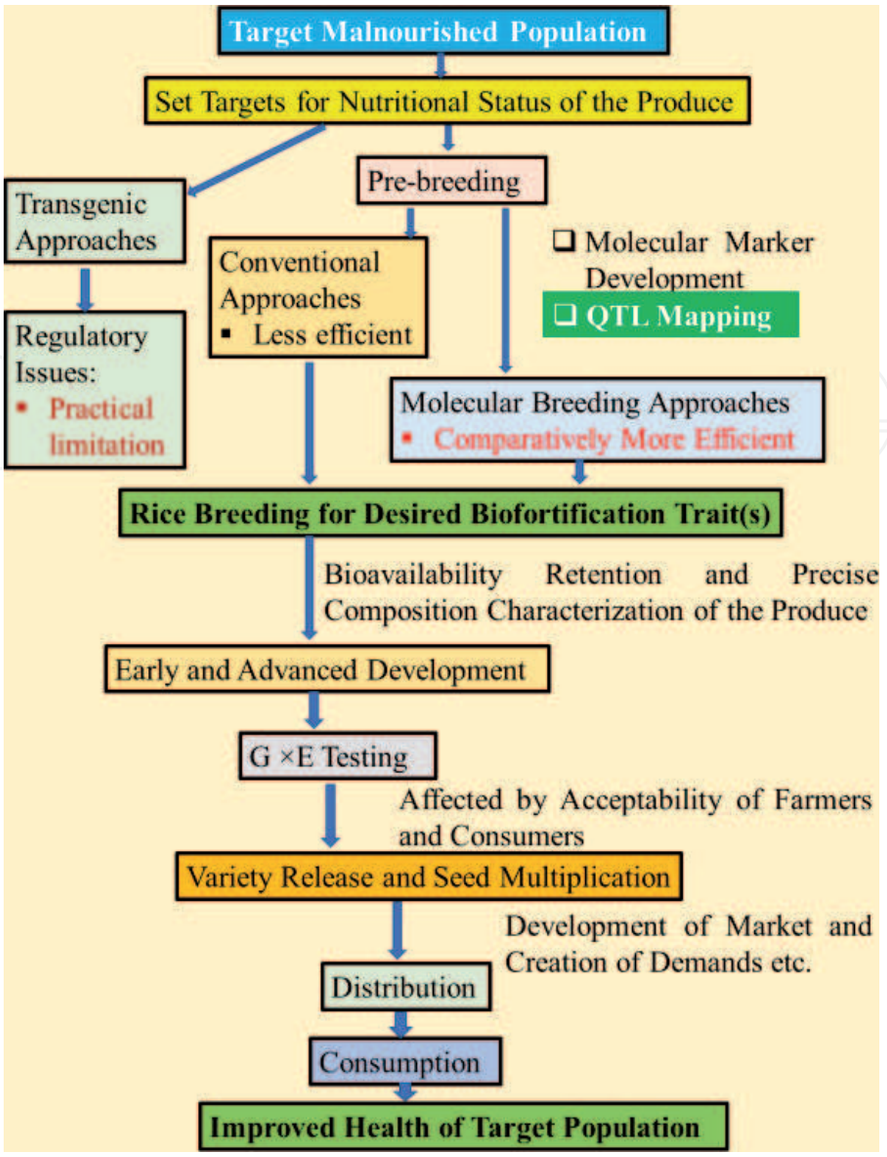


**Figure 3.**
*Flowchart showing development and release of a biofortified variety and its acceptance by farmers and consumers.*

hurdles due to consumer acceptance and costly and time-consuming regulatory approval processes adopted by different countries. Biofortified crops have a very bright future in addition to these challenges, as they have the potential to eliminate micronutrient malnutrition among billions of poor people, particularly in developing countries. Overall developmental process of the biofortified rice variety are presented in **Figure 3**.

## Conflict of interest

The authors declare no conflict of interest.

## Author details

Vinay Sharma[1], Dinesh Kumar Saini[2], Ashish Kumar[3], Hari Kesh[4] and Prashant Kaushik[5,6]*

1 International Crops Research Institute for the Semi-Arid Tropics, India

2 Department of Plant Breeding and Genetics, Punjab Agricultural University, India

3 Division of Genetics, ICAR-Indian Agricultural Research Institute, India

4 Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, India

5 Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain

6 Nagano University, Ueda, Japan

*Address all correspondence to: prakau@doctor.upv.es

## References

[1] GRiSP (Global Rice Science Partnership). Rice Almanac. 4th Ed. Los Baños (Philippines): International Rice Research Institute; 2013

[2] McGuire S, FAO, IFAD, WFP. The state of food insecurity in the world 2015: Meeting the 2015 international hunger targets: Taking stock of uneven progress. Rome: FAO. Advances in Nutrition. 2015;**6**:623

