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Rescheduling zinc fertilization and cultivar choice improve zinc sequestration and its bioavailability in wheat grains and flour

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ABSTRACT

Zinc application may produce grains with increased Zn, but it cannot ensure a higher bioavailable Zn in the ultimate food products prepared from the grain. The aim of the present study was to optimize Zn fertilization protocols for different wheat cultivars with a view to achieve a higher enrichment of Zn in grains, and reduced Zn-Fe antagonism and loss of Zn on processing to achieve enhanced bioavailability of Zn in flour. We evaluated the effectiveness of Zn biofortification in wheat using four criteria viz., i) magnitude of Zn sequestration in flat breads of wheat, ii) Fe retention in it, iii) maintenance of a favourable phytic acid: Zn molar ratio in breads and iv)enhanced grain yield. Zinc fertilization protocols involving its application through soil, foliar spraying or both at critical phenological stages were tested with six cultivars. Both the grains and flat breads were analysed for Zn, Fe and phytic acid. Zinc fertilization yielded Zn dense but Fe- and phytic acid-depleted grains. However, 40–75% of Zn enrichment in grains was lost during processing to prepare the breads. Zinc application through soil + foliar at maximum tillering and flowering stages with cultivar UP 262 excelled in effecting not only higher grain yield but also grain Zn biofortification that ensured a net gain in Zn in breads, and its Zn bioavailability through a reduced antagonism with Fe and phytate.

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). This is mainly because cereals that constitute about two-thirds of the energy intake of humans particularly in developing countries are low in Zn and Fe. Fortification of cereal grains with Zn offers a cost effective solution of the problem of Zn deficiency. This can be pursued through i) fortification of widely used fertilizers with added Zn (Cakmak, 2009), ii) Zn loading in the cereal grains by manipulating Zn-transporters and Zn-ligands (Palmgren et al., 2008; Borrill et al., 2014), iii) selecting germplasm with high bio-availability of Zn in grains or grain products (Blair, 2014), and iv)screening of elite cultivars to identify those efficient in Zn acquisition from soil. Whatever the method, extra Zn in plants can come only from the fertilizer, the soil or both. Soil supplied Zn is, however, limited in plant-availability as manifested by its widespread deficiency in plants throughout the globe. Fertilizer Zn plays a vital role in boosting grain Zn, but Zn-use efficiency of soil-applied Zn by plants hardly exceeds 2% of the applied amount (Mandal et al., 1988; Alloway, 2008). Under such circumstances, development of highly efficient fertilizer molecules (Subbaiah et al., 2016) or tailoring of Zn application to enhance transport of increased amounts of Zn to the edible parts in plants is needed. To achieve enrichment of Zn in grains, we designed Zn application protocols for wheat using key principles of Zn nutrition, namely that: 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 (high pH of phloem sap, chelation processes etc., while, ii) foliar-applied Zn moves more effectively within plants from target leaves to edible parts of the plant, but retranslocation is dependent on plant nitrogen and Zn status, cultivar and plant phenological stage. Immature leaves are physiologically incapable of exporting nutrients, while mature leaves export nutrient directly via phloem to developing grains and other organs but are incapable of importing (Fernandez and Brown, 2013),which determines whether a leaf competes with

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Table 1
Characteristics of the wheat cultivars tested.

Cultivars	Parentage	Duration (days)	Average yield (q ha^{-1})	Zinc in grains (mg kg^{-1})	Zinc Harvest Index	Iron in grains(mg kg^{-1})	Phytic acid in grains (g kg^{-1})
UP 262	S 308/BAJIO 66	126–134	31–35	34.6^{ab}	50.8^{a}	55.3^{a}	8.8^{a}
Sonalika	II-53–388/ANDES//(SIB)PITIC-62/3/LERMA-ROJO-64	126–134	36–40	39.5^{a}	51.4^{a}	48.5^{ab}	8.0^{a}
DBW 39	ATTILA/HUI	110–120	40–45	37.2^{a}	47.1^{ab}	53.3^{ab}	8.2^{a}
HD 2733	ATTILA/3/TUI/CARC//CHEN /CHTO/4/ATTILA	126–134	46–50	33.7^{ab}	48.0^{ab}	53.6^{ab}	8.5^{a}
K 0307	K 8321/UP 2003	120–122	40–48	37.7^{a}	49.9^{ab}	52.8^{ab}	8.1^{a}
PBW 343	NORD-DESPREZ/VG-1944//KALYAN SONA//BLUE BIRD/3/YACO(SIB)/4/VEERY-5	126–134	46–50	33.1^{ab}	53.4^{a}	58.6^{a}	8.7^{a}

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})}].
Different superscripted letters denote significant differences at $P < 0.05$.

