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ZINC BIOFORTIFICATION OF RICE (*Oryza sativa*. L.) BREEDING AND AGRONOMIC APPROACHES: STATUS AND CHALLENGES FOR ITS ADOPTION IN SUB-SAHARAN AFRICAN COUNTRIES.

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ABSTRACT

Zinc (Zn) is among the vital micronutrients fundamental for the growth as well as the development of humans. About 400 million people, mainly pregnant and children, go through Zn deficiency syndromes in sub-Saharan Africa. Though rice is the predominant crop in sub-Saharan Africa and a significant source of energy and micronutrients it does not supply sufficient zinc (Zn) equivalent to peoples dietary needs; rice biofortification has been recognized to be among the plants that need urgent attention to raise Zn concentration. Breeding new rice cultivars with high Zn regarded as a sustainable, cost-effective strategy to ameliorate Zn insufficiency. Agronomic biofortification alone can not solve this problem, but a synergy of agronomic biofortification and genetic approach will be more efficient. Substantial advancement has made toward increasing high Zn rice lines intended for needed countries. That will provide a relatively cost-effective, sustainable, as well as a long-term way of eradicating micronutrients to rural dwellers, the main bottleneck for rice Zn biofortification are well-known, as small transport and Zn uptake, into the grain. Extensively grain Zn accumulation should be explored. Finally, we talk about the tactical usage of Zn fertilizer meant for emergent of biofortified rice.

Keywords: Agronomic; biofortification; Hiding hunger, rice; zinc insufficiency.

INTRODUCTION

The nutritional deficit of zinc (Zn) is universal public health, and the dietary dilemma more than one-third of the human race is in danger owing to low dietary intake of Zn (Myers et al., 2014) About 2 billion populace in Asia as well as 400 million peoples in sub-Saharan African countries. (Myers et al. 2015). Majority of individuals at risk depend largely on legumes as well as C_3 grains as their main nutritional supply of Zn, with high dependence on cereals, mainly rice(Oryza sativa L.) That has a very low amount of Zn and meager bioavailability of Zn when compared with other cereals plants. (Myers et al. 2015) Thus, Zn shortage is a dilemma amongst peoples that depend on a rice-based diet. (Impa and Johnson-Beebout 2012) Rice grains zinc level can be enriched through biofortification with known Zn fertilizers (Cakmak 2009) ii manipulating rice Zn ligands and,transporters (Borrill et al. 2014; Palmgren et al. 2008) and iii screening of efficient germplasm with high Zn bioavailability (Blair 2013) The entire methods depend on soil or fertilizer or both, as Zn source to produce Zn enriched grains. Though, Zn is inadequate depending on soil properties such as organic matter, pH, oxides of Fe, Al, and redox potential,(Tuyogon et al. 2016)

In addition to natural Zn condition within the upper layer of the soil (Mandal and Mandal 1990) Zn availability problem for plants is worsened when growing rice plant in waterlogged. (Tuyogon et al. 2016). Reported that Zn fertilizer application is the most common choice to conquer such problems. However, The improvement when Zn fertilizer applied in rice plant hardly exceeds 2% of the used amount. (Meng et al. 2014) Within the last decade, many researchers have been conducted with a view to biofortify crops using micronutrients, and the outcomes lead to a better perspective of genetic, physiological as well as the molecular origin of high grain Zn concentration in plants. (Impa and Johnson-Beebout 2012) Many research has been done to map-out quantitative trait loci (QTLs) which have high Zn that can be used to develop transgenic plants with high Zn. (Johnson et al. 2011; Slamet-Loedin et al. 2015)

Breeding efforts may perhaps boost Zn proportion with 6–8 mg kg−1(Stomph et al. 2009) Whereas transgenic lines developed for rice also improve Zn proportion by 15–30 mg kg−1.(Olsen and Palmgren 2014) Knowing the physiological base of Zn uptake, as well as its translocation, among different rice part, and proficient load of Zn into panicle is necessary for rice genetic biofortification , but only little about this process is known in rice, (Rose et al. 2013) the Main bottleneck for proficient Zn accumulation in rice grain is identified as (i) barrier from soil-to- rice root; and blockage of transporting Zn into rice grains. (Hacisalihoglu and Kochian 2003) The first stride towards rice Zn accumulation in grains by root uptake, furthermore, Zn uptake is affected by some factors consist of root hairs, root architecture, root surface area, crown root development, root anatomical structures as well as alteration of rhizosphere during exudation of protons. And result in changing the soil pH, thus improve the solubility of Zn within the soil and aid its dispersal into the root. (White and Broadley 2011) While some soil factors that influence Zn plant-availability, like, organic matter content, soil texture, soil pH, microbial populations, as well as soil mineralogy,(White and Broadley2011) Rice Zn availability in flooded condition (anaerobic) is also affected by soil soluble bicarbonate, sulfur content, and redox potential. (White and Broadley 2011) Hence, an amalgamation of genetic strategy, as well as agronomic practices, will progress the topsoil healthiness, in addition, to enhance the root Zn uptake, especially in rice.

