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BIOFORTIFICATION STRATEGIES TO INCREASE GRAIN ZINC IN WHEAT

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ABSTRACT

Wheat is a staple food of world, especially of developing countries, which lacks mechanism of zinc absorption, compared to pulses, more intention is therefore necessary to be given for the same. Micronutrient, zinc deficiency affects one third population of world. Zn is important for different metabolic processes of the human body and controls different enzymatic processes, which are responsible for good human health. Although it is not integral part like nitrogen, phosphorus and potash but recent aroused deficiencies in soil, plants and of course in human beings ranged a bell of danger. Improper and imbalanced application of fertilizers, especially of N, P, and K lack of awareness among farmers about zinc and its application and prospects, are some major primary factors that prevent its application. Application of zinc is therefore necessary in wheat through different sources that are compatible for particular soil, management and its availability. Zn biofortification is a technique in which the inherent Zn status of the edible portion of plants is improved by simply spraying a Zn solution onto the crop o through a soil application at a predetermined stage and a proper dose.

KEYWORDS: Zinc, Wheat, Biofortification

Zinc (Zn) is an essential trace element for the growth and development of humans animals and plants and recent estimates shows that over two billion of the world population is affected by Zn deficiency.Zinc is most often applied to crops through soil and foliar methods. The application of Zn through seed treatments has improved grain yield and grain Zn status in wheat. In cropping systems where legumes are cultivated in rotation with wheat, microorganisms can improve the available Zn pool in soil for the wheat crop (Cakmak *et al;* 2010a).Breeding and molecular approaches have been used to develop wheat genotypes with high grain Zn density options for improving grain yield and grain Zn concentration in wheat include screening wheat genotypes for higher root Zn uptake and grain translocation efficiency, the inclusion of these Zn-efficient genotypes in breeding programs, and Zn fertilization through soil, foliar and seed treatments.

BIOFORTIFICATION OF ZINC IN WHEAT

Zinc is an important mineral needed in small amounts by humans and plants for normal growth and development, immune system function, neurotransmitter function and reproductive health (Hotz and Brown, 2004). Wheat is a major source of daily food intake for masses but has low Zn bioavailability. The concentration of Zn can be enhanced in wheat by using agronomic or breeding/ molecular approaches to develop microelement-dense wheat genotypes (Velu *et al.* 2014). Zinc deposition in wheat grains requires the translocation of Zn from leaves .The major bottlenecks in Zn biofortification are (i) storage of excess Zn in root vacuoles, (ii) dependence of grain Zn concentration on leaf Zn translocation rather than Zn uptake during seed filling, and (iii) discontinuous xylem at the base of each grain in cereals being a major hurdle in the transfer of Zn (Palmgren *et al.* 2008).

AGRONOMIC APPROACHES

Growing wheat on low Zn soil is the principal reason for low Zn concentration in cereals, thereby reducing Zn bioavailability to humans. The agronomic approach involves a fertilizer strategy; it is a rapid solution for reducing Zn deficiency and increasing grain Zn concentration (Cakmak 2008), and it is most effective when Zn fertilizers are applied in combination with nitrogen, organic fertilizers and better crop cultivars. The application of organic/ inorganic Zn fertilizer increases grain Zn concentration. To effectively accumulate Zn in grains, information on the source of Zn and time of foliar application is critical. For instance, foliar fertilization post-flowering is more effective than soil application at improving Zn accumulation in wheat seeds (Cakmak *et al*. 2010a, b). Zinc application through foliar spray increased seed Zn concentration beyond the breeding targets (10 mg kg^{-1} of the baseline of cultivated varieties) suggested by nutritionists.

The effect of Zn biofortification (via application of six rates of Zn fertilizer to soil) on Zn bioavailability in wheat grain and flour and its impacts on human health was evaluated. Zn bioavailability was estimated with a trivariate model that included Zn homeostasis in the human intestine. As the rate of Zn fertilization increased, the Zn concentration increased in all flour fractions, but the percentages of Zn in standard flour (25%) and bran (75%) relative to total grain Zn were constant. Phytic acid (PA) concentrations in grain and flours were unaffected by Zn biofortification. Zn bioavailability and the health

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impact, as indicated by disability-adjusted life years (DALYs) saved, increased with the Zn application rate and were greater in standard and refined flour than in whole grain and coarse flour. The biofortified standard and refined flour obtained with application of 50 kg/ha $ZnSO_4.7H_2O$ met the health requirement (3 mg of Zn obtained from 300 g of wheat flour) and reduced DALYs by >20%. Although Zn biofortification increased Zn bioavailability in standard and refined flour, it did not reduce the bioavailability of iron, manganese, or copper in wheat flour.

