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Harnessing diversity in durum wheat *(Triticum turgidum L.)* to enhance climate resilience and micronutrient concentration through genetic and agronomic biofortification

Anteneh Agezew Melash[1,2*] – Attila Vad[3] – Bekir Bytyqi[1] – Éva Babett Ábrahám[1]

[1]Institute of Crop Sciences, Faculty of Agricultural, Food Sciences and Environmental Management, University of Debrecen, H-4032 Debrecen, Böszörményi str. 138, Hungary.

[2]Debark University, College of Agriculture and Environmental Science, Department of Horticulture, P.O. Box, 90, North Gondar, Debark, Ethiopia

[3]Institutes for Agricultural Research and Educational Farm, Farm and Regional Research Institute of Debrecen, H-4032 Debrecen Böszörményi str 138

*Correspondence: antenehagezew2008@gmail.com

SUMMARY

Huge consumption of wheat-driven food products with low bioavailability and small concentrations of zinc is responsible for zinc-induced malnutrition and associated health complications. The contemporary durum wheat varieties have inherently tiny zinc concentrations in developing grain, which cannot meet the daily human zinc demand. Despite the fact that over two billion people are suffering from iron and zinc-induced malnutrition, various intervention measures have been deployed to reverse the effect of zinc-induced malnutrition on humans. There are evidences that agronomic and genetic biofortification approaches can increase grain yield and nutritional quality (i.e. zinc, iron, protein, and vitamins) of durum wheat to a greater extent. However, there is a lack of direct empirical evidence for which the influence of both biofortification approaches on improving human health. Application of micronutrient-containing fertilizers either in the soil or foliarly is effective in combination with NPK, organic fertilizers coupled with efficient durum wheat varieties, emphasizing the need for integrated soil fertility management (ISFM). Although genetic biofortification is a cost-effective and sustainable approach, agronomic biofortification provides an immediate and effective route to enhancing micronutrient concentrations in durum wheat grain. The application of zinc-containing fertilizers is more effective under drought conditions than in normal growing situations. Hence, this article provides a key information for agronomists and breeders about the potential of biofortification interventions to improve durum wheat yield and enrich the grain qualitative traits to ensure food and nutritional security of the ever-increasing world population.

Keywords: *Biofortification, micronutrients, drought, durum wheat, malnutrition*

INTRODUCTION

Agricultural genetic diversity is a fundamental resource for sustainable crop production, ecosystem functioning and reduction of food and nutritional insecurity, yet only a few have been promoted and extensively used as staple food crops (Frison et al., 2011). This perchance can cause the extinction of crop diversity within the farming system, which would be a principal factor in the prevalence of malnutrition, particularly where cereals are predominantly utilized as a source of food (Melash et al., 2016). In the past decades, agricultural research has been geared towards increasing wheat production at its center. However, despite the abundant food supplies and considerable progress in reducing hunger, the burden of malnutrition in all its forms continues to be a challenge (FAO, 2020). It has been estimated that one out of three people is influenced by micronutrient malnutrition globally (Han et al., 2022). These factors could cause further adverse health consequences in humans, such as poor pregnancy outcomes, stagnant national development efforts, increased risk of morbidity in children, and reduced work productivity in adults (Hess and King, 2009). Hence, there is an urgent challenge and dire which need to increase grain mineral concentration and bioavailability of staple food crops.

Improvement in grain micronutrient composition involves a series of processes to ensure that nutrients, such as zinc and iron, are bioavailable upon consumption. Intervention measurements including dietary diversification, industrial fortification, and pharmaceutical supplementation have been proposed to improve the bioavailability of micronutrients in the edible part of various crop plants (Melash et al., 2016). These approaches are an instant and effective ways to provide micronutrient concentration easily to the human body. However, still, there is a need for other interventions as the aforementioned strategies have not been universally successful because of limited access and affordability for resource-poor farmers, failure to reach all individuals, and non-availability in abundance (Gomez-Galera et al., 2010). In search of alternative measures, agricultural-based interventions such as agronomic biofortification, conventional breeding, and genetic engineering are deployed to improve nutritional security (Hao et al., 2015; Dhaliwal et al., 2022).

The conventional plant breeding approach, also known as genetic biofortification, focuses on screening and selecting the existing crop varieties having high yielding potential and crossbreeding with a variety possessing genetically higher micronutrient concentrations to develop edible food crops with desired micronutrient and agronomic features. Agronomic biofortification which is a complementary

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approach to genetic biofortification, is also a principle of augmenting micronutrient containing fertilizers either into the soil or foliarly with the primary aim of enriching the micronutrient concentration of edible food crops (Górniak et al., 2018). These intervention measures have been observed more effective and sustainable way of agricultural-based approaches to enhance wheat grain nutritional composition (Velu et al., 2014). However, the effectiveness of these approaches highly depends on the genetic diversity of durum wheat. These observations could reinforce the idea that screening large numbers of durum wheat varieties for zinc grain concentration and their adaptability to a set of environmental conditions. Although biofortification offers many advantages, biofortified wheat varieties must deliver sufficiently high yield with minimal use of external resources, be stable across multiple years and be tolerant to biotic and abiotic factors to get accepted by the producers.

