Philip J. White[1]

Biofortification of Edible Crops

[1] The James Hutton Institute, Invergowrie, Dundee (United Kingdom)

introduction

Humans require sufficient amounts of at least 18 mineral elements for their normal development and well-being (White, 2016a). These include the macronutrients, nitrogen, phosphorus, sulphur, calcium, magnesium, potassium, sodium and chlorine, which are required in amounts greater than 100 mg d^{-1}, and the micronutrients, zinc, iron, fluorine, manganese, copper, iodine, selenium, molybdenum, chromium and cobalt, which are required in smaller amounts (White, 2016a).

Edible crops supply the mineral nutrients to the diets of most people (White et al., 2013b; White, 2016a). Unfortunately, it is estimated that the diets of about 2 billion people lack sufficient iron, 1.1 billion lack sufficient zinc, 3.5 billion lack sufficient calcium, 1.9 billion lack sufficient iodine, 1 billion lack sufficient selenium, and 14 million lack sufficient magnesium (White, 2016a). Dietary deficiencies of mineral nutrients in human diets can be addressed in a variety of ways. These include: (1) dietary diversification, to create a 'balanced' diet with sufficient mineral nutrients, (2) the distribution of mineral supplements, such as pills and tonics that contain the mineral nutrients lacking in people's diets, (3) food fortification, through the addition of mineral nutrients to cooking ingredients, such as salt and flour, or to processed foods, and (4) the biofortification of edible crops (White and Broadley, 2009; White, 2016a). Biofortification is defined as the process of increasing the bioavailable concentration of a nutrient in the edible portion of crop plants through agronomic intervention or genetic selection (White and Broadley, 2005). Biofortification is particularly effective where the resources or infrastructures for other interventions are unavailable and, since biofortifification is often used in regions where the availability of mineral nutrients limits crop production, it has the added benefit of increasing crop yields and farmers' income (Cakmak, 2009; White and Broadley, 2009; Stein, 2010).

Bread wheat (*Triticum aestivum* L.) is the staple food for about 35% of the world's population and provides approximately 20% of the calories consumed by humans worldwide (Cakmak and Kutman, 2018). However, the concentrations of mineral nutrients in wheat grain, as in the grain of other cereals, are relatively small when compared to the concentration of mineral nutrients in other edible crops, and reliance on cereal-based diets contributes to mineral malnutrition of humans (White and Broadley, 2005, 2009). Despite this, because large amounts of wheat grain are consumed, increasing the concentrations of mineral nutrients in wheat grain can increase the dietary delivery of mineral nutrients to humans and help alleviate the health issues associated with mineral malnutrition (Cakmak, 2008; Bouis and Welch, 2010). This paper focuses on strategies to biofortify bread wheat with two mineral nutrients commonly lacking in human diets: zinc (Zn) and selenium (Se).

BIOFORTIFYING BREAD WHEAT WITH ZINC

Adults require between 8-13 mg Zn day^{-1} for adequate nutrition (White, 2016a). In the UK, as in other European countries, about 25% of the zinc in human diets is delivered by cereal products (White, 2016a). It is estimated that about 9% of the UK population has dietary intakes of zinc less than the UK Lower Reference Nutrient Intake (LRNI), which is the intake considered likely to be sufficient to meet the needs of only 2.5% of the population (Bates et al., 2014). Worldwide, about 1.1 billion people lack sufficient zinc in their diets (Kumssa et al., 2015). The international HarvestPlus Program (http://www. harvestplus.org) has suggested a target concentration of 38 mg Zn kg^{-1} dry matter (DM) in wheat grain to alleviate widespread zinc malnutrition (Bouis and Welch, 2010).

Human diets with insufficient zinc often collocate with soils that have low zinc phytoavailability (Cakmak et al., 2017). These soils include calcareous or alkaline soils, which comprise 25-30% of the world's agricultural land (Broadley et al., 2007; White et al., 2013a). About half the cereal-growing areas in the world have soils with low zinc phytoavailability (Cakmak, 2008; Cakmak and Kutman, 2018). If sufficient zinc is present in these soils, edible crops with greater zinc concentrations can be produced by increasing soil zinc phytoavailability by applying acidifying fertilisers, such as urea, ammonium salts or elemental sulphur, or by cultivating varieties that secrete more protons, phytosiderophores, organic acids and enzymes that degrade organic chelates of these elements or that interact better with beneficial microorganisms to effect this (Cakmak, 2008; White et al., 2013a). Alternatively, Zn-fertilisers can be applied to crops (Cakmak, 2008; White and Broadley, 2009, 2011; Velu et al., 2014; Cakmak and Kutman, 2018). The application of Zn-fertilisers to increase crop production and grain zinc concentrations in bread wheat was initially demonstrated in Anatolia, Turkey (Cakmak, 2004), and has since been employed elsewhere in the world (Cakmak et al., 2017; Cakmak and Kutman, 2018).

