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Review on

Rice Germplasm: Source of Iron and Zinc for Nutritional Security

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Abstract

Rice is the most important food crop, more than half (½) of the world's population depends on it for their sustainable livelihood. Population growth is increasing day by day and it will reach more than > 9 billion by 2050, and to feed the overpopulation we need to produce nearly double amount of food grains to fulfil the demand. It was projected that Global rice yields and consumption rate will rise by 12% and 13% respectively by the year 2027 (FAO 2018). The Green Revolution has played a prime role in the 1960s -1970s to increase agricultural productivity worldwide to make many countries in food self-sufficiency leading to food secured world. The present situation is posing serious challenge for global food security in coming decades due to climate change, limited availability of arable land and water, more over other natural resources are continued to exhaustion. Rice is consumed as sole source of energy mainly in South and Southeast Asia, Africa, and Latin America which causes micronutrients deficiency leading to chronic malnutrition. Malnutrition due to inadequate intake of micronutrients mostly iron and zinc can lead to 'Hidden Hunger', which is responsible for many diseases. Important micronutrients Fe and Zn deficiencies in rice promoting the hidden hunger and causes anemia, stunted growth, poor cognitive development for iron deficiency and for zinc deficiency that causes reduced immunity, diarrhea, lesions on skin, mental lethargy. Approximately 2 billion people are suffering from malnutrition deficiencies for iron and zinc. Micronutrient elements Fe and Zn are available in various local rice varieties which ranged from 6.3-24.4 mg/kg Fe and 13.53- 58.4 mg/kg Zn. Biofortification of rice can assist to alleviate malnutrition associated diseases among the poor people those who are depended on rice as staple food for 40-70% daily caloric intake. Nutritional studies recommended that 24–28 mg/ kg Zn and 13 mg/ kg Fe concentration in polished grain is vital to attain the 30% of human estimated average requirement. Biofortification of cereal foods through conventional breeding can be a good opportunity to improve micronutrient deficiency in the diets. Wild rice accessions (*Oryza rufipogon, O.nivara, O. latifolia* and *O. officinalis*) may be used to improve the mineral nutrition in rice grain through breeding and conserve as important resources.

Key-Words: Rice germplasm, Nutritional security, iron zinc content, wild rice.

Introduction

Rice (*Oryza sativa* L.) is the most important staple food for livelihood of half of the world's population. Global rice production was 782 million tons (paddy rice) produced from an area of 167 million produced 172 Mt rice (paddy rice) from 44.5 Mh of cultivated land. Yield rate and total production must be increased to meet the future demand due to population explosion. India is the second largest producer of rice after China with a production of 112.76 million tons in kharih 2017-2018(USDA report 2019). Recently it has been reported that (irristat-2018), cultivated area of rice over 161 million

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hectares globally and produced 488.3 million tons milled rice in 2018 (Dixit et al 2019). Approximately 8–10 million tons rice is to be added more each year to meet future demands for sustainable global food and nutritional security (Trijatmiko et al. 2016; Tripathy et al. 2017). It is estimated that more than 820 million people in the world are in starvation and under nourishment which resulted in poor health and disease susceptible (FAO 2019). The United Nations Organization has fixed target to achieve the Zero Hunger by 2030 under SDGs agenda. Rice (*Oryza sativa* L.) is a most important staple food crop and more than half of the World population are dependent on it (Wang and Li 2005). Based on genetic analysis rice (*O. sativa*) is classified into two subspecies, japonica and indica (Kato 1928), but belongs to five groups such as indica, aus, aromatic, temperate japonica and tropical japonica (Garris et al. 2005). Cultivated rice was domesticated around 10,000 years ago from common Asian wild rice O. rufipogon and O. nivara (Kovach et al. 2007; Chen et al. 2019). Study showed that two subspecies japonica and indica have undergone considerable amount of phenotypic changes compared to the wild rice progenitor *O. rufipogon* and *O. nivara*. The *O. rufipogon* is considered as proto-japonica and *O. nivara* as proto-indica (Fuller et al. 2010).