Nevertheless, Zn transport is limited as a result of some barriers to root-to-shoot transport. In addition to Zn internal allocation as well as re-allocation among vegetative and reproductive tissues, and this result in decrease grain Zn accumulation in rice grains. (Jiang et al. 2008)The present review focused on developing Zn biofortified rice which can be used to overcome hidden hunger or malnutrition to rural dwellers especially in sub-Saharan African countries as well as thrash out the challenges linked with the adoption of rice Zn-enriched germplasm. We preferred rice to be our plant for the reason that it is one of the primary edible plants in sub-Saharan African regions affected by Zn malnutrition.

Rice Zn uptake with translocation.

Depending on the need, but Zn is allocated first into the leaves of the plants during the vegetative phase, and later it will be in the panicle during grain filling phase (Jiang et al. 2008). Toward the later step of plant development, Zn partition involving the shoots leaf, as well as, grains varied. (Seneweera 2011)

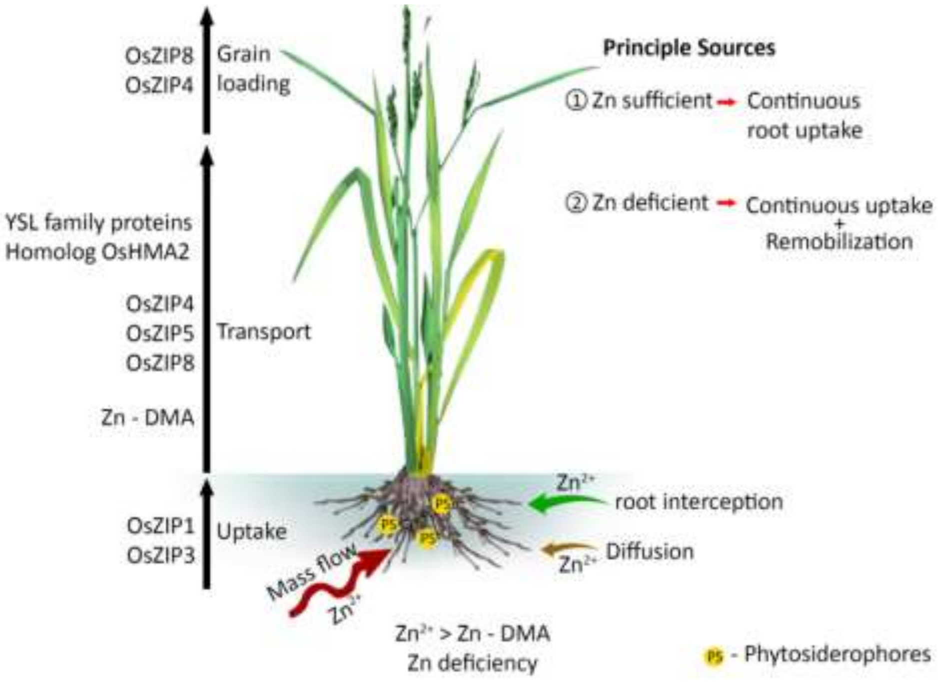
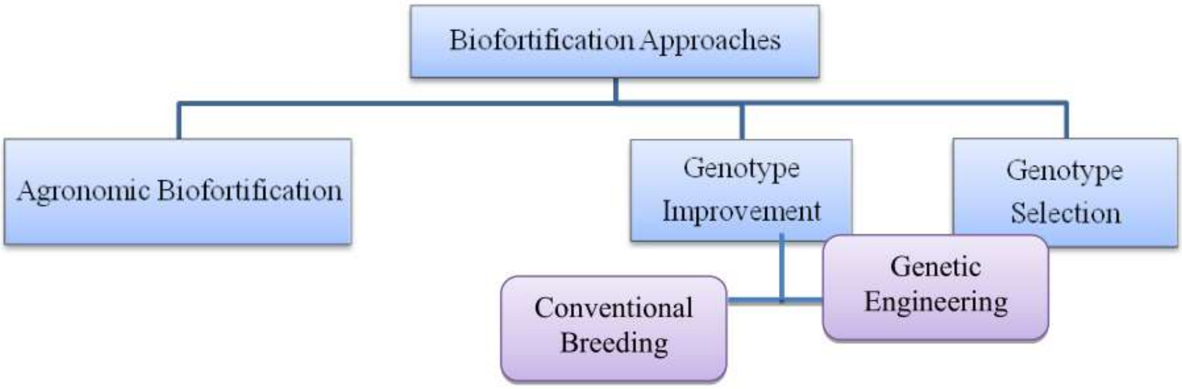


