



A brief review on Early Seedling Vigour and Rice Biofortification

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ABSTRACT- Seedling vigour for the canopy establishment and production of increased yield under aerobic circumstances is vital. Iron (Fe) and zinc (Zn) are essential trace elements affront people's energy and stamina worldwide and refrain from nutritional deficiencies arising from iron/zinc. Iron (Fe) plays a crucial role in chlorophyll and is essential for photosynthesis and energy generation through the electron transport chain. It is also important for root growth and nitrogen fixation, especially in legumes. In contrast, Zinc (Zn) serves as a cofactor for many enzymes that participate in metabolic processes, carbohydrate and protein synthesis, and the regulation of hormones. It is vital for photosynthesis, root growth, and important reproductive processes such as flowering and seed production. The biological enrichment of iron, zinc, manganese, and vitamins in rice provides seeds with power-packed nutrition and also economical and accessible to supplement the diet of poor people with this biofortified food. This review discusses progress and strategies to increase grain Iron, Zinc, Manganese and vitamins.

1. INTRODUCTION

Minerals like iron, manganese, copper, and zinc play crucial roles in the development of living organisms and humans. Different dietary sources provide iron, which is vital for the proper functioning of our metabolism. Regrettably, iron (Fe) and zinc (Zn), the two most significant metals, are present only in limited quantities in staple foods such as rice and wheat [1]. A lack of iron is a major contributor to disability and mortality around the globe. About 30% of the global population is thought to experience deficiencies in zinc, iron, and vitamins. The grain-filling phase is the concluding stage of the rice life cycle, where fertilized ovaries transform into caryopses. The rates and extent of filling play a crucial role in determining the weight of the rice grain, which in turn affects both grain yield and quality. There is a significant demand for enhancing rice yields, as well as for improvements in grain filling and quality. Numerous theories have been suggested that attribute the causes of inadequate grain filling to a decrease in source and sink sizes, hormonal imbalances, decreased gene expression, and diminished enzyme activity responsible for converting sucrose to starch, leading to less efficient assimilation and transportation [2]. The process of grain filling is also connected to Early Seedling Vigour. However, the mechanisms driving the differences observed in the rates

of grain filling between the top and bottom portions of the panicle have yet to be clarified. This topic is discussed in the following section.

2. EARLY SEEDLING VIGOUR

Rice seed containing phosphorus produces good seedling vigour has been noticed [3]. Rice is the most cultivated cereal in the world and serves as the primary food for more than one-third of the world's population. The growing areas worldwide majorly require seedling transplantation in puddled soil. Anaerobic condition generation, transplanted rice has the advantage of easy seedling establishment, restraining weed growth, and increased nutrient availability. In this scenario, DSR could be an alternate strategy for sustainable rice cultivation, overcoming labour, shortage, and energy production. Suggested reports indicate that DSR has the advantage of saving input water from 35% to 57%, reducing labour costs, and reducing methane emissions [4]. In addition, early seedling vigour is an imperative trait imparting weed competitiveness using early seedling growth and establishing a uniform crop.

Direct seeding leads to rapid germination and biomass buildup are crucial during the early seedling establishment phase and are regarded as early seedling vigor. The role of increased carbohydrate, amylase, growth hormone, an antioxidant enzyme, and ascorbic acid changes vigour and seedling phenotype. Seedling establishment, dominates the surroundings and provides edge over weed competitiveness. Crop plants with better growth compete with weed plants suppressing growth. One trait of high-quality seeds is their early vigor, which refers to the quick, consistent germination and establishment of robust seedlings for the given environment. Modern rice cultivars undergo phenotypic alterations to become more transplant-tolerant. Decreased availability of freshwater, and labour cost increase, give rise to a suitable alternative management system leading to enhanced grain yield, producing for increment in world population. Shoot length and coleoptile lengths are essential for early seedling vigour in addition to amylase, ascorbic peroxidase, and nitrate reductase activity [3,4].