It was estimated that at least two billion people are micronutrient deficient (Trijatmiko et al. 2016; Tripathy et al. 2017). Micronutrients mainly zinc (Zn) and iron (Fe) is essential element for the normal growth and development. Iron is necessary for the synthesis of oxygen-transporting proteins like hemoglobin and myoglobin and maintenance of immune functions (Stoltzfus 2001; Bollinedi et al. 2020). Iron deficiency in humans causes many diseases like anemia, premature births, impaired cognitive and motor normal growth and improvement. Other mineral element zinc is necessary for biological functions such as cell division, reproduction and immunity maintenance (Brown et al. 2004) and deficiency causes immune system dysfunction, anorexia, delayed wound healing, cognitive disorder, hypogonadism (Salgueiro et al. 2000; Bollinedi et al. 2020).

Rice Germplasm for Zinc and Iron Content

Different rice germplasm were tested and quantified for iron and zinc content in the polished rice, about 2-8 µg/g iron (Fe) and 16 µg/g zinc (Zn) was detected in rice (Gregorio et al. 2000; Graham 2003). Initiative to augment iron (Fe) in the rice grain through conventional breeding is inhibited by the limited natural variation of iron in diverse rice germplasm. But recommended dose for human intake of Fe and Zn is 15 mg per day (Calayugan et al. 2020). Therefore, breeding program has been initiated by Harvestplus to biofortify rice at the rate 13 µg/g Fe and 28 µg/g Zn to meet approximately 30% of the estimated average requirement (EAR) for mineral nutrients. Rice germplasm are the good source of iron and zinc and these resources can be utilized for the improvement of mineral content in the rice grain through breeding (Figure 1).

Inadequate intake of micronutrients mostly iron and zinc can lead to ‘Hidden Hunger’, which is responsible for many diseases affecting about 2 billion people worldwide. Iron (Fe) deficiency anemia (IDA) may hamper physical development, and reduce immunity mainly in the women and children (Ludwig and Slamet-Loedin 2019). Harvest Plus program has estimated minimum Fe 13 µg/g in polished rice to fulfil the 30% estimated average requirement (EAR) (Bouis et al. 2011).

Therefore, biofortification for iron and zinc element (micronutrients) is needed to combat the mineral deficiency related human diseases. Bioavailability of micronutrients (Fe and Zn) in the edible parts of staple food rice can be enhanced by many ways- conventional breeding program, genetic engineering technology and agronomic practices (Bouis and Saltzman 2017). Biofortification of rice can assist to alleviate malnutrition associated diseases among the poor people those who are depended on rice as staple food for 40-70% daily caloric intake (Bouis and Saltzman 2017). Zinc deficiency is the most important factor for illness and diseases in developing countries according to the report of World Health Organization causes diarrhea and respiratory diseases, impaired immune response, leading to 400,000 deaths annually across the world. The zinc content in brown rice of the different germplasm of India including landraces, breeding lines, ranged from 7.3 to 52.7 mg/kg (Rao et al. 2020) mean value ranged from 15.9 to 27.3 mg/kg. In some cases it has reported that grain zinc content ranges 13.5 to 58.4 mg/kg. The zinc content in polished rice ranged from 4.8 to 40.9 mg/kg in rice germplasm of India. The zinc content in the Farmers' rice varieties of India ranged from 5 - 25 mg/kg in brown rice (Babu et al. 2014; Anandan et al. 2011), in polished rice zinc content varies 12-14 mg/kg in popular varieties of India. Other report showed that zinc content in polished rice ranged from 4.8 - 40.9 mg/kg (Rao et al. 2020). Harvest Plus program has estimated the threshold value of zinc content in polished rice is 28 mg/kg. It was also observed that overall mean percentage of loss of zinc content is around 19.0% during polishing, which means 1.9 mg/kg loss of zinc and 10 mg/kg of brown rice at the time of polishing. Zinc percentage loss ranged from 5 to 30% in different germplasm during polishing, the variation may be due to the presence of diverse magnitude of thickness in the aleurone layer which also depends on rice varieties' genetic makeup (genotypes) (Gregorio et al. 2000; Sellappan et al. 2009). They showed wide range of iron from 6.9 to 22.3 mg/kg, and zinc ranged from 14.5 to 35.3 mg/kg in brown rice (Maganti et al. 2019). Loss of iron during polishing is more (16 to 97%) compared to zinc ranged from 1 to 45 % (Maganti et al. 2019). Rice germplasm can be considered as promising donors of zinc if they content ≥35 mg/kg in brown rice (Rao et al. 2020). Nutritive value mainly zinc and iron cab be improved through technological advancement in polished rice to alleviate malnutrition related to micronutrients deficiencies because per-capita consumption ranges from 62kg/year to 190 kg/year in rice consuming Asian and African countries (Dixit et al 2019), suffer from chronic micronutrient malnutrition even called as 'hidden hunger' (Muthayya et al. 2013). Fe and Zn deficiencies are in principal widespread micronutrient deficiencies in humans, affecting two billion people and 0.8 million deaths occurs annually due to malnutrition (WHO 2011). World food production was increased in 1996s significantly through the application of advancement of science and technology in agriculture leading to make many countries food self-sufficiency and globally referred to as 'Green Revolution' (Ortiz 2011) which also helped to prevent occurrence of frequent famines and reduced socio-economic disturbances (Khush 1999) in the developing countries. Green Revolution produced at least 20% more food grains in the countries of Asia and Latin America and kept food prices at least 19 % less. Although it is significantly