Fig. 1: Zn - transport, and uptake into the rice panicle, source: Nakandalage (2016) Front. Plant Sci. 7:764.

Rice Zn bioavailability

Rice nutrients bioavailability determined through the number of chelating molecules within the rice grain, like Phytic Acid. (Sperotto et al. 2013)It is the major plentiful anti-nutrient within cereal crops, about 80% of P was invested in Phytic Acid that binds metallic ions among which are Zn Fe, Mg, K, as well as Mn, and consequently reduce its bio-availability. (Cakmak 2008) Even though Phytic Acid suppresses Zn bioavailability, as well as increasing its concentration will affect plant growth as well as development. (Yoneyama et al. 2015). Throughout the kernel germination process, Phytic acid is hydrolyzed to discharge phosphate, inositol, in addition to micronutrients that sustain seedling grow. PA, along with derivative are concerned in DNA repair, RNA export, signaling, and endocytosis along with cell vesicular trafficking. Thus, Phytic Acid consumption safeguards humans against a mixture of cancers mediated during the disruption of cellular signal transduction in addition to cell cycle inhibition movement. (El-Sherbiny et al. 2001). Phytic Acids situated within the aleurone layer. (Iwai et al. 2012). Thoughtful knowledge on the genetic and physiological mechanism regulating tissue localization meant for both Phytic acid with Zn is crucial to attaining Zn biofortification with no lose or effect on Phytic acid. There is a need for a better understanding of genetic factors as well as environmental interactions scheming Zn homeostasis (Welch and Graham 2004).

Approaches for biofortification of crop plants.



Source: Meena. (2017).

Agronomic practices

Agronomic practices are the easiest and the fastest technique used for biofortification of cereals grains with, Zn, Fe or any other nutrients in developing countries of African as well as Asia, whom mostly depend on cereals as their primary food. And it is also a convenient means that will reach rural dwellers that don't have enough or afford a mineral supplement. (Farooq et al. 2009). Zn is not homogeneously in agricultural fields, and its concentrations also differ from 10 to 300 mg/kg(Noulas et al. 2018). The overall stand for Zn concentrations in soils is approximately 5–55 mg/kg.

Additionally, it has been reported that 64 mg/kg is the mean rate used for Zn in the ground (Noulas et al. 2018). Application of fertilizers with Zn by foliar means has revealed enhanced outcomes than when applying it directly into the soil. However, the extent of this rise is not consistent among some cultivars(Mabesa et al. 2013; Wei et al. 2012).

Nevertheless, the discovery of Zn threshold level within the soils that can be declared as Zn insufficiency soils is intricate owing to overall changeable Zn. Unreliable Zn bioavailability within the grounds dependent on numerous environmental factors (Noulas et al. 2018). Many studies have reported that improving growth and yield, with enhancement of plants Zn absorption will be achieved via fertilization with Zn for various plants, together with rice. (Fageria et al. 2011; Shehu 2010)Therefore, it is significant to make sure that sufficient Zn can be supplied, through foliar Zn application or by soil Zn fertilization especially during growth stage like heading, as well as the beginning of the grain-filling stage (Boonchuay et al. 2013). Seed priming or seeds treatments before sowing with various nutrients are usually done to improve germination in addition to tolerance against diverse abiotic stresses at an early stage of growth (Farooq et al. 2009). Suggest that synchronizing Zn plus Nitrogen fertilization leads to attaining good results than when applying a sole (Alloway 2008). Though the application of high rates of P possibly will progress the growth of shoot as well as grain yield of rice (Fageria et al. 2011)