Zinc fertilisers can be applied either to foliage or to the soil, but it is generally observed that applying Zn-fertilisers to foliage is more effective than applying Zn-fertilisers to the soil for biofortifying grain of bread wheat with zinc (Cakmak, 2008; Zhang et al., 2012ab; Zou et al., 2012; Barunawati et al., 2013; Prasad et al., 2014; Velu et al., 2014; Cakmak and Kutman, 2018). It has also been observed that greater N-fertiliser applications, either to the soil or to foliage, can increase grain zinc concentrations, provided there is sufficient zinc supply to the crop (Shi et al., 2010; Xue et al., 2012, 2016; Li et al., 2015; Liu et al., 2018; Pascoalino et al., 2018). It is likely that this reflects the close correlation between protein and zinc concentrations in grain (Cakmak, 2008; Zhao et al., 2009; Cakmak et al., 2010; Velu et al., 2014). In addition to inorganic Zn-fertilisers, composts and manures can also be used to increase soil zinc concentrations, and appropriate crop rotations, intercropping, or the introduction of beneficial soil microorganisms can increase zinc phytoavailability in soils (White and Broadley, 2009; Prasad et al., 2014; Brooker et al., 2015; Pellegrino et al., 2015; Coccina et al., 2019).

There is considerable genetic variation in the zinc concentration of wheat grain (e.g. Graham et al., 1999, Cakmak, 2008; White and Broadley, 2009, 2011; Zhao et al., 2009; Velu et al., 2014, 2017; Pascoalino et al., 2018) and varieties with seed zinc concentrations approaching the HarvestPlus target value have been achieved by conventional breeding strategies, provided plants have access to sufficient phytoavailable Zn in the soil (Bouis and Salzman, 2017). Several chromosomal loci (QTLs, quantitative trait loci) have been identified that affect grain zinc concentration in wheat (Velu et al., 2014, 2017; Liu et al., 2019). Among these is a gene encoding a NAC transcription factor (NAM-B1, GPC-B1) that accelerates senescence and increases nutrient remobilization from leaves to grain (Velu et al., 2014, 2017; Tabbita et al., 2017). It is expected that knowledge of allelic variation in genes underlying QTL will enable the design of molecular markers for breeding wheat varieties with greater potential for Zn biofortification.

The amount of zinc delivered to the diet from biofortified wheat grain is influenced greatly by its processing. Since zinc is located mainly in the embryo and aleurone layer of the seed, much can be lost during milling or polishing of grains (Cakmak, 2008; Shi et al., 2010; Velu et al., 2014; Ciccolini et al., 2017). In addition, the bioavailability of zinc in human diets is influenced by the presence of antinutrients, such as fibre, tannins and phytate, and promoter substances, such as inulin, ascorbic acid and various amino and carboxylic acids, in food (White and Broadley, 2009; White, 2016a). Although much of the zinc in wheat grain is associated with phytate, there appears to be genetic variation in the concentrations of both antinutrients and promoter substances in wheat grain (White and Broadley, 2009; Velu et al., 2014) and there has been some effort to generate low-phytic acid (*lpa*) mutants and identify and breed wheat genotypes with reduced seed phytate concentrations (Raboy, 2009; White and Broadley, 2009). The application of foliar N-fertilisers also appears to reduce phytate concentrations in cereal grain (Li et al., 2015). In addition, to this genetic strategy to improve zinc bioavailability in food, some processing techniques, such as soaking, malting and fermentation, can reduce phytate concentrations in seeds and improve zinc bioavailability in the human diet (Kumar et al., 2010).