Figure 1. Local rice varieties are conserved through *in situ* on-farm conditions (more than 65 rice varieties conserved) and evaluated for the quantification of minerals like zinc and iron.

reduced the proportion of under nourished people worldwide, still the problem of malnutrition existed due to lack of quality grain (micronutrients) which is also associated with the socio-economic saddle (Pingali 2012) of the poor people. At least 2 billion people affected due to micronutrient deficiencies in staple food (called as hidden hunger) mainly in South Asia, Latin America and sub-Saharan Africa (FAO 2015), and urgently demand for solution of this chronic problem of hidden hunger. Biofortification of cereal foods through conventional breeding can be a good opportunity to improve micronutrient deficiency in the diets (Bouis and Salzman 2017). Successful breeding program depends on some preconditions such as availability of genetic variation within the gene pool of specific traits. Breeders generally target the additive genetic effects, transgressive segregation pattern, or heterosis principles of the target traits for their improvement in the breeding lines; it is possible if numerous genetic variations exist in the germplasm of the crop (Garcia-Oliveira et al. 2018). In rice, polishing of grain removes up to 50% iron from the raw brown rice (Gregorio et al. 2000). Micronutrient zinc (Zn) concentration is two-fold higher in *indica* rice type compared to japonica rice type, but contains less amount of iron (Fe) (Yang et al. 1998). Modern rice varieties are with less amount of Fe and Zn compared to farmers' varieties and landraces (Anandan et al. 2011) because breeders have not given prime importance to the enrichment of micronutrients, instead yield and other traits.

Zinc and iron availability in the soil

Level of these nutrients in cereal grains depends on the availability of these nutrients elements in the soil conditions and other environmental factors, infertile soils and obviously the germplasm types (Velu et al. 2014). Wild rice accessions may be used to improve the mineral nutrition in rice grain through breeding and conserve as important resources (*Oryza rufipogon, O.nivara,O. latifolia* and *O. officinalis*) (Anuradha et al. 2012).

Detection methods of mineral elements zinc and iron

Screening and evaluation of micronutrients contents in the available germplasm is necessary for the possibility of introgression of favorable gene through recombination in breeding lines for high zinc and iron content without compromising yield and grain quality. Biofortified rice varieties with high zinc and high iron can help to achieve nutritional security in the country. The ICP-AES technique is used to quantify mineral elements zinc and iron content in rice grain. Fully quantitative methods are employed in the detection of microelements in the rice grains such as inductively coupled plasma-optical emission spectrometry (ICP-OES), ICP-mass spectrometry, the energy-dispersive X-ray fluorescence spectrophotometry (Pfeiffer and McClafferty 2007).

Genetic Technology for Biofortification of Iron and Zinc in Rice

Transgenic rice overexpressing the nicotianamine synthase genes of rice (OsNAS1) and barley nicotianamine aminotransferase gene (HvNAAT) can increase the accumulation of Fe in the endosperm (up to 55 µg/g) (Diaz-Benito et al. 2018). Overexpression (OE) of SoyFerH1 gene has also increased the Fe (up to 38 µg/g) in the rice endosperm (Swarna, BR29, IR64) using endosperm specific promoters such as OsGluB1, OsGtbl, OsG1b) (Slamet-Loedin et al. 2015). Overexpression of the the rice ferritin gene (OsFer2) increased the Fe concentrations in T3 rice grain (up to ~15.9 µg/g) compared to the control cultivar Pusa-Sugandh II with approximately 7 µg/g (Paul et al 2012). Iron uptake and translocation can be increased by OE the OsYSL15, which is accountable for the uptake of Fe (III)–DMA (Lee et al. 2009a) and OsYSL2 for the uptake of Fe (II)–NA from the rhizosphere (Ishimura et al. 2010) resulting in higher Fe content up to 7.5 µg/g in polished rice compared to control cultivar (~1.8 µg/g) (Senoura et al. 2017). YS1 (Yellow Stripe) gene functions as a proton-coupled symporter for phytosiderophore (PS)-chelated metals which has high affinity Fe3+-MA transporter. Better results were attained in the OsNAS2 plants with a Fe level-up to 19 µg/g in polished rice (Johnson et al. 2011) compared to wild type (4.5 µg/g).