Tremendous agronomic approaches such as choice of suitable varieties, proper cropping system, and exogenous amendment of multiple nutrients have also been implemented to enhance soil nutrient status and improve the bioavailability of micronutrients. However, under the existing scenario, food products provide an insufficient amount of multiple micronutrients (Dhaliwal et al., 2022), highlighting that biofortified crops with a single micronutrient may not hit the required target, particularly where the monotonous wheat-based food product is more dominant. In compression, the concentration of zinc in animal-based food products is much higher than in cereal-based meals (Cakmak and Kutman, 2018). Indeed, translocating the target level of micronutrient concentration into the cereal grains is determined by multiple genetic and other extraneous factors, such as ant-nutritional compounds. This problem is more pronounced with zinc and iron biofortification, where absorption of Zn and Fe is inhibited by the grain phytate accumulation (Dhaliwal et al., 2022). Furthermore, foliar application of multiple cations, such as Fe^{2+} Cu^{2+} and Zn^{2+} has been observed to reduce the bioavailability of other nutrients in the grain (Dhaliwal et al., 2022). These imply that synergistic and antagonistic associations between nutrients being applied in biofortification programs should be intensively considered, as far as nutritional security is concerned.

There are a number of indicative evidences that highlight the prominence of ameliorating wheat grain zinc concentration. Hence, keeping in view of these issues, this article attempts to bring together various aspects of genetic and agronomic practices and their role in ameliorating grain nutritional concentration and in alleviating drought-induced stress under the current climate change scenarios. Additionally, it highlights significance of a mechanistic understanding of agronomic-based measures, in particular, integrating knowledge, if the universal aim is greater crop productivity with minimal use of micronutrients through safeguarding the environment are to be achieved. With an increasing amount of literature on some selected implementations of biofortification programs in the alleviation of malnutrition, and sustainable durum wheat production, the overall aim of the review work is to provide a state-of-knowledge review of selected refereed scientific publications, which report on genetic and agronomic biofortification (fertilizer strategies) and their effects in the improvement of grain micronutrient concentration enough for the human body under the current climate change scenarios.

Methodology

Data search engines such as, Google® Scholar, PubMed and Scopus, were used one after the other, to generate the published articles around the subject matter. The online searches using key terms such as agronomic biofortification, genetic biofortification, and genetic engineering of crop varieties in combination with the terms malnutrition, and other agronomic based crop management practices. Most of the search results were downloaded through the University license (subscription). The articles were then screened for relevancy and were included in the review when they fulfilled three criteria **(a)** accessibility, **(b)** written in English, and most importantly **(c)** have primary data on the aforementioned stated terms generated by either quantitative or qualitative research methods.

WHAT IS BIOFORTIFICATION – PART OF A NUTRITION REVOLUTION?

In a universal term, biofortification is a process by which the micronutrient concentration of edible crops is enhanced through conventional plant breeding, agro-technical measures, and modern biotechnology strategies without sacrificing any features of crops which are preferred by the farming community and end-users as well (Klikocka and Marks, 2018; Nestel et al., 2006). This process is considered a nutrition-sensitive agricultural-based intervention that provides an ample amount of micronutrients and vitamins essential for humans (Ruel and Alderman, 2013). A number of studies confirm that biofortification could be a sustainable solution potentially to reduce malnutrition and associated health complications, because once planting materials (varieties) are developed, they could be reserved, recycled, and further disseminated to the end-users (Hotz, 2013). It is true that the initial development and dissemination are completed; recurring costs of maintaining the production of biofortified crops are estimated to be low (Hotz, 2013). These practices could universally provide foods with higher micronutrient concentrations that offer optimal vitamins and minerals essential for the human body, consequently reducing the risk of inadequate mineral intake. This means that enhanced grain micronutrient concentration achieved by biofortification programs will be modest as compared with amounts augmented in supplements, and in some cases fortified food. As a result, the potential magnitude of the effect of biofortification on nutrient status of the targeted group

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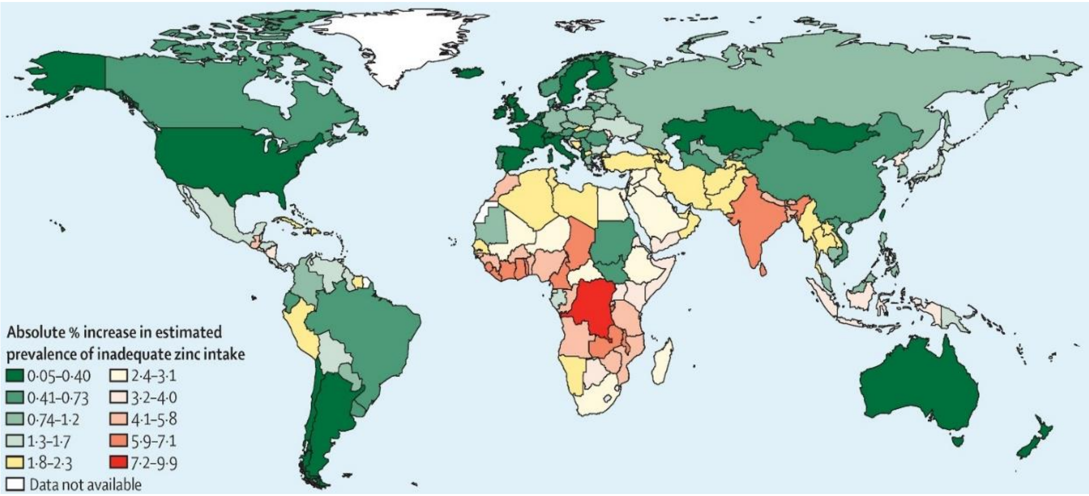
could be commensurately modest, particularly where mineral malnutrition is profoundly prevalent.