[3] Muthayya A, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The global hidden hunger indices and maps: An advocacy tool for action. PLoS One. 2013;**8**(6):e67860

[4] Gearing ME. Good as gold: Can golden rice and other biofortified crops prevent malnutrition. Science in the News Harvard University. 2015. Available form: http://sitn.hms.harvard.edu

[5] Xu JH, Messing J. Amplification of prolamin storage protein genes in different subfamilies of the Poaceae. Theoretical and Applied Genetics. 2009;**119**:1397

[6] Shobharani N, Pandey MK, Prasad GSV, Sudharshan I. Historical significance in grain quality features and precision breeding for improvement of export quality basmati varieties in India. Journal of Crop Science. 2006;**1**:29

[7] Raboy V. Approaches and challenges to engineering seed phytate and total phosphorus. Plant Science. 2009;**177**:281

[8] Gemede HM. Potential health benefits and adverse effects associated with phytate in foods. Food Science and Quality Management. 2014;**27**:45

[9] Tamanna S, Sayma P, Sanjay K, Alak KD, Aysha FM, Ali S, et al. Content of some minerals and their bioavailability in selected popular rice varieties from Bangladesh. International Journal of Current Microbiology and Applied Sciences. 2013;**2**:35

[10] Institute of Medicine Food and Nutrition Board (IMFNB). Dietary Reference Intakes for Vitamin a, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Washington: National Academy Press; 2001

[11] Gregorio GB, Senadhira D, Htut H, Graham RD. Breeding for trace mineral density in rice. Food and Nutrition Bulletin. 2000;**21**:382

[12] Yadava DK, Choudhury PR, Hossain F, Kumar D. Biofortified Varieties: Sustainable Way to Alleviate Malnutrition. New Delhi: Indian Council of Agricultural Research; 2017

[13] Yadava DK, Hossain F, Choudhury PR, Kumar D, Singh AK. Biofortification of crops: A sustainable tool for nutritional security in India. Indian Farming. 2018;**68**:37-42

[14] Perez-de-Castro AM, Vilanova S, Canizares J, Pascual L, Blanca JM, Diez MJ, et al. Application of genomic tools in plant breeding. Current Genomics. 2012;**13**:179

[15] Zhang M, Pinson SR, Tarpley L, Huang XY, Lahner B, Yakubova E, et al. Mapping and validation of quantitative trait loci associated with concentrations of 16 elements in unmilled rice grain. Theoretical and Applied Genetics. 2014;**127**:137

[16] McCouch SR, Wright MH, Tung CW, Maron LG, McNally KL, Fitzgerald M, et al. Open access resources for genome-wide association mapping in rice. Nature Communications. 2016;**7**:10532

[17] Garcia-Oliveira AL, Tan L, Fu Y, Sun C. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. Journal of Integrated Plant Biology. 2009;**51**:84

[18] Stangoulis JC, Huynh BL, Welch RM, Choi EY, Graham RD. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. Euphytica. 2007;**154**:289

[19] Jain BT, Sarial AK, Prashant K. Stability analysis utilizing AMMI model and regression analysis for grain yield of basmati rice (*Oryza sativa* L.) genotypes. JEBAS. 2018;**6**:522

[20] Jain B, Sarial A, Kaushik P. Understanding GxE interaction of elite basmati Rice (*Oryza sativa* L.) genotypes under north Indian conditions using stability models. Applied Ecology and Environmental Research. 2019;**17**:5863

[21] Hefferon KL. Can biofortified crops help attain food security? Current Molecular Biology Research. 2016;**2**:180

[22] Matsue Y, Odahara K, Hiramatsu M. Differences in amylose content amylographic characteristics and storage proteins of grains on primary and secondary rachis branches in rice. Japanese Journal of Crop Science. 1995;**64**:601

[23] Wang L, Zhong M, Li X, Yuan D, Xu Y, Liu H, et al. The QTL controlling amino acid content in grains of rice (*Oryza sativa*) are co-localized with the regions involved in the amino acid metabolism pathway. Molecular Breeding. 2008;**21**:127

[24] Zheng X, Jian-Guo WU, LOU X-Y, Hai-Ming XU, SHI C-H. QTL analysis of maternal and endosperm genomes for histidine and arginine in rice (*Oryza sativa* L.) across environments. Acta Agronomica Sinica. 2008;**34**:369

[25] Lu K, Li L, Zheng X, Zhang Z, Mou T, Hu Z. Genetic dissection of amino acid content in rice grain. Journal of Food Science & Agriculture. 2009;**89**:2377

[26] Zhong M, Wang LQ, Yuan DJ, Luo LJ, Xu CG, He YQ. Identification of QTL affecting protein and amino acid contents in rice. Rice Science. 2011;**18**:187