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

Zinc application may produce grains with high Zn, but it cannot ensure a higher bioavailable Zn in the ultimate food products prepared from it since during processing a good amount of Zn may be lost (Cakmak et al., 2010a; Saha, 2014). In addition, enrichment of Zn in grains occurs concomitantly with changes in other important nutritional or anti-nutritional properties viz., a possible depletion in Fe concentration (Giordano and Mortvedt, 1972; Saha et al., 2015), lowering of phytic acid:Zn molar ratio (Hussain et al., 2012), and enhanced loss on grain Zn on processing (Saha, 2014). Capturing all these changes in response to Zn enrichment is necessary for a comprehensive evaluation of the net gains in nutritional quality of grains from Zn biofortification. The aim of the present study was to optimize Zn fertilization protocols with proper choice of wheat cultivars with a view to achieve a higher enrichment of Zn in grains, and reduced Zn-Fe antagonism, and loss of Zn on processing to achieve enhanced bioavailability of Zn in ultimate food products for humans.

2. Materials and methods

2.1. Experimental sites

The experiment was conducted on the University Research Farm (22°58′N, 88°29′E) located 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 any Zn) for analysis of pH(soil:water::1:2.5), oxidizable organic C (Walkley and Black, 1934), KMnO4 extractable N, Olsen extractable P, 1.0 M ammonium acetate extractable K (Jackson, 1973) and DTPA extractable Zn and Fe (Lindsay and Norvell, 1978) following standard methods. The soil was Aeric Endoaquept with loamy texture, neutral in reaction (pH 6.5–6.7), medium in organic C (6.0–8.0 g kg^{-1}) and extractable N, P and K (426–450, 28–36 and 238–290 kg ha^{-1}, respectively), low in DTPA extractable Zn (0.6–0.7 mg kg^{-1}) but adequate in Fe (38–51 mg kg^{-1}).

2.2. Cultivars used

The key characteristics of the six wheat cultivars (UP 262, Sonalika, DBW 39,HD 2733, K 0307 and PBW 343) used for the study are given in Table 1. The seeds, raised on the same production field, were taken from a gene bank of a National wheat project operating at the university with funding from the Indian Council of Agricultural Research, New Delhi. The selected cultivars represent those most commonly grown and cover almost the entire wheat area of the eastern India.

2.3. Management practices

Seeds of the six cultivars were sown with 6 treatments of Zn (Table 2) in the field both in 2010–11 and 2011–12 during 20 to 25th November at 22.5 cm row to row spacing in plots of 30m^2 (6.0 × 5.0 m) with three replications. Fertilizers were applied (N:P:K – 120:26.2:46.5 kg ha^{-1}) on the basis of soil test values. One-half of the N and the entire amounts of P and K were applied at the time of sowing and the other half of N at maximum tillering stage (~40 days after sowing) of the crop. Irrigations were given (80 L of water/m^2/irrigation) at the time of sowing, crown root initiation, maximum tillering, flowering and grain filling stages. All other recommended practices, as advocated by the Indian Institute of Wheat and Barley Research for the eastern part of India (www.dwr.res.in/node/80) were followed for raising the crop.

Zinc deficiency in soil severely limits yield of wheat in this region due to low plant-available soil Zn (Mandal and Mandal, 1986). Accordingly, Zn treatments were designed to boost Zn supply at sowing, maximum tillering or flowering stages and their combinations to study the effects of root uptake and remobilization of Zn from source tissues to grains for its Zn enrichment. Basal (soil) application of Zn was made to avoid Zn deficiency at initial growth stage; while its foliar application(s) was done for loading Zn in grains and straw. Zinc was applied through soil [basal at 25.0 or 22.5 (to supply the same total Zn after foliar application) kg $ZnSO_4 \cdot 7H_2O$(Merck, 99.9% purity) ha^{-1}] by broadcasting at the time of final land preparation and by foliar spray (3.3–6.6 kg ha^{-1} as a solution applied at 660–1320 l ha^{-1} using a Knapsack sprayer) at maximum tillering and/or flowering stages. The six treatments of Zn are described in detail in Table 2.

Two levels of farmyard manure (FYM) i.e. no FYM (OM_0) and FYM at 5.0 t ha^{-1} (OM_1) were also applied (two weeks before the sowing of seeds) along with the Zn treatments. The experiment was conducted in a strip-strip design for the two seasons mentioned. For