Early seedling vigour traits have been identified in six accessions of *O. glaberrima* (EC861820, EC861819, EC861795, EC861792, EC861790, and EC861787). All the accessions are promising with early seedling vigour based on mean seedling establishment in both seasons under direct-seeded rice, increasing weed competitiveness

[5]. Early seedling vigor (EV) and eight characteristics related to the cross between Swarna and Moroberekan were used to phenotype a set of 253 BC3F4 lines viz. constant rate of emergence, length of shoot, length of the stem, fresh weight of shoot, fresh weight in total, dry weight of shoot, total dry weight, dry weight of root. 194 SNP markers were identified with six genomic regions in association with traits on chromosomes 3, 4, 5, and 6 with phenotype variance ranging from 2.5% to 18.6%. There are two QTL hotspot areas found on chromosome 3 (id3001701-id300833) on chromosome 5 (wd5002636-id5001470).

The hotspot regions identified as qEV_{3.1} (Early vigour), qEUE_{3.1} (Early uniform emergence), qSHL_{3.1} (shoot length), qSL_{3.1} (Stem length), qSFW_{3.1} (Shoot fresh weight), qTFW_{3.1} (Total fresh weight), qRDW_{3.1} (Root dry weight) and (qEV_{5.1}, qEUE_{5.1}, qSHL_{5.1}, qSL_{5.1}, qSFW_{5.1}, qSDW_{5.1}, qTDW_{5.1}). Mutations were also identified in the coding region of ethylene-insensitive 2-glutamate decarboxylase playing (a positive role in providing oxygen in anoxia conditions), 1-alpha-amylase precursor (hydrolyses α bonds of large, α -linked polysaccharides), 3-expansin precursor (causing cell wall expansion), 1-ethylene-insensitive 3 (causing ethylene-dependent hypocotyl elongation) and 3-phenylalanine ammonia-lyase (helping in the synthesis of salicylic acid) which were putative candidates associated with the target traits [6]. A non-destructive scanner mediated by whole-plant area (WPA) as the flatbed scanner estimated was highly correlated to seedling vigour. That helped the cause by saving labour [8].

With the help of GD1, OsIPMS1, and OsMYBS1, GA signalling proteins are responsible for seed germination by the influence of α Amy expression through the GA pathway. In addition, agronomic features like seed development and storage also play vital roles in enhancing seed vigour [9]. Increased seedling vigour is a quality of high-quality seed, thus improving the speed and uniformity of seedlings and good yield. In experiments concerning 31 rice genotypes, Vandana was followed by Bala, AUS 276, Addaysel, N22, and IR64 in decreasing order when performed based on root length, shoot length, mesocotyl length, coleoptile length, dry weight of seedlings [10]. Another study derived seedling vigor form 132 (RILs) from CSSL lines 93-11 and PA64s in BC₃F₂ lines. A major QTL (qSSL1b) was identified as present in 80.5 kb between two InDel markers in the long arm of chromosome 1 [11].

With the aid of particular transport proteins, zinc is moved from the xylem to the xylem parenchyma cells in the leaf symplast. From there, it travels to the leaf apoplast and, finally, to the phloem, which is the vascular pathway through which zinc is translocated into developing seeds. OsFRDL1, an iron reductase-deficient 1-like transporter, mediates the transfer of iron-citrate complexes from the root to the xylem. OsYSL15, which is expressed in phloem partner cells, is believed to carry Ferric-DMA for phloem trafficking in addition to the transporter OsYSL2. After that, iron is either linked to proteins and citrates or transferred from the phloem to the grain as Iron-DMA. In phloem sap, zinc is primarily attached to NA, whereas in xylem sap, zinc is a free ion that is partially bound to a chelator. Early seedling growth depends on OsYSL expression because it facilitates the intake of nutrients that

are necessary for growth in the early stages [12, 13, 14]. When the seedling's dry weight is taken into consideration, the biomass-influenced culm strength is also shown. The seed vigour is also influenced by strong culm nature of rice.