[27] Yoo SC. Quantitative trait loci controlling the amino acid content in rice (*Oryza sativa* L.). Journal of Plant Biotechnology. 2017;**44**:349

[28] Tan YF, Sun M, Xing YZ, Hua JP, Sun XL, Zhang QF, et al. 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. 2001;**103**:1037

[29] Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard JH. QTL mapping of grain quality traits from the inter-specific cross between *Oryza sativa* × Oryza glaberrima. Theoretical and Applied Genetics. 2004;**109**:630

[30] Hu ZL, Li P, Zhou MQ, Zhang ZH, Wang LX, Zhu LH, et al. Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. Euphytica. 2004;**135**:47

[31] Li J, Xiao J, Grandillo S, Jiang L, Wan Y, Deng Q, et al. QTL detection for rice grain quality traits using an inter-specific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. Genome. 2004;**47**:697

[32] Wada T, Uchimura Y, Ogata T, Tsubone M, Matsue Y. Mapping of QTLs for physicochemical properties in japonica rice. Breeding Science. 2006;**56**:253

[33] Takeuchi Y, Nonoue Y, Ebitani T, Suzuki K, Aoki N, Sato H, et al. QTL detection for eating quality including glossiness stickiness taste and hardness of cooked rice. Breeding Science. 2007;**57**:231

[34] Zhang X, Zhang G, Guo L, Wang H, Zeng D, Dong G, et al. Identification of quantitative trait loci for Cd and Zn concentrations of brown rice grown in Cd-polluted soils. Euphytica. 2011;**180**:173

[35] Lou J, Chen L, Yue G, Lou Q, Mei H, Xiong L, et al. QTL mapping of grain quality traits in rice. Journal of Cereal Science. 2009;**50**:145

[36] Yu YH, Li G, Fan YY, Zhang KQ, Min J, Zhu ZW, et al. Genetic relationship between grain yield and the contents of protein and fat in a recombinant inbred population of rice. Journal of Cereal Science. 2009;**50**:121

[37] Shi CH, Shi Y, Lou XY, Xu HM, Zheng X, Wu JG. Identification of endosperm and maternal plant QTLs for protein and lysine contents of rice across different environments. Crop & Pasture Science. 2009;**60**:295

[38] Qin Y, Kim SM. Sohn JK. QTL analysis of protein content in double-haploid lines of rice. Korean Journal of Crop Science. 2009;**54**:165

[39] Zheng L, Zhang W, Chen X, Ma J, Chen W, Zhao Z, et al. Dynamic QTL analysis of rice protein content and protein index using recombinant inbred lines. Journal of Plant Biology. 2011;**54**:321

[40] Zheng L, Zhang W, Liu S, Chen L, Liu X, Chen X, et al. Genetic relationship between grain chalkiness protein content and paste viscosity properties in a backcross inbred population of rice. Journal of Cereal Science. 2012;**56**:153

[41] Lee GH, Yun BW, Kim KM. Analysis of QTLs associated with the rice quality related gene by double haploid populations. International Journal of Genomics. 2014

[42] Leng Y, Xue D, Yang Y, Hu S, Su Y, Huang L, et al. Mapping of QTLs for eating and cooking quality-related traits in rice (*Oryza sativa* L.). Euphytica. 2014;**197**:99

[43] Yun BW, Kim MG, Handoyo T, Kim KM. Analysis of rice grain quality-associated quantitative trait loci by using genetic mapping. Asian Journal of Plant Sciences. 2014;**5**(09):1125

[44] Xu F, Sun C, Huang Y, Chen Y, Tong C, Bao J. QTL mapping for rice grain quality: A strategy to detect more QTLs within sub-populations. Molecular Breeding. 2015;**35**:105

[45] Yang Y, Guo M, Li R, Shen L, Wang W, Liu M, et al. Identification of quantitative trait loci responsible for rice grain protein content using chromosome segment substitution lines and fine mapping of qPC-1 in rice (*Oryza sativa* L.). Molecular Breeding. 2015;**35**:130

[46] Bruno E, Choi YS, Chung IK, Kim KM. QTLs and analysis of the candidate gene for amylose protein and moisture content in rice (*Oryza sativa* L.). 3 Biotechnology. 2017;**7**:40

[47] Chattopadhyay K, Behera L, Bagchi TB, Sardar SS, Moharana N, Patra NR, et al. Detection of stable QTLs for grain protein content in rice (*Oryza sativa* L.) employing high throughput phenotyping and genotyping platforms. Science Reporter. 2019;**9**:3196

[48] Lu K, Li L, Zheng X, Zhang Z, Mou T, Hu Z. Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. Journal of Genetics. 2008;**87**:305

[49] Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, Price AH. Genetic mapping of the rice genome in leaves and grain: Identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant and Soil. 2010;**329**:139