WHY WHEAT VARIETIES ARE BIOFORTIFIED?

Wheat is a common form of cultivated cereal crop which provides calories, proteins, and bioavailable micronutrients as well. However, an excessive intake of wheat-based food consumption monotonously could be a chief source of zinc-associated malnutrition because of its inherently poor bioavailable grain zinc concentration and high phytate accumulation (Welch and Graham, 2004; Cakmak et al., 2010). Although micronutrients provide huge health benefits, a commonly cultivated form of crop species such as rice, wheat, and maize contained inadequate amounts of essential elements including vitamin A, iron, zinc, calcium, manganese, copper, iodine, and selenium (Garg et al., 2018). This means that crop varieties with poor grain nutritional composition couldn't support a healthy life and could cause poor health outcomes, sickness, increased morbidity and disability, impaired development, stunted mental and physical growth, diminished livelihoods, and reduced national socioeconomic development (Chizuru et al., 2003). Hence, biofortification could circumvent these and associated health problems by enhancing both the concentration and bioavailability of micronutrients into the grain of target staple crops (Borrill et al., 2014).

Hidden hunger, also known as "mineral malnutrition" is results from the insufficient acquisition of micronutrients and vitamins from every daily meal (Melash et al., 2016). It has been reported that, over two billion people are suffering from malnutrition due to shortcomings of zinc, iron, vitamin A, and folates, particularly in developing countries (Bailey et al., 2015). Preschool children and pregnant women are the most vulnerable group to mineral malnutrition since they need required higher micronutrient intake than any other stages (UNICEF, 2019). As stated in the literature, agricultural products are not sufficiently providing vitamins and minerals at an affordable price, particularly in developing countries (Mostafa, 2019). The decreased micronutrient concentration could also aggravate the vulnerability of humans to infectious diseases, including COVID-19 (Huizar et al., 2021). The existence global pandemic also highlights the need to enhance the nutritional status, particularly in the rural household, to improve their nutritional self-sufficiency through biofortification approach (Huizar et al., 2021). However, as illustrated in *Figure 1*, this aim could be influenced by the ever changing climate such as elevated atmospheric carbo dioxide oxide through decreased the grain zinc concentration (Samuel et al., 2015).

Figure 1: **Illustrates an absolute percentage of increase in the risk of zinc deficiency in response to elevated atmospheric (Samuel et al., 2015)**



HARNESSING WHEAT GENOTYPES TO REDUCE DROUGHT EFFECT AND HIDDEN HUNGER

There have been marked increases in grain yield of major food crops by about twofold due to the breeding effort and improved agronomic based soil management measures (Cakmak and Kutman, 2018). This huge increment in grain yield has caused a considerable reduction in the concentration of essential micronutrient including zinc through the dilution effect (Kamaral et al., 2022; Shewry et al., 2016), although the extent that varied with source of the cultivars. A study on thousands of wheat cultivars has been evidenced that the improved varieties, landraces, adopted cultivars, and wheat progenitors showed wider genetic variation from 12 mg kg^{-1} to 117 mg kg^{-1} (Velu et al., 2014). Hence, the landraces, wild emmer wheat (*Triticum turgidum* L), and wheat progenitors could be a promising genetic resources to enhance grain zinc concentration of modern wheat varieties (Kamaral et al., 2022) (*Figure 2*). Although wild relatives, landraces, and old cultivars offered many benefits, they were replaced by modern high-yielding varieties which

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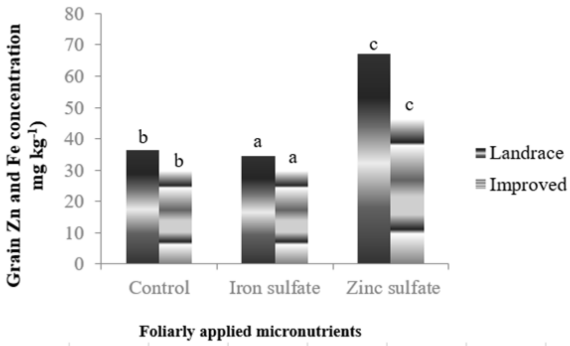
sidelining the nutritional concentration of the grain (Singh et al., 2016). These substantiate the need for revisiting the orphan and neglected wheat genotypes to reverse the adverse consequences that the crop production sector is faced.