BIOFORTIFYING BREAD WHEAT WITH SELENIUM

Recommended selenium intakes for humans range from 25-75 $\mu g\ d^{-1}$, depending upon age, gender and authority (Fairweather-Tait et al., 2011). The concentrations of selenium in edible crops receiving no Se-fertiliser often correlate directly with selenium phytoavailability in the soils on which they are grown (Ihnat, 1989; Garvin et al., 2006; Fairweather-Tait et al., 2011; Lee et al., 2011; Garrett et al., 2013; Joy et al., 2015; Lazo-Vélez et al., 2015; Kumssa et al., 2017). Soils with insufficient selenium to produce edible crops with selenium concentrations adequate for human nutrition occur worldwide (White and Broadley, 2009; White, 2016b). However, dietary selenium deficiencies can be addressed effectively through the application of Se-fertilisers to edible crops (White, 2016b; Bañuelos et al., 2017).

The natural soils of many countries in Northern Europe, including Finland and the United Kingdom, lack sufficient selenium to produce selenium-rich grain (Broadley et al., 2006; Fairweather-Tait et al., 2011; Lazo-Vélez et al., 2015). The mandatory inclusion of selenium into inorganic fertilisers to address selenium deficiencies in Finnish diets increased the selenium concentrations in produce and improved the selenium status of the Finnish population (Alfthan et al., 2015). In the United Kingdom, about 26% of the selenium in human diets is delivered by cereal products (Fairweather-Tait et al., 2011; White, 2016a) and methods have been developed to deliver approximately 20% of the UK Reference Nutrient Intake, the mean population intake required to protect 97.5% of the population from deficiency (UK-RNI = 60 μg d^{-1} for females and 75 μg d^{-1} for males), in two slices of Se-biofortified bread (Broadley et al., 2010; Hart et al., 2011). Bread and cereal products also provide much of the selenium in peoples' diets in many other countries (Lazo-Vélez et al., 2015).

Selenium fertilisers can be applied either to foliage or to the soil. Although the application of Se-fertilisers to foliage is generally more efficient in increasing concentrations in produce than applications of Se-fertilisers to the soil (Ros et al., 2016), application of Se-fertilisers to soil is recommended because it poses less risk of phytotoxicity (Lyons et al., 2005b; Bañuelos et al., 2017). In general, there is a linear relationship between the amount of Se-fertiliser applied to a non-seleniferous soil and the selenium concentrations in wheat grain (Broadley et al., 2010). The application of soluble selenate salts, such as sodium selenate and potassium selenate, is generally more effective in increasing grain selenium concentrations than the application of selenite salts or less soluble selenate salts, such as barium selenate, although the latter provides a longer lasting selenium source (Broadley et al., 2006, 2010; Ros et al., 2016). The addition of Se-rich organic material to soils can also be used a selenium source to biofortify crops and provides phytoavailable selenium for several years after its application (Bañuelos et al., 2017).

Genetic variation in the selenium concentration of wheat grain has been reported in a number of studies (Garvin et al., 2006; Murphy et al., 2008; Rodríguez et al., 2011; Pu et al., 2014), although this is not always observed (White 2016). It has been suggested that the lack of genetic variation observed might be a consequence of large environmental effects on grain selenium concentration (Lyons et al., 2005b; Garvin et al., 2006; Zhao et al., 2009; Lee et al., 2011; Nelson et al., 2011; White, 2016). In addition, there appears to be a negative relationship between grain selenium concentration and grain yield among bread wheat cultivars (Zhao et al., 2007; Fan et al., 2008; Murphy et al., 2008), although, again, this is not always observed (Lyons et al., 2005b; Zhao et al., 2009; Nelson et al., 2011). Chromosomal loci (QTLs) influencing grain selenium concentration have been identified in crosses between Chinese wheat varieties (Pu et al 2014, 2018; Wang et al., 2017), but no genes affecting grain selenium concentrations have yet been identified.

Relatively little selenium is lost during food preparation (Lyons et al. 2005b). However, although selenium is fairly evenly distributed throughout the grain, the selenium concentrations in wholemeal flour are slightly greater than white flour made from the same grain (Lyons et al., 2005a; Moore et al., 2010; Hart et al., 2011; Lazo-Vélez et al., 2015). No genetic variation in the distribution of selenium within wheat grain has been reported (Lyons et al., 2005a). Selenomethionine (SeMet) is the main selenium species in wheat grain, which also contains smaller concentrations of selenocysteine (SeCys), selenomethylselenocysteine (SeMeSeCys), selenohomolanthionine (SeHLan), selenite, selenate and other selenium species (Lyons et al., 2005a; Hart et al., 2011; Lazo-Vélez et al., 2015; Duncan et al., 2017). Selenomethionine (SeMet) is an effective selenium source for human nutrition (Fairweather-Tait et al., 2011; Hart et al., 2011).