The majority of Zn fertilizer applied to the soil is zinc sulfate fertilizer, and also it is widespread Zn fertilizer used in rice production, and it is the least efficient up to date. (Shivay et al. 2008; Wei et al. 2012) Noticed that the quantity of Zn in brown rice increased from 30.28 mg kg -1 to 33.75 mg kg -1 through foliar Zn-EDTA application to 35.07mg kg-1 by foliar Zn-Citrate application, to 38.45mg kg-1 by foliar ZnSO 4 application, to 39.84 mg kg -1 by foliar Zn-AA application. It might be due to the diverse ability of leaf penetration. Foliar fertilizer among low molecular weight like Zn-AA as well as ZnSO4 might penetrate the leaves easily than when compared with those that have higher molecular weight like Zn-EDTA and Zn- Citrate fertilizers. (Bharti et al. 2013) The report that application of pooled soil and foliar use of Zn fertilizer increased grain Zn concentration considerably then when compared with other treatments. It may be due to increasing Zn levels and also obtained lowest values of phytic acid because PA is a P storage composite and therefore high P uptake may be due to the antagonistic association of phytic acid with Zn. (Shivay and Prasad 2014)

Soil application of Zn-coated urea gives high result then than use as Zn sulfate. (Shivay et al. 2016).The consequence of applying fertilizer containing nitrogen in rice grain, for increase Zn level revealed unfavorable outcomes, However, increasing nitrogen fertilizer miserably affect grain Zn content(Moraghan et al. 1999; Zhang et al. 2008). Application of gypsum take away bicarbonate from the soil solution, and lower the pH and rising the accessibility of micronutrients together with Zn within alkaline as well as sodic soils (Rengel et al. 1999) Bio-fertilizers application like Azospirillum, Rhizobacter, Azolla, Zn-solubilizing bacteria, Mycorrhiza, in addition to organic manures improve the concentration of Zn bioavailable in waterlogged environment by means of increasing the Zn content in rice panicle. (Singh and Prasad 2014; Tariq et al. 2007)

Breeding strategy to boost grain Zn in rice

The genetic procedure for biofortification uses crop breeding methods to produce food that contain a high amount of micronutrient. (HarvestPlus 2014).Provide a sustainable way out to undernourishment troubles through exploring common genetic variant with the aim to extend high nutrient varieties. (Bouis 2003; Pfeiffer and McClafferty 2007) Since rice germplasm has a broad dissimilarity in favor of grain Zn it would-be easy to breed high Zn rice through exploration of donor parents with high-Zn. The Zn breeding goal aim to raised Zn concentration began with an earlier mark of 24 mg $kg^{-1}$of Zn to the latest intention of 28 mg kg^{-1}(HarvestPlus 2014). Biofortification possibly will be achieved through a variety of conventional, non-conventional and integrated approaches. (Rawat et al. 2013; Shahzad et al. 2014).Breeding approaches such as quantitative genetics, mutation breeding along with marker-assisted breeding help in exploiting the genetic multiplicity for increasing the genetic gains into zinc biofortification. The improvement, as well as the exploitation of molecular markers plus linkage maps as well, helps in discovering QTLs for traits of interest. Marker-assisted breeding has also been found to play an imperative role in zinc biofortification (Collard and Mackill 2008).Single-nucleotide polymorphism. (SNP),high-throughput meant for genotyping were developed in other cereals crops like maize (McMullen et al. 2009), thus open a new gap for Zn genetic biofortification. Development of Transgenic plants also is another critical milestone for versatile genetic improvement in plants. Breeding criterion is vital to discover the roadmap intended for varietal improvement through the fulfillment of enriched micronutrients, which is prerequisite for obtaining biofortification. The breeding strategies used for nutrient bio-fortification are (Welch and Graham 2004)

