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Agronomic biofortification of zinc in rice: Influence of cultivars and zinc application methods on grain yield and zinc bioavailability

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ABSTRACT

Zinc biofortification in rice can be improved by altering Zn application timing and placement and cultivar choice. We made a comprehensive assessment on this, analysing Zn, Fe and phytic acid in whole grains and processed brown, white and cooked rice obtained from six cultivars raised with Zn applied through soil and/or foliar supply at different phenological stages of the crop and measuring Zn bioavailability in cooked rice. Pathways for Zn enrichment (27.4–92.6% over control) by Zn fertilization with different application protocols and cultivars were elucidated. Such enrichment of Zn was associated with depletion in Fe (6.5–29.4%) and phytic acid (14.8–30.4%). However, the loss of Zn on processing of rice grains increased on Zn fertilization (12.6–28.7 mg kg^{-1}) because of a preferential allocation of applied Zn into bran and aleurone of the grains. Despite such loss, application of Zn caused a net increase in Zn bioavailability (52.2% over control) in the cooked product. Using the ranksum scoring technique, we found cultivar GB 1 and Zn supply through soil (basal) + 2 foliar applications achieved the most effective biofortfication of Zn in rice by optimizing grain yield, and enriching Zn and its bioavailability in cooked grain with least antagonism of Fe availability.

1. Introduction

Dietary deficiencies of Zn and Fe are a serious global public health problem affecting over two billion people and causing a loss of 63 million life-years annually (Myers et al., 2014). These cases of malnutrition are more acute in populations of Africa, South and South East Asia where cereals, the major staple foods, are low in dietary Zn and Fe. Rice is of major importance particularly in South and South East Asia because it contributes more than two thirds of the energy intake of its population (Timsina et al., 2010). Zinc concentration in rice grains can be enriched by: i) biofortification with popular Zn fertilizers (Cakmak, 2009), ii) manipulating Zn transporters and ligands in rice plants (Palmgren et al., 2008; Borrill et al., 2014) and iii) efficient germplasm screening for higher bioavailable Zn (Blair 2014; Trijatmiko et al., 2016). All these methods depend on fertilizer or the soil or both as the source of Zn to produce Zn enriched grains. Soil supplied Zn is, however, limited depending upon soil properties such as pH and redox potential, contents of CO_3^{2-} and HCO_3^{-}, oxides of Fe and Al, and organic matter (Mandal and Mandal, 1990) and inherent Zn status in the upper soil layer (Tuyogon et al., 2016). The problem of low Zn availability to plants is exacerbated when rice is grown in submerged soils (Meng et al., 2014). Application of Zn fertilizer is the most common option to overcome such problems. But recovery of applied Zn by rice hardly exceeds 2% of the applied amount (Alloway, 2008).

Tailoring Zn application protocols may help to enhance transport of applied Zn to the edible parts in plants and thus its use-efficiency. We, therefore, designed Zn application protocols using key principles of Zn nutrition of rice namely: (i) soil applied Zn after undergoing reactions with soil components, is absorbed by roots, travels through xylem to storage tissues, leaves and subsequently to grains via phloem (Pottier et al., 2014) despite a number of impediments like high pH of phloem sap, chelation processes etc. (Impa and Johnson-Beebout, 2012); (ii) on the contrary, foliar applied Zn moves faster within plants but retranslocation is dependent on plant nutritional status, germplasm and plant phenological stage (Sperotto, 2013). Immature leaves are physiologically incapable of exporting nutrients until they mature, while mature leaves export nutrient directly via phloem to developing grains and other organs but are incapable of importing (Fernandez and Brown,

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2013). Leaf maturity determines whether a leaf competes with grain as a sink for Zn or whether it can act as a source for Zn translocation to grains. Hence an application of Zn both through soil and foliar methods may help to overcome the above difficulties and improve the efficiency of loading of Zn into grains from applied Zn. Zinc application either in soils or onto leaves at different phenological stages or both, may influence Zn enrichment in grains. However, cultivars are known to respond differently to Zn application depending upon their inherent grain Zn density (Hegelund et al., 2012; Saha et al., 2015a). Cultivars with high Zn density in grain are resistant to further enrichment; while those with low density are more responsive to Zn enrichment (Saha et al., 2015a). To capture the actual differences among the Zn application protocols, the tested cultivars thus need to be relatively similar in their native Zn density.

Efficient Zn application protocols and cultivars may enrich grains with Zn but cannot ensure higher Zn bioavailability in the ultimate food products prepared from them. This is particularly true for rice, since it undergoes three steps of processing viz., hulling involving removal of the husk or the seed coat from the paddy grains yielding the brown rice, milling/polishing that removes the bran containing the embryo and aleurone from the brown rice producing white rice and cooking which involves removal of the boiled water soluble Zn from the white rice through gruel to yield low Zn cooked rice for consumption. A substantial loss of Zn may occur in each of the steps from the grains. Spatial distribution of Zn in those components of the grain ultimately determines the magnitude of loss of Zn during the processing.