ACKNOWLEDGMENTS

This work was supported by the Rural & Environment Science and Analytical Division of the Scottish Government.

RIASSUNTO

Gli esseri umani hanno bisogno di sufficienti quantità di almeno 18 elementi minerali per il loro normale sviluppo e benessere. Le colture agrarie forniscono questi elementi con la dieta. Sfortunatamente, si ritiene che molte persone non dispongano di quantità sufficienti di nutrienti minerali nella loro dieta. Le carenze alimentari di nutrienti minerali potrebbero essere affrontate attraverso una varietà di approcci tra cui (1) la diversificazione dietetica, per creare una dieta "equilibrata" con sufficienti nutrienti minerali, (2) l'utilizzazione di integratori minerali, come pillole e tonici che contengono il nutriente minerale carente nella dieta, (3) la fortificazione alimentare, attraverso l'aggiunta di nutrienti minerali agli ingredienti per cucinare, come sale e farina, o agli alimenti trasformati e (4) la biofortificazione delle colture. La biofortificazione è definita come il processo di aumento della concentrazione biodisponibile di un nutriente nella porzione commestibile delle piante coltivate attraverso l'intervento agronomico o la selezione genetica. Questo discorso si concentrerà sulle strategie per biofortificare le colture commestibili con due nutrienti minerali comunemente carenti nella dieta: zinco e selenio. Sarà usato il frumento come esempio principale. Nel discorso saranno descritti innanzitutto i ruoli di questi nutrienti minerali nella fisiologia umana e le quantità richieste nella dieta.

Inoltre, sarà mostrato che le carenze di questi nutrienti nella dieta sono spesso correlate alla limitata disponibilità di questi elementi nel suolo e che l'applicazione di fertilizzanti fogliari o del suolo contenenti questi elementi può essere utilizzata per biofortificare le colture commestibili con zinco e selenio. Saranno descritti la variabilità genetica nelle capacità delle colture di accumulare zinco e selenio nelle loro porzioni commestibili ed i loci cromosomici che potrebbero essere utilizzati per il miglioramento genetico assistito del frumento per un maggiore accumulo di zinco e selenio. Infine, saranno presi in considerazione gli effetti degli antinutrienti, come il fitato, e la posizione dei nutrienti di zinco e selenio nella granella di frumento sulla biodisponibilità di questi nutrienti nei prodotti alimentari.

ABSTRACT

It is thought that the diets of over half the humans in the world lack sufficient mineral nutrients for their normal development and well-being. One strategy to address this is to increase the bioavailable concentrations of mineral nutrients in edible crops. Since bread wheat (*Triticum aestivum* L.) is the staple food for about one in three people, increasing the concentrations of mineral nutrients in wheat grain could help alleviate mineral malnutrition and improve human health. This paper focuses on strategies to biofortify grain of bread wheat with two mineral nutrients commonly lacking in human diets: zinc and selenium. It is observed that applying Zn-fertilisers, either to soil or, more effectively, to foliage, can increase grain zinc concentrations and that there is appreciable genetic variation in grain zinc concentrations. Similarly, the application of Se-fertilisers to soil or to foliage can increase grain selenium concentrations and, although the effects of the environment on grain selenium concentrations are large, there is also genetic variation in grain selenium concentrations. Thus, the application of fertilisers containing zinc and selenium to varieties that can accumulate these elements in their grain efficiently could increase the delivery of these mineral nutrients to peoples' diets to improve their health.

BIBLIOGRAPHY

Alfthan G., Eurola M., Ekholm P., Venäläinen E.R., Root T., Korkalainen K., Hartikainen H., Salminen P., Hietaniemi V., Aspila P., Aro A. (2015): *Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: from deficiency to optimal selenium status of the population*, «Journal of Trace Elements in Medicine and Biology», 31, pp. 142-147.

Bañuelos G.S., Lin Z.-Q., Broadley M. (2017): *Selenium biofortification*, in *Selenium in plants: molecular, physiological, ecological and evolutionary aspects*, edited by E.A.H. Pilon-Smits, L.H.E. Winkel, Z.Q. Lin, Springer, Cham, Switzerland, pp. 231-255.

Barunawati N., Giehl R.F., Bauer B., Von Wirén N. (2013): *The influence of inorganic nitrogen fertilizer forms on micronutrient retranslocation and accumulation in grains of winter wheat*, «Frontiers in Plant Science», 4, pp. 320.