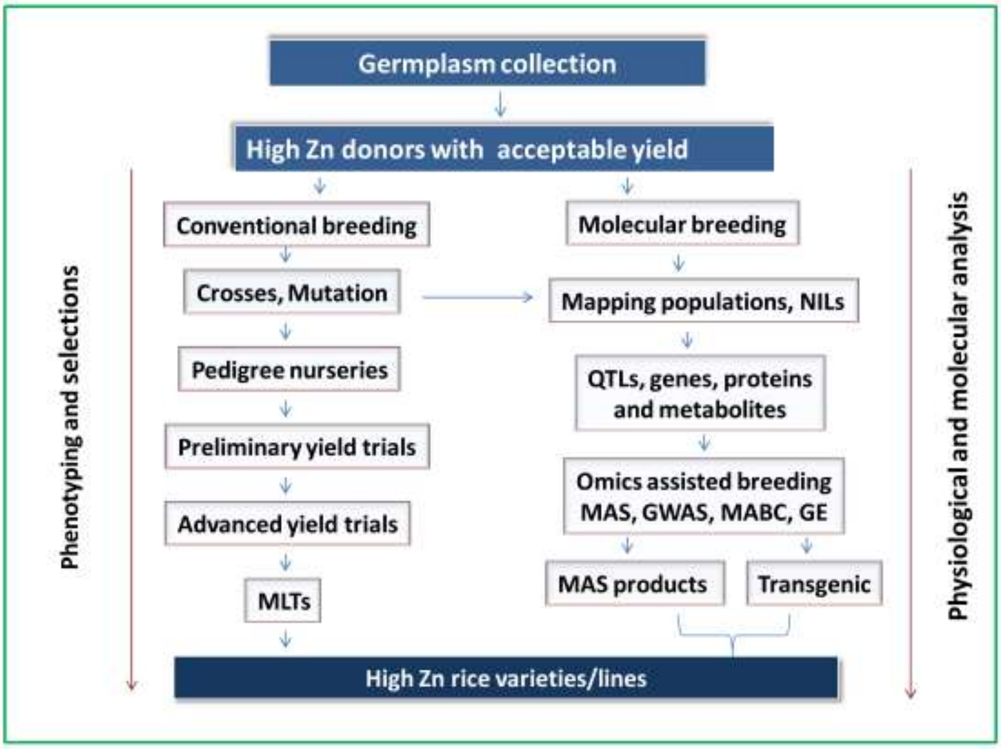


Fig. 3: Schematic Diagram of Breeding Strategies to Develop Biofortified Rice, Source: B. P. M. Swamy. (2016)

(a) The yield of the developed plants should be increased or maintained to certified consumers preference to the farmers.

(b) The established plants through biofortification must be sufficient-enough and have a considerable impact on human being health.

(c) Characters associated with nutrient biofortification among varied environment be found.

(d) The biofortified germplasms must be assessed for bioavailability of micronutrients meant for people.

(e) Consumer suitability, appearance quality, and cooking quality must be evaluated, for gaining the total impact on the dietary outline of the consumers.

Because the genetic basis for grain, Zn is multifaceted through association of various genes and QTLs as well as the environment, the selection of suitable methods of breeding, choices of individual crops, along with field assessment is indispensable meant for the steady improvement of rice with high-Zn. Plant with high-Zn concentration used as donor plants, and they crossed with known high-yielding. However, low-Zn rice cultivars also the choice was conducted in favor of agronomic character in the segregating generations. (Welch and Graham 2002)Multiple crosses including some recipient parents and donors like a three-way, and four-way crosses, as well as reciprocal crosses exceptionally high Zn crossed with high Zn, crosses improve Zn concentration as well as yield. Multi-parent advanced generation inter-cross (MAGIC) is another technique aimed at pooling the genes with high Zn; many magic populations developed at an international rice research institute (IRRI) like magic-japonica， magic-indica and magic-global (Bandillo et al. 2013) which are excellent resource meant for choosing high Zn lines(Bandillo et al. 2013; Nagesh P 2012). Reported that wild type of rice like *O.barthii*, *O. rufipogon*, *O. nivara*, and.*O.glaberrima* contained a higher amount of Zn that can serve as donor parents (Garcia-Oliveira et al. 2009; Sarla N and AP 2012) Mutation breeding is also another move toward increasing Zn level into a rice plant. Both physical, as well as chemical mutagens, have been used many mutants with high Zn level were identified. Like IR64 mutants formed through the use of sodium azide reported having elevated Zn level (Jeng et al. 2012). Three IR64 mutant lines MIR-49, MIR-175, MIR-180, all have <26 mg kg−1Zn content in polished rice as having 16 mg kg−1 in IR64.and can be use for Zinc breeding programs as donor parents. (Jeng et al. 2012) So far there is no report of using marker-assisted for breeding high Zn in rice. (Collard and Mackill 2008) Subsequently, a lot of QTLs and genes for rice grain Zn positioned in the different chromosome, Marker-assisted recurrent selection (MARS) and, QTL pyramiding; methods could also be used to increase high-Zn rice. (Collard and Mackill 2008).There is need for exploiting, genome-wide association studies (GWAS) in addition to genomic selections (GS) method with the aim of improving grain micronutrients. However, there is also need for paying attention when breeding high Zn rice, because of some anti-nutrients that will likely affect bioavailability of Zn like Phytate which is among the significant anti-nutrient impeding the amount of Zn absorbed. (Alexandrov et al. 2015)

Up to date information on breeding high Zn rice.