3. MANAGEMENT APPROACHES FOR EARLY SEEDLING VIGOUR AND BIOFORTIFICATION

Iron is found in the scutellum of the embryo and in the dorsal vascular bundle, aleurone layer, and endosperm of rice seeds. Zinc is shipped to the seed and is stored aleurone layer and embryo controlled by phytate [15]. Low iron and zinc contents in rice are regularly restrained because of the soil's low concentration of iron or zinc. Enriching iron or zinc in grains through fertilization or water management is called agronomic biofortification. Although there is a significant amount of iron in mineral soil, aerobic conditions might result in a deficit. The primary hassle with Fe uptake is solubility. Under aerobic circumstances, iron (Fe) in the soil—typically in ferrous form, either chelated or as a sulfate salt—is transformed into ferric form that is unavailable. Therefore, the utility of Fe as fertilizer is not an effective method for fortifying seed Fe. In another scenario, the foliar application or Iron-fortified bacteriosiderophore is a good alternative for overcoming iron deficiency, fortifying grains with iron, and its bioavailability in rice [15, 16, 17].

The utility of Zinc as fertilizer is effective in increasing rice fortification with zinc. However, the supply of Zinc decreases with an anaerobic cultivation system as precipitation as insoluble zinc sulfide or as insoluble carbonate mixtures. Zinc sulfate is not very effective at giving plants zinc, and plants that receive zinc applied to calcareous soils improve by less than 1% of the zinc applied. Zinc reactions in soils are attributed to their low efficiency, which restricts their availability. to plants or the diffusion of Zinc in certain soils. The tremendous positive effects of soil Zinc fertilization on grain Zinc have been observed with aerobic water management. In addition, compared to soil Zinc fertilization, foliar Zinc application has enhanced grain Zinc conditions in anaerobic conditions [18, 19].

Even though the foliar application of iron or zinc is more effective than soil application for growing Fe or Zinc than soil utility for increasing Iron or Zinc content in rice grains, the efficiency of foliar implemented Iron or Zinc depends on the application range. Foliar application of Zinc or Iron at flowering or early grain filling is more potent in enhancing grain Zinc or Iron and simultaneous seedling vigour, respectively than application in the early season. Even though the level of Zinc in grains is related to iron, research showed that foliar fertilization of combined iron and zinc fertilizers enhanced both grain iron and zinc content material, indicating that fertilization of one element does not affect the grain concentration of the others [20,21].

N fertilizer application has been said to be associated with Iron and Zinc content in rice grains. Optimized N fertilizer application could enhance grain iron and zinc content in rice grains and in several crop species, including rice, besides sufficient zinc delivery. The motives are: i) N nutrition promotes protein synthesis, that's a first-rate sink for Iron and Zinc; ii) N nutrition complements the expression of Zinc and Iron transporter protein, inclusive

of ZIP family transporters; iii) N nutrition complements the production of N compounds along with NA (nicotianamine) and DMA (2 deoxymugineic acid); iv) N nutrition enhances Iron and Zinc accumulation time by growing accumulation of Iron and Zinc in grain filling intervals. N fertilizer can lower rice grain Zinc content in low Zinc conditions through increased biomass production. Optimized N fertilization utility in rice production is essential to adjust Iron and Zinc accumulation and efficient water management [22-23]. Recent experiments on hybrid rice at IIRR, Hyderabad, further proved that aerobic cultivation has efficiently built-up grain zinc and iron better than irrigated rice [23].

4. Breeding and Transgenic Approaches for Biofortification

Since the last decade, IRRI (International Rice Research Institute) studies have evaluated the genetic variability of iron and zinc concentrations in rice and genotype x environment interactions carried out at ICRISAT, India [25, 26]. Promising breeding lines with higher zinc or iron content, or both, are presented (Table 1).