Figure 2: **Illustrated that the landrace durum wheat varieties are more micronutrient accumulator than the modern improved varieties (Melash et al., 2019)**



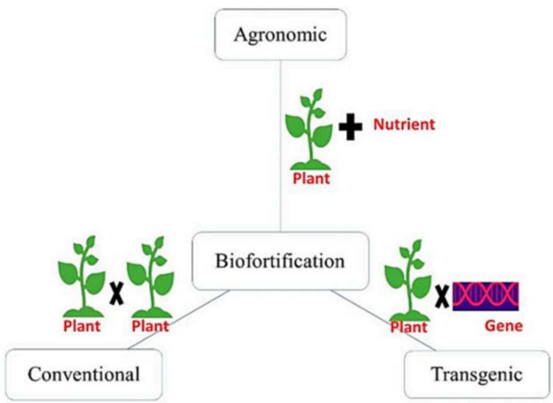
Following the green revolution, different high yielder wheat varieties are developed, but a significant reduction in grain zinc and iron concentration has been observed to the extent even that varied with the plant stature. This extent of nutritional reduction is more profound for dwarf wheat cultivars than for tallest wheat cultivars (Sharma et al., 2021). This substantiates the need to include the orphan durum wheat varieties, their wild relatives, landraces, and old cultivars in the modern durum wheat cultivation system. There has been wider genetic variability in wheat germplasm, particularly in wild relatives, yet there is no full utilization of these resources. In comparison, the wild relative, landraces, diploid progenitors of bread wheat, and wild emmer wheat cultivars are characterized by higher grain zinc and iron concentration than the modern wheat varieties (Gupta et al., 2020; Sharma et al., 2021). However, the ploidy level of a cultivar could significantly influence the grain zinc concentration. It has been evidenced that higher (64%) zinc efficiency was observed from the hexaploid wheat cultivars followed by diploid (60%) and tetraploid (36%) wheat varieties (Sharma et al., 2021). Hence, considering durum wheat varieties based on their ploidy level could offer significant benefit as far as higher grain micronutrient concentration is concerned.

Grain zinc and iron biofortification are also significantly influenced by the pedoclimatic conditions of the growing environment, such as soil type, soil fertility status, and their interaction with the environment (Khokhar et al., 2018; Trethowan et al., 2010). In wheat, variation in growing environment has been frequently reported as a determinant factor for grain zinc and iron concentration (Badakhshan et al., 2013; Velu et al., 2012). This effect is more significant when the environment interacts with the biological potential of wheat genotypes (G×E), which substantiates the screening of wheat varieties across multiple environments and growing seasons (Khokhar et al., 2018; Trethowan et al., 2010). It is also worth mentioning that genotype by environment (G×E) interaction has been found significant for modern wheat varieties and their wild relatives (Khokhar et al., 2020; Gómez-Galera et al., 2010). Evaluation of biofortified wheat cultivars across multiple environments revealed high genetic heritability and a strong association between growing location and grain zinc concentration (Sharma et al., 2021). Nevertheless, caution is needed, as variation in grain zinc concertation is observed due to a strong association with environmental variability of the growing location.

METHODS OF DURUM WHEAT BIOFORTIFICATION

Biofortification involves the enhancement of grain micronutrient concentration in targeted food crops without dictating basic agronomic features, i.e., potential yield, pest and drought tolerance ability. Hence, alternatives such as classical, agronomic and transgenic approaches have been suggested as a practical solution to improve nutritional composition and ensure the nutritional security (*Figure 3*). These approaches are initially designed with a primary aim of enhancing the grain nutritional composition and essential elements including zinc, iron, iodine and etc. (Klikocka and Marks, 2018). As a practical solution, a number of cereal and horticultural crops such as sweet potatoes, maize, orange, cassava, cowpeas, iron enriched beans, squash, lentils, sorghum, zinc enriched rice have been disseminated (Steur et al., 2017). However, information about the direct health outcome of these biofortified crops on the targeted population is still limited.

Figure 3: **Illustrates modes of biofortification to improve grain micronutrient concentration in the edible (This figure is adopted from Dhaliwal et al., 2022)**



Agronomic biofortification through the mode of mineral fertilization

The modern wheat cultivated area faces with a low bioavailability of micronutrients in the soil and this enforces the agricultural sector for exogenous application of terrace elements (White et al., 2009). This implies that, there is a need to improve the soil

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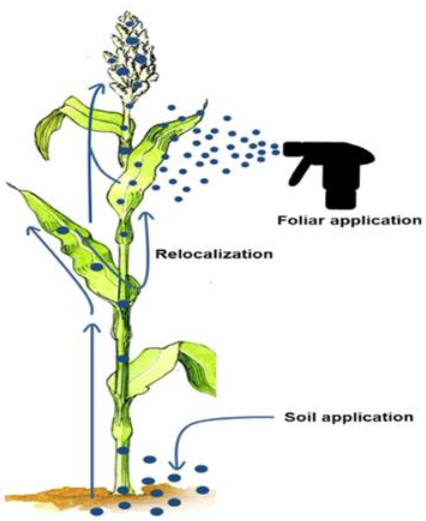
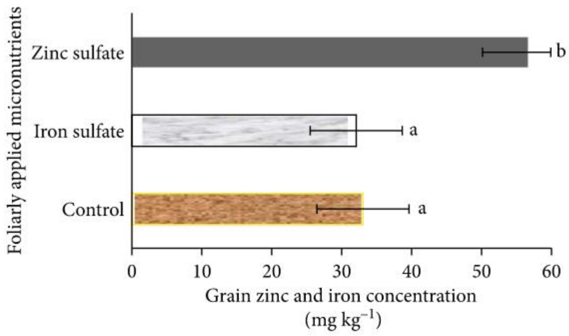
fertility status, as low soil micronutrient concentration could decrease grain mineral concentration. Alternatives such as agronomic biofortification (*Figure 4*) has been suggested as a practical solution to enhance micronutrient concentration into the grain (De Valença et al., 2017). This approach can be also used as a complementary strategy to the breeding approach and possibly reduce micronutrient malnutrition through provision of adequate grain micronutrient composition (Melash et al., 2020). However, the effectiveness of fertilizer-based interventions is significantly influenced by the source of fertilizers and their mode of application (Velu et al., 2014). There have been different sources of zinc nutrient application suggested for wheat crop including EDTA-chelated zinc and $ZnSO_4$. Nevertheless, foliarly applied zinc as a sulfate form has been frequently reported as the most suitable and cost-effective source of zinc fertilizer as compared to EDTA-chelated zinc (Cakmak and Kutman, 2018). This result confirms that fertilizer formulation could determine the bioavailability of micronutrients, as the nutrient form and their interaction with the environment can have positive and neutral or even negative effects on grain yield and nutrient use efficiency of crops (De Valença et al., 2017). Hence, the selection of a proper application method with suitable fertilization sources provides adequate nutrient and satisfies crop nutrient demand in the durum wheat fortification program. Foliar-based application of micronutrients often stimulates more nutrient uptake and efficient allocation in the edible plant parts than in the soil (Lawson et al., 2015).