Multigene cassette can be inserted in to the plant genome for the overexpression of the genes for desired enhancement of minerals in the grain endosperm. Storage gene PvFER, chelator gene AtNAS1, and iron storage gene AtNRAMP3 has been expressed in rice resulting in enriched grain with 13.65 µg/g iron in greenhouse condition (Wu et al. 2019). Other result showed little higher amount of Fe 15 µg/g concentration in polished grain (Trijatmiko et al. 2016).

Agronomic Biofortification

Agronomic biofortification can be opted to enrich iron in the rice grain through soil agronomic practices using fertilizer-based procedure that is easy and cost effective (Cakmak and Kutman 2017; (Ludwing and Slamet-Loedin 2019), because rice mainly cultivated in lowland irrigated areas where Fe is accessible in high amount. It can be an alternative of genetic biofortification methods. Main obstacle is that the translocation of the mineral from the vegetative part to the grain (Slamet-Loedin et al 2015). The Zn fertilizers may be used in the rice fields deficient in available Zn levels to enrich grain with high Zn content (Johnson-Beebout et al. 2009). Additionally a combination of genetic and agronomic approaches can be required to enhance grain Zn concentration. Prevalence of zinc deficiency throughout the world has been assessed to be ~20% in soils (Hotz and Brown 2004).

Conventional Breeding as an Effective Tool for Zn Biofortification

During Green revolution 1960s developed HYV rice varsities but grains of HYVs contain lesser amounts of nutrients; and polishing further diminishes the nutrients concentration mainly iron and zinc (Rao et al. 2020). Biofortification has been initiated by CGIAR through the launching of Harvest Plus Challenge Program in 2003 (Harvest Plus 2003) to enrich rice grain with iron and zinc using breeding system. The achievement of biofortification depends on the existence of diversity for the desired trait available in the germplasm, to be used in breeding program for successful recombination of the trait (iron and zinc content) with yield stability. To phenotype zinc content in the rice grain X-ray fluorescence spectroscopy (XRF) or ICP-AES can be used. Conventional breeding principles have been used to develop one improved breeding line with enriched iron in brown rice (21 mg/kg) by crossing IR72 (HYV) with traditional variety (ZawaBonday) (Gregorio et al. 2000). Protein content in milled rice is approximately 7% w/w (8.5% in unpolished brown rice) which is low compare to other cereal foods (wheat, barley and millets). Major protein is glutelin about 60-80% and prolamine consisting only 20-30% of the total amount (Xu and Messing 2009). Rice supplies around 40% of the total protein consumed by peoples in developing countries and protein quality is high, because the protein has essential amino acid lysine richness (3.8%) (Shobharani et al. 2006). Anti-nutritional factor as like as phytic acid make complex structure with seed proteins and essential minerals mainly Zn, Fe, and Ca, ultimately reduced the bioavailability of these micronutrients. Nutritional studies recommended that 24–28 mg/ kg Zn and 13 mg/ kg Fe concentration in polished grain is vital to attain the 30% of human estimated average requirement (Bouis et al. 2011).

Micronutrients uptake depends on the plant age, tissue specific demand and root system but overall mechanism accountable on genotype constitution of the rice varieties (Fageria 2013).