Genotypes	Zn	Fe
IR 83294-66-2-2-3-2	22–37–41.97 mg kg ⁻¹	Fe: 9.43–17.16 mg kg ⁻¹
IR 82475-110-2-2-1-2	Zinc: 20.24–37.33 mg kg ⁻¹	Fe: 7.47–14.65 mg kg ⁻¹
IR 83668-35-2-2-2	Zinc: 27.15–42.73 mg kg ⁻¹	Iron: 6.01–14.71
RP Bio 5478-185M7	Zinc: 22.60–40.07 mg kg ⁻¹	
IR 68144-2B-2-2-3-1-166	Zinc: 23.53–40.30 mg kg ⁻¹	Iron: 10.53–17.80 mg kg ⁻¹
IR 68144-2B-2-2-3-1-166	Zinc: 23.53–40.30 mg kg ⁻¹	Iron: 10.53–17.80 mg kg ⁻¹
High yielding rice varieties (more than 3.75 tonnes/hectare):		
IR82475-110-2-2-1-2	Zinc: 20.24–37.33 mg kg ⁻¹	Iron: 7.47–14.65 mg kg ⁻¹
Dzuluorhe	Zinc: 17.48–39.68 mg kg ⁻¹	Iron: 7.89–19.90 mg kg ⁻¹
Kelhrrie Cha	Zinc: 17.76–36.45 mg kg ⁻¹	Iron: 7.17–14.77 mg kg ⁻¹
Nedu	Zinc: 18.97–43.55 mg kg ⁻¹	Iron: 8.01–19.51 mg kg ⁻¹
Mima	Zinc: 17.10–45.64 mg kg ⁻¹	Iron: 9.97–17.40 mg kg ⁻¹
Kuhusoi-Ri-Sareku	Zinc: 17.37–44.14 mg kg ⁻¹	Iron: 8.99–14.30 mg kg ⁻¹

Table 1: List of rice genotypes having high iron and high zinc

The IR IR82475-110-2-2-1-2, Dzuluorhe, Kelhrrie Cha, Nedu, Mima, Kuhusoi-Ri-Sareku were traditional genotypes possessing high grain Iron and Zinc content were used in the crossing program and enhanced the grain zinc and iron in new rice varieties.

[26] showed that ferritin overexpression increased Iron levels in rice endosperm and caused high iron increases of up to 10.46 µg/g dry weight. Overexpression of HMA3 upregulates ZIP family genes in roots, increasing zinc uptake. OsHMA2 is involved in the transport of zinc to developing tissues. QTLs and candidate genes for Iron and Zinc transport are OsYSL1 and OsMTP1 which are responsible for Iron transport. At the same time, OsARD2, OsIRT1, OsNAS1, and OsNAS2 are responsible for Zinc transport. Facilitated diffusion of iron and zinc mediated by citrate increases grain iron and zinc concentrations [27,28].

5. Biofortification Importance from Seed Vigour Perspective

The ability of the seed to function under different condition s determines whether or not it will germinate.

Water intake during the first few hours of imbibition, which can continue for up to twenty hours, is what defines the germination period. The emersion of the radicle follows this. The embryo moves into a metabolically active stage from the quiescent stage. The metabolically active stage involves the metabolism of carbohydrates, synthesis of nucleic acids, regulation by redox homeostasis, and gene expression, DNA repair, ultimately leading to seedling establishment [29].

Distribution of minerals during seed germination observation using synchrotron X-ray microfluorescence

(XRF) was observed iron (Fe), zinc (Zn), and Manganese (Mn) accumulated more in elongating tissues rather than in rice grain [30,31]. Iron and zinc are required for germination for protein synthesis, expression, respiration, and tolerance to oxidative stress. As evidenced in earlier studies, potassium and calcium are required for better plant growth and stress tolerance. Biofortified rice, on many occasions, has shown a slow germination rate. This is because phytic acid content, a chelating agent binding Ca, Fe, and Zn, makes them unavailable for germination [32]. However, low phytic acid containing mutants were developed but failed to achieve great yields. High amounts of ascorbate also diminish germination rates [33]. As described earlier, foliar spray in soils devoid of much fertility may serve the purpose of early germination and supports early seedling vigor [34].