Figure 4: **Mode of agronomic biofortification to improve micronutrient concentration in the edible portion of staple food crops including durum wheat (De Valença et al., 2017)**



Application foliar based zinc and iron fertilizers at different developmental stages improves grain zinc concentration, although the extent that varies with absorption efficiency of the varieties (Dhaliwal et al., 2013). In wheat varieties, superior results have been recorded due to foliar application of zinc that soil based fertilizer application (Ram et al., 2015). Agronomic biofortification has been observed to be more effective when one or more application methods has been employed. With reference to application methods, grain zinc content, biomass yield, grain yield, protein content and fodder quality has been enhanced due to combined use of foliar and soil based zinc application method (Khattak et al., 2015; Cakmak and Kutman, 2018). This could be potentially due to the fact that soil based fertilization of zinc fertilizer could enhance bioavailability of zinc particularly in the rhizosphere, while foliarly applied zinc nutrition enable rapid translocation of zinc into storage organs by enhancing absorption capacity (Dhaliwal et al., 2022). Universally, agronomic biofortification has been frequently reported as a practical solution to improve grain mineral content, and the grain zinc concentration was enhanced from 33.04 mg kg^{-1} to 56.73 mg kg^{-1} (*Figure 5*). This approach could also exert a significant change in grain micronutrient concentration when multiple application methods (i.e. soil plus foliar) is deployed than a single application method (Phattarakul et al., 2012; Cakmak et al., 2010).

Figure 5: **Influence of foliarly applied zinc and iron sulfate on grain micronutrient concentration (mg kg^{-1}) of durum wheat varieties. The bars indicated with different letters are significantly different from each other at 0.05 level of probability (Melash and Dejene, 2020)**



Nowadays, agronomic biofortification is successfully implemented for different nutrients such as selenium, iodine, and zinc, probably due to their better mobility in the soil and crops (Ambuj and Thomas, 2020). Although application of zinc containing fertilizers are a center for agronomic biofortification approaches, implementation of an integrated nutrient management strategies could offer a significant benefit in translocating micronutrient into the developing grains. It has been clearly observed that application of nitrogen, phosphorus and potassium combined with zinc, boron and sulphur could improve nutrient uptake and productivity of cereal crops (Rao et al., 2012). A number of studies verified that, under adequate nitrogen application, nutrients such as grain zinc and iron concentration have been improved due to enhanced uptake and utilization efficiency of the applied inputs (Kutman et al., 2011). This has been verified that sufficient fertilization of nitrogen and

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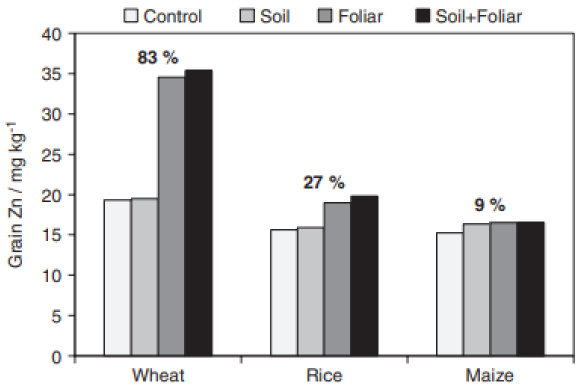
phosphorus could have a positive effect on root development, shoot transport and re-localization of nutrients from vegetative tissue to the grain, which improves grain nutrient concentration (Prasad et al., 2014). However, the major production problem associated with augmenting phosphorus-containing fertilizer could incipient soil zinc deficiency through precipitation of insoluble zinc phosphate (Zingore et al., 2008). These imply the need for proper phosphorus application dose and considering its interaction with other ions could be very important in biofortification program.