Overcoming Zn famine is earnest support in favor of kids as well as pregnant and nursing mothers especially in developing nations. Even though various techniques are accessible, but crop biofortification remains a suitable way. Zn biofortified rice has a considerable role to play, to curtail the menace of malnutrition especially in developing nations of sub-Saharan Africa. At present, there is a concerted effort between the International rice research institute (IRRI) and other agricultural organization in many countries researching evolved rice cultivars with high Zn (Bouis 2003) like Philippines, Bangladesh, India, and, Indonesia, and the, IRRI. Working on developing genotypes meant for micronutrient enhancement as part of its breeding program, already they have identified a number of high Zn rice germplasm that will serve as a donors parents, besides they have already developed and released cultivars to other research partners for multi-location trials , among which are Ciherang, IR64, NSICRc222, Sub1, Swarna, PSBRc82, BR28, BR11, and BR29, (Swamy BPM 2015) Presently numerous lines for elevated Zn that is undergoing trials in many research station within and outside Philippines like,Philippines rice research institute (Inabangan-Asilo MA 2015) Beside Philippines, other countries such as India and Indonesia, have gone very far in developing high Zn rice lines, their research status reach advanced stage of on station and multi- trials. The earliest high Zn lines contain 18–22 mg kg^{-1} of Zn with pleasing yield (Swamy BPM 2015)The first achievement of high Zn rice along with great Zn variety developed provide useful information to raise the biofortified breeding program to other emergent nations of Africa and Asia.

The bottleneck for deployment and adoption of Zn biofortified rice.

Yield reduction of biofortified rice

It is believed that dietary enhancement in any plants possibly will cause yield reduction sometimes total yield loss. Thus there is an urgent need to enriched inbred lines enough compared to the genetic base of non Biofortified rice.there is a need for reinforce our research institutes to engage in biofortification programmes; (Gupta et al. 2015).

Phenotyping and quality assurance of boiofortified rice

Breeding for biofortification require extra care throughout, because of contamination either genetic or physical possibly will weaken the quality and the traits genetic basis. Though, there is a need to quantify and evaluate the dietary contents at different breeding stages to guarantee the genetic gains within the plant. Therefore, it is imperative when setting any bio-fortification programmes to have well-equipped nutritional quality assurance laboratory. (Andersson et al. 2017)

Suitability of biofortified rice

The technique used in developing the biofortified plant also, determine its adoption. Biofortified Varieties developed using traditional breeding methods are fit for consumption, whereas varieties developed via genetic engineering is not acceptable for consumptions due to fear of GMO. There is also conflict among the scientific community. Furthermore, enrichment of nutrient associated with price hike especially to needy families, who suffers malnutrition problem, will be deprived of accessing bio-fortified crops. Thus there is a need to consider them to ensure that they can afford it.

Implementation of policies

There is an urgent need for passing policies at the national as well as states for smooth consumption of biofortified rice. Also to find a way for increase seed system to make sure that the superior and high-quality seed is available at a subsidized rate, additional incentives besides grain like inputs will also help in adoption of biofortified rice genotypes.

CONCLUSION

The concentration of Zinc within the rice grain inclined through crop-associated factors along with environmental factors, for better conscious of how they act together to affect rice grain Zn gathering is imperative for elevating the concentration of Zn in rice. Also will enhance efficient Zn uptake, as well as remobilization, is recognized as a critical log jam for Zn biofortification. These barriers can be solved using exploiting the hereditary collection of rice germplasm.

Future perspective and outlook

The use of wildtype with cultivars that have positive genetic dissimilarity of high Zn absorption.will increase Zn concentration, as well as its bioavailability. For this reason, Zn proficient and inept cultivars should be examined in different Zn conditions both adequate as well as undersupplied at a distinct phase of plant growth to discover the heritable ability for Zinc loading, Zn uptake, Zn utilization. Exploitation of Zn transporters plus Zn ligands into the aleurone coat is an essential goal for rice biofortification

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