Zinc bioavailability is more important than grain Zn concentration as most of the Zn is present in the aleurone and embryo of wheat seeds. Likewise, phytic acid concentration is higher in the germ and aleurone, and accounts for 75% of P stored in seeds. For instance, foliar fertilization of Zn not only improved the whole grain Zn concentration, but it enhanced the endosperm Zn concentration (Cakmak *et al*. 2010b) suggesting the high bioavailability of Zn to the human body. Moreover, application of Zn at later stages of grain development increased the bioavailable Zn concentration in endosperm as very low phytate is present in this seed fraction (Cakmak 2012). Similarly, Bharti *et al*. (2013) found that grain Zn concentration increased in wheat with a lower phytate concentration and phytate:Zn ratio. Recently, Saha *et al*. (2017) demonstrated that soil application + two foliar sprays of Zn or two post-flowering foliar applications of Zn reduced the phytate:Zn ratioto <10 which is well belowthe breeding target. Agronomic biofortification is a time-saving and effectiveapproach for improving Zn bioavailability in wheat grains. Soil and foliar Zn fertilization improve the Zn concentration and bioavailability to humans by reducing the phytate concentration and phytate: Zn ratio.

SELECTIVE AND CONVENTIONAL BREEDING

Cereal crops have large variation in terms of sensitivity to Zn deficiency, which can be used to select and develop Zn-efficient genotypes. Amiri *et al*. (2015) reported that Zn concentrations in bread wheat genotypes (*Triticum aestivum* L.) range from 31.64–55.06 mg kg^{-1} DW. Among relatives of wheat, *Triticum turgidum* ssp. *dicoccoides* (wild emmer wheat) has a wide range of variability for Zn (14–190 mg kg^{-1} DW) and Fe (15– 109 mg kg^{-1} DW), and the highest concentration of these two microelements surpass those in modern wheat cultivars (Cakmak *et al*. 2004). In fact, of the studied populations of wild emmer wheat, Tabigha (*Terra rossa*) has great potential in breeding programs with its high Zn, Fe and seed protein concentrations. The link between seed protein, Fe and Zn concentrations provides an opportunity to develop these three traits simultaneously (Chatzav *et al*. 2010). Furthermore, Znefficient wheat genotypes can be developed by transferring the genes in rye responsible for Zn efficiency (Rengel 1999).

Precision phenotyping is important for the development of high-Zn wheat genotypes. However, environmental factors, particularly soil physiochemical properties, influence breeding for high Zn cultivars. This can be overcome by applying Zn fertilizer and maintaining a homogenous Zn concentration in soil. In wheat, Zn and Fe are quantitative traits. There is a strong positive correlation between modern, wild and spelt wheat for grain Zn and Fe concentrations showing that genetic and physiological factors responsible for Zn and Fe are similar. In conclusion, genetic diversity among different wheat genotypes and wild and cultivated relatives for grain Zn concentration should be exploited to develop Zn-dense wheat cultivars.

MOLECULAR APPROACHES

Molecular markers are useful for identifying genotypes with high mineral concentration without the need for field testing. In this regard, QTL mapping is effective in the identification of QTLs responsible for grain Zn concentration in wheat. Moreover, QTLs responsible for protein, Zn and Fe significantly overlap suggesting a positive correlation between Zn, Fe and grain protein concentrations. Distelfeld *et al*. (2007) cloned the TtNAM-B1 gene from *Triticum dicoccoides*, which influences seed Zn, Fe and protein concentrations. Quantitative trait loci are mapped using two methods viz. association and linkage mapping. In a recent study, Sadeghzadeh *et al*. (2015) identified QTLs for Zn in barley (*Hordeum vulgare* L) and reported that two regions (2HL and 2HS) are linked with grain Zn concentration. They found that 45% and 59% of the variation in seed Zn concentration and seed Zn content, respectively, was linked with these two regions. This QTL can help in the identification of genes, and their transfer in plants for improving crop Zn status as transgenic wheat genotypes have high grain Zn concentration with more bioavailable Zn.

Molecular/transgenic approaches offer a more rapid solution than conventional breeding approaches to identify and transfer genes and QTLs responsible for grain Zn concentration or phytase activity, and their transfer and expression in newly developed wheat genotypes has proved effective in increasing grain Zn concentration and Zn bioavailability.

At present, studies in wheat are restricted to the endosperm-specific expression of wheat or soybean ferritin which led to increases in grain iron content of 1.5 to 1.9-fold and 1.1 to 1.6-fold respectively and increasing phytase activity. These studies give proof of concept that grain Fe and Zn can be modified in wheat through transgenic approaches.

Using knowledge from model species, it is possible now to identify more rapidly and with higher confidence candidate genes that might play a role in Fe and Zn transport. Access to relatively complete genomic sequence for polyploid wheat will allow more comprehensive phylogenetic studies for putative wheat homologs of large gene families. Wheat candidate genes with putative Fe and Zn transporter function inferred from phylogeny, can be taken forward and characterized in yeast mutants, with a view eventually to expressing these in transgenic wheat plants to increase vacuolar export and ultimately nutrient content in the grain.

Zinc biofortification appears as an innovative technology to alleviate the good zinc deficiency in human health, especially on the Indian subcontinent, by applying Zn either as a foliar or soil application. Zinc transfer to seeds via increased expression of genes is associated with transporter proteins and biosynthesis/exudation of Zn chelates, which is yet to be fully characterized. The genetic variability among different wheat genotypes and their wild relatives for grain Zn concentration should be exploited to develop wheat genotypes with better yield and grain Zn concentration under specific environments.

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