Fertilization method of target micronutrients

Several application methods, such as seed dressing, foliar application, and soil based fertilization and their effectiveness in improving grain iron and zinc concentration have been evaluated (Mathpal et al., 2015). These methods of targeted nutrient application may affect effectiveness of agronomic biofortification in different way (Velu et al., 2014). It has been evidenced that soil based fertilization of micronutrients enhances grain yield rather than the content of micronutrient in the grain (Narwal et al., 2010). While micronutrient was applied foliarly, grain zinc concentration was much greater than nutrients applied in the soil (Cakmak et al., 2010). A threefold increment in grain zinc concentration in durum wheat was also observed due to foliarly applied zinc containing fertilizers (Yilmaz et al., 1997). This could be the readily translocation of foliarly applied zinc fertilizers into the developing wheat grain, as zinc is more phloem mobile nutrient (Erenoglu et al., 2011). These imply the potential of biofortification to tackle health complication associated with micronutrient deficiency, and could be a practical solution to minimize malnutrition and associated health consequences (Welch, 2002). Adequate fertilization of commercial crops including durum wheat could enhance its market acceptance can bring economic benefits concomitantly the nutritional security.

Figure 6: **Indicates the average changes in grain concertation of zinc in grains of different crops (wheat, rice and maize) due to different mode of zinc fertilization i.e. soil based, foliarly and combination of both methods (Cakmak and Kutman, 2018)**



Wheat has been reported more responsive to zinc fertilization and in terms of grain zinc accumulation than other cereal crops such as rice and maize (*Figure 6*). Wheat grain micronutrient concentration has been improved due to foliarly applied zinc containing fertilizers (Mao et al., 2014). However, grain zinc concentration could not be linearly improved in durum wheat either through soil based or foliar application due to multiple factors such as initial soil zinc concentration and application dose (Cakmak et al., 2010). In other perspective, improvement in micronutrient concentration could enhance market value of industrial crops including durum wheat and can ensure economic sustainability of durum wheat producing farmers.

Time of nutrient application: an agronomic implication

The timing of micronutrient fertilization such as zinc, is an important determinant factor which dictates effectiveness of agronomic biofortification. Application of zinc containing fertilizers is more effective when nutrients fertilized at a later stage (e.g. grain filling stage) than early in the growing season (Abdoli et al., 2014). The effectiveness of foliar application in translocating zinc into the developing grain is significantly influenced by crop developmental phases. It has been evidenced that application of zinc containing fertilizers at early milky, heading and dough stages were more effective in translocating micronutrients into the grain than nutrients applied at booting and stem elongation stages (Cakmak et al., 2010; Melash et al., 2016). Effective translocation of micronutrients, particularly at the milking stage could be due to easily remobilization of the nutrients due to allocation of active photo-assimilates into the sink. These means understanding the critical growth phases during which foliar fertilization of micronutrients is very important.

In the implementation of agronomic biofortification, understanding of crop developmental stages at which trace elements should be foliarly fertilized is fundamental for maximum translocation of zinc into the grain (Cakmak, 2012). An increment of grain iron concentration by about 64.4% has been reported because of foliar application of zinc containing fertilizers during the crop life cycle (Maralian, 2009). It is worth mentioning that, zinc can mobilize at higher amount at milky stage than any other developmental stages of wheat (Ozturk et al., 2006). A strong mobility of zinc in the phloem has been also reported due to foliar application of zinc containing fertilizers particularly during reproductive phases (Haslett et al., 2001).

Genetic biofortification: A conventional plant breeding technique

Following the significant influence of malnutrition, agricultural researchers enforces to develop bio-fortified crops rich in grain mineral concentration either by conventional breeding or contemporary biotechnological interventions (Garg et al., 2018; Nestel et al., 2006). In biofortification program, the

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conventional plant breeding intervention has been suggested as the most powerful, and promising technique which entails the crossbreeding of existing genotypes to enhance grain microelements (Zhao and Shewry, 2011). This technique is considered as economically sustainable in addressing global malnutrition, because once the crop has developed with the target level of essential elements there will be no further cost for buying fortified products or their addition to foods (Gómez-Galera et al., 2010). As a complimentary to the breeding programs, biotechnological approaches such as molecular marker assisted section are also employed (Collard and Mackill, 2008). However, the selective breeding approaches are significantly influenced by a number of factors including lack of genetic diversity, low heritability and linkage drag, which enforce the genetic engineering techniques as a deliberate approach in biofortification program (Malik and Maqbool, 2020). Hence, then the genes would be incorporated into the genome of the crop being biofortified with the primary aim of enhancing micronutrient concentration of grain crops (Paine et al., 2005a).

Indeed, the productivity, and grain quality of wheat, as in all cereals, could be influenced by the genetic constitute of a cultivar. A number of studies stated that wild and primitive wheat cultivars offered a promising genetic resource for grain zinc concentration that the common cultivated wheat. Cakmak et al. (2004) has been observed an important genetic diversity in wild emmer wheat varieties at which highest (14 to 190 mg Zn kg^{-1}) grain zinc content was recorded. It is worth mentioning that, in wild emmer wheat accessions a simultaneously improvement in grain yield, drought tolerant ability, high (139 mg kg^{-1}) in grain zinc and (88 mg kg^{-1}) iron concentration has been observed under zinc deficient soil condition (Peleg et al., 2008). Additionally, a wheat crop derived from *Aegilops tauschii* has been found to be a significant source of genetic potential for enhancing grain zinc concentration (Calderini and Ortiz-Monasterio, 2003). All these outcomes are also consistent for other grain crops including maize and rice where this genetic variation could be used in various breeding programs (Graham et al., 1999). These means that there is an existence of significant genetic variation in grain micronutrient concentration of wheat crops which in fact allows crop scientists to enhance the level of grain micronutrients and vitamins as well (Hirschi, 2009). However, most breeding strategies are largely depending on the presence of genetic variation in the germplasm which could be sourced from sexually compatible crops.