Environmental factors are also responsible for Zn accumulation in rice grains such as Zn availability in the soil, temperature and atmospheric carbon dioxide level (Welch and Graham 2002; Fernando et al. 2014b). During Green Revolution prime focus was to develop high yielding varieties to increase yield without considering quality (Graham and Welch 1996). At present, emphasis has been given to develop high grain quality rice varieties specially zinc and iron to alleviate hidden hunger related diseases (Kant et al. 2012; Myers et al. 2014). Germplasm screening is the most excellent choice before begin the genetic approach (breeding program) to enrich Zn concentration in the grain. Through which we will know the availability of unique genetic variation existed in the gene pool of local landraces, traditional varieties and even in wild species to accomplish breeding targets of high zinc in rice endosperm. Enormous germplasm collection of IRRI has been analyzed for Zn concentration in brown rice, which showed considerable genetic variation in zinc content (13.5–58.4 mg/Kg), with an average of 25.4 mg/Kg (Boonchuay et al. 2013). Range of zinc varies from 7.3 to 52.7 mg/kg in some Indian rice germplasm (Rao et al. 2020). BRRI dhan-62 has been developed and released by Bangladesh (2013) containing zinc concentration 20-22 mg/kg in brown rice; it is the world's first rice variety with enriched Zn. Harvest Plus program has suggested 30 mg/kg Zn concentration in brown rice, BRRI dhan-62 contains Zn amount is below the target value (Shahzad et al. 2014). The Zn biofortification can be enhanced by using the under-utilized genetic resources of rice germplasm (genetic factors) including environmental aspects, and field management policies (agronomic factors). Therefore, it is to understand completely that what roles played by these factors in Zn accumulation in the rice endosperm. These factors may be the vital bottleneck for Zn absorption and remobilization in the different plant tissues and ultimately to grain leading to biofortification of Zn by enriching concentration (Nakandalage et al 2016).

The amount of micronutrients (zinc and iron) in rice grain is a major factor for determining its nutritional value (Anuradha et al. 2012). Rice grain (brown) is consisting of endosperm (90 % w/w), bran (6-7 % w/w) and embryo (2-3 % w/w) (Chen et al. 1998). Bran layer is the key storehouse for dietary fiber, proteins, vitamins, minerals, and lipids compared to the central endosperm layer (Shahzad et al. 2014). Rice grain comprises of 80% starch, 7.5% protein, 0.5% ash, and 12% water. On average 300 g rice is ingested per day by adult people of China and India and about 62-190 kg annual consumption (Lu et al. 2008). The daily Zn requirement is 15 mg for adult (Lu et al. 2008; Lu et al. 2013) and children above 4 years old.

The Zn concentration is 3 times more in the bran layer than that of hulls and endosperm (Lu et al. 2013). Polished rice delivers only one fifth amounts of daily Zn requirements, because during polishing bran layer is removed which depletes zinc from rice grain leading to zinc deficient rice grain (Sharma et al. 2013). Therefore, Zn deficiency is a global health problem specifically in Asia and Africa where rice has take as staple diets (Impa and Johnson-Beebout 2012; Myers et al. 2015; 2015). More than 2 billion people in Asia and 400 million in Sub-Saharan Africa are at risk of Zn deficiency associated diseases.

It is important to increase the Zn concentration in the rice endosperm to combat the zinc deficiency related diseases. That can be accomplished by understanding the genetic mechanism of Zn uptake by root system, its transport and remobilization in the different plant tissues and interactions to the environmental factors to raise Zn concentration in rice endosperm.

Conclusion

New improved breeding lines can be developed using the promising local germplasm (>35 mg/kg Zn) as donor through recombination breeding and selection to fulfil the goal of HarvestPlus to alleviate zinc deficiency related diseases. It has been observed that approximately > 70% of micronutrients is removed during polishing of rice grain. External application of zinc in the soil during cultivation can be a crucial to maintain the nutrient performance potential of a biofortified rice variety. Recommended daily allowance (RDA) of zinc is 12 mg/kg for male and 10 mg/kg for female. Biofortified rice intake with enriched zinc content can meet 38 to 47% of the RDA for male and 46 to 57% of the RDA for female to keep healthy us. In order to meet the RDA value of zinc, as sole source of diet, it must have 54.5 to 68.2 mg/kg zinc in polished rice. Available rice germplasm does not content such amount of zinc in the grain. Advanced transgenic technology has been utilized to enrich rice grain with high quantity of zinc 34.9 to 55.5 mg/kg by inserting soybean ferritin gene. Transgenic IR64 rice lines with nicotianamine synthase (OsNAS2) and soybean ferritin (SferH-1) genes inserted has increased zinc content to 45.7 mg/kg in polished rice without changing any other important traits such as yield and quality.

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