[50] Anuradha K, Agarwal S, Rao YV, Rao KV, Viraktamath BC, Sarla N. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar× Swarna RILs. Genetics. 2012;**508**:233

[51] Kumar J, Jain S, Jain RK. Linkage mapping for grain iron and zinc content in $F_2$ population derived from the cross between PAU 201 and Palman 579 in rice (*Oryza sativa* L.). Cereal Research Communications. 2014;**42**:389

[52] Indurkar AB, Majgahe SK, Sahu VK, Vishwakarma A, Premi V, Shrivastatva P. Identification characterization and mapping of QTLs related to grain Fe, Zn and protein contents in Rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding. 2015;**6**:1059

[53] Hu BL, Huang DR, Xiao YQ, Fan YY, Chen DZ, Zhuang JY. Mapping QTLs for mineral element contents in brown and milled rice using an *Oryza sativa* × *Oryza rufipogon* backcross inbred line population. Cereal Research Communications. 2016;**44**:57

[54] Ishikawa R, Iwata M, Taniko K, Monden G, Miyazaki N, Orn C, et al. Detection of quantitative trait loci controlling grain zinc concentration using Australian wild rice (*Oryza meridionalis*) a potential genetic resource for biofortification of rice. PLoS One. 2017;**12**:e0187224

[55] Swamy BM, Descalsota GIL, Nha CT, Amparado A, Inabangan-Asilo MA, Manito C, et al. Identification of genomic regions associated with agronomic and biofortification traits in DH populations of rice. PLoS One. 2018;**13**:e0201756

[56] Descalsota-Empleo GI, Amparado A, Inabangan-Asilo MA, Tesoro F, Stangoulis J, Reinke R, et al. Genetic mapping of QTL for agronomic traits and grain mineral elements in rice. The Crop Journal. 2019

[57] Kumar N, Jain RK, Chowdhury VK. Linkage Mapping of QTLs for Grain Minerals (Iron and Zinc) and Physio-Morphological Traits for Development of Mineral Rich Rice (*Oryza sativa* L.) Indian Journal of Biotechnology. 2019;**18**:69-80

[58] Xu Q, Zheng TQ, Hu X, Cheng LR, Xu JL, Shi YM, et al. Examining two sets of introgression lines in rice (*Oryza sativa* L.) reveals favorable alleles that improve grain Zn and Fe concentrations. PLoS One. 2015;**10**:e0131846

[59] Friedman M, Brandon DL. Nutritional and health benefits of soy proteins. Journal of Agricultural and Food Chemistry. 2001;**49**:1069-1086

[60] Panthee DR, Pantalone VR, Saxton AM, West DR, Sams CE. Genomic regions associated with amino acid composition in soybean. Molecular Breeding. 2006;**17**:79-89

[61] D'Mello JPF. Amino acids as multifunctional molecules. In: Amino acids in animal nutrition. 2nd ed. Cambridge: CABI Publishing; 2003. pp. 1-14

[62] Hambidge M. Human zinc deficiency. The Journal of Nutrition. 2000;**130**(5):1344S-1349S

[63] FAO/WHO. Vitamin and Mineral Requirements in Human Nutrition. 2nd Ed. Geneva: World Health Organization; 2005

[64] Kühn LC. Control of cellular iron transport and storage at the molecular

level. In: Iron Nutrition in Health and Disease. London: John Libbey; 1996. pp. 17-29

[65] Harland BF, Morris ER. Phytate: A good or a bad food component. Nutrition Research. 1995;**15**:733-754

[66] Erdman JW. Bioavailability of trace minerals from cereals and legumens. Cereal Chemistry. 1981;**58**:21-26

[67] Febles CI, Arias A, Hardisson A, Rodriguez-Alvarez C, Sierra A. Phytic acid level in wheat flours. Journal of Cereal Science. 2002;**36**:19-23

[68] Liu Z, Cheng F, Zhang G. Grain phytic acid content in japonica rice as affected by cultivar and environment and its relation to protein content. Food Chemistry. 2005;**89**:49

[69] Liu QL, Xu XH, Ren XL, Fu HW, Wu DX, Shu QY. Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.). Theoretical and Applied Genetics. 2007;**114**:803

[70] Saini DK, Devi P, Kaushik P. Advances in genomic interventions for wheat biofortification: A review. Agronomy. 2020;**10**(1):62

[71] Kaushik P. Application of Conventional, Biotechnological and Genomics Approaches for Eggplant (*Solanum melongena* L). Breeding with a Focus on Bioactive Phenolics. March 11, 2019 [Cited: 17 July 2019]. Available from: https://riunet.upv.es/handle/10251/122295

[72] Srivastava A, Kaushik P, Singh SP. Physiological traits and their importance in wheat breeding. Trends in Biosciences. 2018;**11**(**15**):2499-2503