As a genetic approach controlled by numerous factors, genetic biofortification can significantly dictate grain mineral concentration. The most important factor that control the grain micronutrient is varietal difference and their utilization efficiency to the applied inputs. In compression, durum wheat varieties have been observed to accumulate more micronutrients than bread wheat verities (Conti et al., 2000). This means that crop ploidy level could determine the nutritional composition being translocating in the grain. Through comparing the inter accessional variation, the landrace, old wheat cultivars and their wild relatives showed wider genetic difference in translocating micro elements than the contemporary wheat varieties (Velu et al., 2012). In a certain case, considering the soil fertility status of the cultivation environment is very important, as far as biofortification is a primary aim. Since wider range of grain zinc and iron composition was observed due to variation in soil fertility status (Alloway, 2009), and nutrient uptake efficiency of the variety (Impa et al., 2013). Grain micronutrient concentration could be therefore, enhanced via improving crop nutrient uptake efficiency, absorption and storage (Ramaswami, 2007).

Although biofortification through breeding approach is frequently stated as a suitable and sustainable intervention to counterattack malnutrition and associated health problems, development of micronutrient enriched varieties involves a protracted process which often hampered by low available micronutrient pool in the soil solution (Cakmak, 2010). This approach requires fairly prolonged period and having higher grain yield combined with grain micronutrient concentration needs much more time as well (Prasad et al., 2014). This is clearly implying the need for alternative and complimentary approaches to improve micronutrient concentration in durum wheat cultivation system. Transgenic approaches have been therefore, suggested as a complimentary to the breeding strategy, if the target level of grain micronutrients could not be achieved by the breeding approaches (Zhao and Shewry, 2011). Because, a developed micronutrient enriched varieties through breeding approach would be encountered some problems such as adoption resistance by the producers in a sense that this crops are genetically modified (Melash et al., 2016).

Increasing multiple minerals using genetic engineering: A recombinant DNA technology

In a certain case genetic engineering could be used as complimentary strategy especially when there is narrow genetic variation of a desired trait, poor bioavailability of specific micronutrient, and any form of modification which cannot be employed through conventional breeding approaches (Mayer et al., 2008). Along with enhanced grain micronutrient concentration, genetic engineering deployed with a primary aim of both avoiding anti nutritional compounds and/or promotors that can enhance bioavailability of micronutrients in the grain, in a simultaneously manner (Carvalho and Vasconcelos, 2013). Although development of genetically engineered crops required substantial investment especially in the first instance, it could offer sustainable approach having a potential targeting large populations (Hefferon, 2016).

Edible food crops have been successfully modified through recently transgenic approach aim at reduction in micronutrient deficiency and associated health complications in humans. As evidenced in rice, grain iron concentration was enhanced through iron-storage protein expression (Vasconcelos et al., 2003).

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Additionally, to combat vitamin A deficiency, genetically engineered rice varieties has showed greater amount of β-carotene (Paine et al., 2005b). This trend also observed in a transgenic multivitamin corn which has been produced through modification of three different metabolic pathways aimed at ameliorating the level of selected vitamins such as β-carotene, ascorbate, and folate as well (Naqvi et al., 2009). These means vitamins including folate could be improved through a process called metabolic engineering (Blancquaert et al., 2014). Previously, an improvement in folate concentration by about hundreds of folds has been reported through overexpression of para-aminobenzoate and *Arabidopsis thaliana* pterin genes, precursors of the folate biosynthesis pathway (Storozhenko et al., 2007).

SYNERGETIC EFFECT BETWEEN GENETIC AND AGRONOMIC BIOFORTIFICATION

It has been previously discussed that agronomic biofortification is a short term and practical solution to enhance bioavailability of grain micronutrients (Velu et al., 2014). It could be capitalizing on this benefit through hybridize the agronomic and genetic biofortification to further improve concentration of micronutrients into a grain target crops. Hence, understanding of varietal difference in response to the applied inputs is very important. Mathpal et al. (2015) has been clearly observed a significant difference of wheat hybrids in response to the applied zinc fertilizer and translocation of zinc into their grain.

Nutrient use efficiency and accumulation capacity could dictate the effectiveness of grain mineral concentration. This effect has been observed more profound when old and modern durum wheat varieties compared where old cultivars found to be higher efficient and conversion capacity of the applied inputs (Cakmak et al., 2004; Zhang et al., 2010). This indicated that agronomic biofortification could be determined by the genetic landscape of wheat varieties due to variation in their nutrient use efficiency. This results universally calls for an integrated approach, as a singleton agronomic measures could not be hit the required target. Genetically efficient varieties could therefore make the agronomic biofortification more successful (Bouis and Welch, 2010). Hence, cultivation of durum wheat with acceptable grain micronutrient begins with selection of suitable varieties followed by agronomic practices that can increase nutrient availability at the latter stage.