The enrichment of Zn in grains needs to be assessed in relation to changes in other important nutritional traits of grain viz., concentrations of Fe (Giordano and Mortvedt, 1972) and phytic acid, the phytic acid:Zn molar ratio (Cakmak et al., 2010; Hussain et al., 2012), protein (Cakmak, 2008) etc. Such changes in composition of grains or cooked rice may influence their Zn bioavailability, since it is widely understood that the two primary factors affecting dietary Zn absorption in adults are the quantities of Zn and phytate in the diet (Miller et al., 2007). Capturing all the above changes in nutritional traits in grains in response to Zn enrichment is thus necessary for a comprehensive evaluation of the net gains from Zn biofortification in crops. In our present study, we have used a model proposed by Miller et al. (2007) to estimate Zn bioavailability from cooked products for more accuracy rather than phytic acid:Zn molar ratio. Estimation of Zn bioavailability through Caco-2 cell model is another option in this regard (Jou et al., 2012). In our previous study on agronomic biofortification of Zn in wheat, we have shown i) the best timing for application of Zn along with suitable cultivars, ii) the magnitude of Zn induced Fe and phytic acid depletion with different treatments and cultivars, iii) the extent of loss of Zn on processing (milling and cooking i.e. two step processing) of wheat grains and iv) the net magnitude of bioavailability of Zn from flat breads made from Zn-loaded wheat grains (Saha et al., 2017). Rice differs from wheat in many ways particularly its i) growing environments (submerged, low redox potential soil causing significant variations in nutrient availability), ii) native tendency for enriching seed endosperm with Zn by lowering Zn concentration in vascular tissues (Stomph et al., 2014) and iii) grain processing involves three steps viz., i.e. hulling, milling and cooking to make consumable products. All these may profoundly influence biofortification of Zn in rice compared to that in wheat (Stomph et al., 2009; Mabesa et al., 2013). Keeping the above in view, a series of experiments were conducted to test the hypotheses formulated to ascertain i) a suitable Zn fertilization protocol and cultivar for maximizing Zn loading in grains, ii) the loci (hull, bran or endosperm) of such enrichment of Zn in the grains and the extent of its loss during processing, iii) the amount of Zn bioavailability in cooked rice with compositional changes on Zn application, and iv) the best cultivar and Zn application protocol optimizing Zn loading with increased grain yield, reduced Zn-Fe antagonism, favourable phytic acid:Zn molar ratio and enhanced Zn bioavailability in ultimate food product (cooked rice) for human consumption.

2. Materials and methods

2.1. Experiment 1

2.1.1. Experimental sites

The experiment was conducted in the University Research Farm located (22°60′N, 88°23′E) under hot and humid climate with annual average rainfall of about 1480 mm, and maximum and minimum monthly temperature of 36.2 ± 2.0 °C and 12.5 ± 1.0 °C, respectively. After primary land preparation, soil samples were collected (0–0.2 m layer) from the experimental fields (two adjacent Zn-deficient fields were chosen for consecutive years to avoid residual effect of applied Zn) for analysis of pH (soil: water: 1:2.5), oxidizable organic carbon (Walkley and Black, 1934), 0.32% $KMnO_4$ extractable N, 0.5 M $NaHCO_3$ (pH 8.5) extractable P, 1.0 M $NH_4CH_3CO_2$ (pH 7.0) extractable K (Jackson, 1973) and 0.005 M DTPA extractable Zn and Fe (Lindsay and Norvell, 1978) following standard methods. The soil was an Aeric Endoaquept with loamy texture (sand, silt and clay = 35–38, 43–45 and 23–25%, respectively), neutral reaction (pH 6.7–7.5), medium organic C (5.0–6.0 g kg^{-1}) and extractable N, P and K (340–360, 20–24 and 180–200 kg ha^{-1} respectively), low DTPA extractable Zn (0.5–0.6 mg kg^{-1}) but adequate Fe (30–40 mg kg^{-1}).

2.1.2. Cultivars used

The major characteristics of the six selected rice cultivars viz., Gobindobhog, GB 1, MTU 7029, KRH 2, Satabdi and Lalat used for the study (Table 1) were determined before the commencement of the present experiment. The seeds, raised with same management practices (without Zn application), were taken from a gene bank of the university and considered to be true to type with their native characteristics including similarity in Zn density. The cultivars are widely grown and cover almost the entire rice-area of this region of the world.

2.1.3. Management practices

Twenty one day-old seedlings of the six cultivars were transplanted with 6 treatments of Zn (Table 1) in the fields both in 2011–12 and 2012–13 during 10 to 15th July at 25.0 cm × 25.0 cm (row to row and

Table 1
Characteristics of the rice cultivars tested before the commencement of the experiment.

Cultivars	Parentage	Duration (days)	Average yield (t ha^{-1})	Zn in grains (mg kg^{-1})	Zn Harvest Index*	Fe in grains (mg kg^{-1})	Phytic acid in grains (g kg^{-1})
Gobindobhog	Folk Rice	130–135	2.5^{c}	18.6^{b}	0.09^{c}	49.8^{ab}	17.5^{c}
GB 1	Selection from Assam Rice Collection	110–115	5.0^{a}	20.6^{b}	0.19^{a}	43.6^{b}	27.0^{a}
MTU 7029	Vasistha × Masuri	140–145	5.2^{a}	21.0^{b}	0.17^{a}	44.6^{b}	21.9^{b}
KRH 2	IR 58025A × KMR 3R	130–135	5.5^{a}	19.8^{b}	0.19^{a}	38.8^{b}	27.4^{a}
Satabdi (IET 4786)	CR-10–114 × CR-10-115	120–125	4.5^{b}	20.8^{b}	0.13^{b}	59.0^{a}	26.3^{a}
Lalat	Obs 677 × IR–207 × Vikram	125–130	4.5^{b}	28.1^{a}	0.18^{a}	56.7^{a}	26.6^{a}

Different superscripted letters denote significant differences at $P < 0.05$.
* Zn Harvest Index = [Zn in grains (mg kg^{-1}) × grain yield (t ha^{-1})]/[{Zn in grains (mg kg^{-1}) × grain yield (t ha^{-1})} + {Zn in straw (mg kg^{-1}) × straw yield (t ha^{-1})}].