For improvement of seed vigor, OsIPMS1 (isopropylmalate synthase 1) gene encoding for biosynthesis of leucine (Leu) plays a role in seed dormancy release. Disruption mediated by CRISPR/Cas9 system led to seed dormancy and low germination and growth rate [33]. OsIPMS1 expression has helped mobilize starch, amino acid biosynthesis is demonstrated by 1209 DEGs. Real-time PCR experiments also suggested that gene may be an important indicator for determining priming interval in rice seeds [34]. In addition, PARP genes involved in chromatin remodelling also play key roles in the determination of seed vigour. Disruption of the PARP gene delayed the germination rate and showed poor amino acid metabolism, nucleic acid degradation, poor lipid metabolism, cell wall metabolism, and poor ROS scavenging mechanism [35]. Deep sequence analysis of Nipponbare after 12 and 24 hours of germination revealed

289 miRNA loci, of which 230 were novel. Among special mentions, miR319, miR168, miR156, miR166, and miR159 were upregulated in imbibed seeds. Transcription factors identified were SPLs, ARFs, TCPs, MYBs, and hormone regulatory pathways included (auxin, ABA, GA) [36].

Rice seedlings' elongation of coleoptile occurs over root development. The coleoptile reaches the water's surface and supplies oxygen to the developing tissue. Microarray transcriptome profile of rice genotype embryos incubated at 24 hours in aerobic/anaerobic treatments and the presence/absence of sugar starvation in cultivar Dongiin. Anoxic conditions lead to downregulating more genes (434), whereas in aerobic conditions, these genes were upregulated 3 -fold more times. The energy-requiring processes of enzymes (α -amylase, hexokinase, ATP-dependent phosphofructokinase, pyruvate orthophosphate dikinase, pyruvate decarboxylase, alcohol dehydrogenase) and oxidative phosphorylation were downregulated in hypoxic conditions [37,38]. The transcriptomics profile revealing the machinery of the Sub1 genotype under anoxic conditions revealed protein from the DNA repair department, chromatin modification, cell elongation, carbohydrate metabolism, and ethylene response factor to be expressed in the genotype and hence showed greater vigour under anoxic conditions [35, 39,40].

Equipping rice with manganese Mn deficiency in rice is not a common problem in irrigated field conditions. OsNRAMP1 transports manganese. OsNRAMP5 was expressed in the root epidermis, outer cortex, and tissues surrounding the xylem. Upregulation of OsNRAMP5 regulates manganese (Mn) uptake. (Fe–manganese (Fe–Mn) oxide-modified biochar (BC-FM) ameliorated toxic materials from soil and enhanced the uptake of Mn from the roots of rice. Knockout of OsNRAMP5 by CRISPR-

Cas9 technology led to promising hybrid lines with increased Mn. It supplied a practical approach for developing Mn-loaded rice. [41].

Vitamin rice

In a trial to reconstruct vitamin A produced in rice, under the control of the endosperm-particular glutelin promoter together with a bacterial phytoene desaturase (crtI, from *Erwinia uredovora* under constitutive 35S promoter control) could produce vitamin A containing yellow rice at T0 stage [42]. Earlier, to create transgenic japonica rice (Taipei 309), the daffodil (*Narcissus pseudonarcissus*) phytoene synthase (PSY) gene was expressed under an endosperm-specific promoter. Using *Agrobacterium*-mediated transformation, the same daffodil PSY gene was combined with a bacterial phytoene desaturase (CRTI) gene from *Erwinia uredovora*. It was introduced into the same japonica rice variety (Taipei-309). The highest total carotenoid expression (37 g/g) by introducing maize ZmPSY and *Erwinia uredovora* CRTI genes under an endosperm-specific promoter. The *Arabidopsis thaliana* genes AtDXS (encoding 1-deoxy-D-xylulose 5-phosphate synthase for a continuous supply of metabolic precursors) and AtOR (the ORANGE gene for the formation of a metabolic sink) were combined with maize phytoene synthase (ZmPSY1), bacterial phytoene desaturase (PaCRT1), and produced up to 31.78 g/g total carotenoids in rice [55]. Vitamins are present in the rice endosperm. Rice contains thiamine and riboflavin. Thiamine is synthesized by genes *thi1* and *thiC*. These genes were overexpressed, and cysteine-containing motifs were added to *thi4*. Overexpression of these genes *thiD*, *thi5* and *thiC* have increased thiamine contents in grains and leaves [43,44].