AGRONOMIC BIOFORTIFICATION OF ZINC ALLEVIATES DROUGHT INDUCED STRESS EFFECT

As facing with climate change effect on agricultural productivity different efforts have been made to overcome drought effect. This effect could be much more when the soil is limited is essential growth resources such as nutrient. In the absence of drought stress, foliar based application of zinc containing fertilizers has not been effective, however, enhanced grain yield by about 15% and improved the grain zinc concentration under drought condition (Karim et al., 2012). However, a grain yield was reduced in a greater extent under zinc deficit soil particularly during drought condition (Bagci et al., 2007). This implies the need for adequate fertilization of zinc and their coexistence could influence the grain yield as well as mineral concentration either positively or negatively. These necessities the need for adequate zinc fertilization particularly under drought condition, although the nutrient requirement of the crop depends on the soil water status. It has been evidenced that, zinc containing fertilization was found to be effective in alleviating drought stress (Ma et al., 2017).

Higher yield and grain zinc concentration due to exogenous application of zinc containing fertilizers could be due to its critical role in detoxifying the reactive oxygen species (ROS) generation and increasing antioxidant enzymes (Wang and Jin, 2007). At physiological level, zinc fertilization also improves the photochemical reactions which often occurring in thylakoid membrane, electron transport through PSII, increases the photosynthetic rate and chlorophyll content (Younes et al., 2016). However, to the best of our knowledge, zinc fertilization effect under varying drought stress levels at various phenological periods of durum wheat are not clearly understood. An investigation conducted on the impact of drought stress under different zinc fertilization doses on qualitative and quantitative agronomic traits can provide important insights to develop drought tolerant durum wheat varieties. We therefore, suggest that an adequate evaluation of the combined effect of zinc deficiency and drought stress impact on the morphological, physiological, and grain quality characteristics can provide valuable information, and understanding of durum wheat performance under stress condition.

POSSIBLE CHALLENGES AND STRATEGIES FOR BIOFORTIFICATION APPROACHES

The proliferation of world population coupled with climate change effect enforces the agricultural sector to improve grain yield combined with acceptable qualitative traits. Although biofortification provides adequate nutrient and reverse numerous negative consequence in humans, biofortified crops must delivered higher grain yield and micronutrient concentration which would offer higher adoption and consumption rate in a given community (Bouis et al., 2011). However, different challenges such as mainstreaming biofortified features into breeding approaches, building consumer demand and linking biofortification program into public and private policies have been outlined previously (Bouis and Saltzman, 2017). As far as biofortification is concerned, factors such as ant nutritional compounds, improvement in promotor substances including amino acid concentrations (e.g. cysteine, lysine, methionine), ascorbic acid (e.g. vitamin C), which enable absorption

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of important nutrients and high yielding ability should also be considered (White and Broadley, 2009).

Although genetic biofortification is suitable and sustainable solution in combating malnutrition, it is strongly influenced by narrow crop genetic base, prolonged time of varietal development, higher dependency on bioavailability soil nutrient (Garg et al., 2018). In fact, challenges associated with narrow genetic variation in grain micronutrient concentration could be resolved through reutilization of wild relatives, and landraces (Carvalho and Vasconcelos, 2013). It is being known that, biofortification programs are focused on development of high micronutrient concentration of some selected food crops including durum wheat. Nevertheless, anti-nutritional compounds such as phytate and certain polyphenols have negatively influence certain micronutrient concentration on the grain (White and Broadley, 2009). Therefore, improvement in promotors which enable absorption of micronutrients and reduction ant nutrient compounds are very important (White and Broadley, 2009). Various vitamins (i.e. E, D, and C), choline and provitamin A has been suggested as promotor substances which enable absorption of some selected elements including Zn, Fe, Se, Ca and P (Gopalakrishnan et al., 2016).

A substance called "phytate" refers to a form of phosphorus stored in the grain when could not be digested by humans and/or monogastric animals as well (Rengel et al., 1999). In the process of digestion, phytate could bind to zinc and iron which in turn influences their absorption (Smith and Read, 2008). This implies the need for low phytate accumulated durum wheat varieties and ameliorating grain micronutrient concentration. Hence, development of low grain phytate concentration durum wheat genotypes could resolve this negative consequence on grain quality traits. Higher grain micronutrient concentration through decreasing phytate concentration has been observed in other crops has been Reduction phytate concentration has been (Shunmugam et al., 2015).

CONCLUSIONS

The linkage between the crop production sector and nutrition suggested a better insight that biofortified wheat varieties, either agronomically or genetically, could provide an ample amount of micronutrients required in the daily intake of humans. However, there is a lack of direct evidence on the effectiveness of biofortification in improving human health. This linkage should be investigated for further evidence. Biofortification of wheat through conventional plant breeding provides a comparatively cost-effective, and sustainable solution to deliver micronutrients in abundance. However, this approach largely relies on crop genetic resources, the source of nutrients being fertilized, and methods of nutrient application. Although biofortification approaches are focused on improvement in grain mineral concentration, the selected varieties being fortified should have preferred agronomic features, such as high yielding potential and disease resistance. Agronomic biofortification could be used as a complementary approach to genetic biofortification. Hence, sustainable durum wheat cultivation should begin with the selection of suitable varieties, followed by proper agronomic practices that can increase both yield and grain nutritional composition simultaneously. While much effort has been made in understanding the interaction of zinc in the soil, a long-term investigation is required to understand the effectiveness of residual zinc-based fertilizers.

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