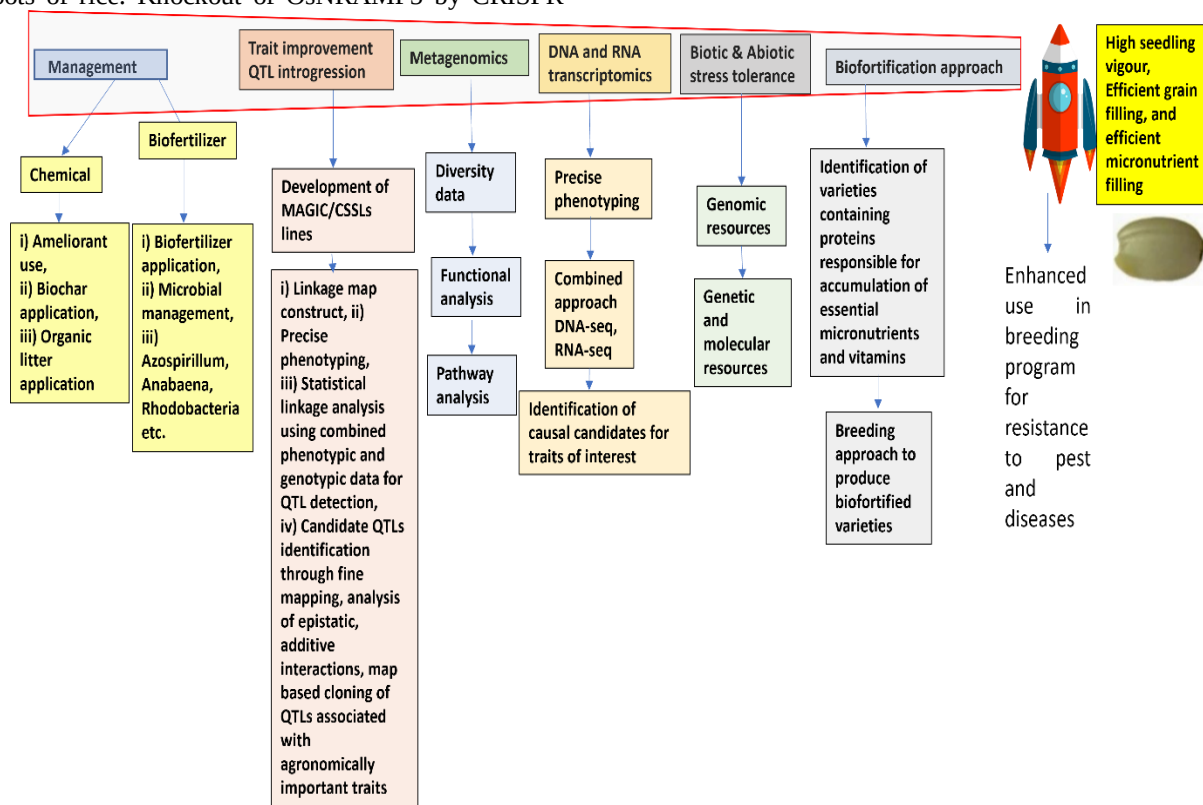


Figure. 1 Various approaches for crop improvement

CONCLUSION

Effective grain filling, along with the efficient absorption of micronutrients in rice plants, is illustrated (Fig. 1). Rice equipped with manganese and vitamins is the requirement for the growing population. The availability and absorption capacity of essential minerals in the soil influence the vigor of rice seeds. Furthermore, the stages of grain filling play a crucial role in yield determination, so enhancing these stages through various methods to boost yield is essential.

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