Bates B., Lennox A., Prentice A., Bates C., Page P., Nicholson S, Swan G. (2014): *National Diet and Nutrition Survey: Results from Years 1-4 (combined) of the Rolling Programme (2008/2009-2011/2012)*, Public Health England, London.

Broadley M.R., White P.J., Bryson R.J., Meacham M.C., Bowen H.C., Johnson S.E., Hawkesford M.J., McGrath S.P., Zhao F.-J., Breward N., Harriman M., Tucker M. (2006): *Biofortification of UK food crops with selenium*, «Proceedings of the Nutrition Society», 65, pp. 169-181.

Broadley M.R., White P.J., Hammond J.P., Zelko I., Lux A. (2007): *Zinc in plants*, «New Phytologist», 173, pp. 677-702.

Broadley M.R., Alcock J., Alford J., Cartwright P., Foot I., Fairweather-Tait S.J., Hart D.J., Hurst R., Knott P., McGrath S.P., Meacham M.C., Norman K., Mowat H., Scott P., Stroud J.L., Tovey M., Tucker M., White P.J., Young S.D., Zhao F.-J. (2010): *Selenium biofortification of high-yielding winter wheat (*Triticum aestivum *L.) by liquid or granular Se fertilisation*, «Plant and Soil», 332, pp. 5-18.

Bouis H.E., Saltzman A. (2017): *Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016*, «Global Food Security», 12, pp. 49-58.

Bouis H.E., Welch R.M. (2010): *Biofortification - a sustainable agricultural strategy for reducing micronutrient malnutrition in the global South*, «Crop Science», 50, pp. S20-S32.

Brooker R.W., Bennett A.E., Cong W.-F., Daniell T.J., George T.S., Hallett P.D., Hawes C., Ianetta P.P.M., Jones H.G., Karley A.J., Li L., McKenzie B.M., Pakeman R.J., Paterson E., Schöb C., Shen J., Squire G., Watson C.A., Zhang C., Zhang F., Zhang J., White P.J. (2015): *Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology*, «New Phytologist», 206, pp. 107-117.

Cakmak I. (2004): Proceedings of the International Fertiliser Society 552. Identification and correction of widespread zinc deficiency in Turkey – a success story, The International Fertiliser Society, York, UK.

Cakmak I. (2008): *Enrichment of cereal grains with zinc: agronomic or genetic biofortification?*, «Plant and Soil», 302, pp. 1-17.

Cakmak I. (2009): *Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India*, «Journal of Trace Elements in Medicine and Biology», 23, pp. 281-289.

Cakmak I., Kutman U.B. (2018): *Agronomic biofortification of cereals with zinc: a review*, «European Journal of Soil Science», 69, pp. 172-180.

Cakmak I., Kalayci M., Kaya Y., Torun A.A., Aydin N., Wang Y., Arisoy Z., Erdem H., Yazici A., Gokmen O., Ozturk L., Horst W.J. (2010): *Biofortification and localization of zinc in wheat grain*, «Journal of Agricultural and Food Chemistry», 58, pp. 9092-9102.

Cakmak I., McLaughlin M.J., White P. (2017): *Zinc for better crop production and human health*, «Plant and Soil», 411, pp. 1-4.

Ciccolini V., Pellegrino E., Coccina A., Fiaschi A.I., Cerretani D., Sgherri C., Quartacci M.F., Ercoli L. (2017): *Biofortification with iron and zinc improves nutritional and nutraceutical properties of common wheat flour and bread*, «Journal of Agricultural and Food Chemistry», 65, pp. 5443-5452.

Coccina A., Cavagnaro T.R., Pellegrino E., Ercoli L., McLaughlin M.J., Watts-Williams S.J. (2019): *The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain*, «BMC Plant Biology», 19, pp. 133.

Duncan E.G., Maher W.A., Jagtap R., Krikowa F., Roper M.M., O'Sullivan C.A. (2017): *Selenium speciation in wheat grain varies in the presence of nitrogen and sulphur fertilisers*, «Environmental Geochemistry and Health», 39, pp. 955-966.

Fairweather-Tait S.J., Bao Y., Broadley M.R., Collings R., Ford D., Hesketh J.E., Hurst R. (2011): *Selenium in human health and disease*, «Antioxidants and Redox Signaling», 14, pp. 1337-1383.

Fan M.-S., Zhao F.-J., Poulton P.R., McGrath S.P. (2008): *Historical changes in the concentrations of selenium in soil and wheat grain from the Broadbalk experiment over the last 160 years*, «Science of the Total Environment», 389, pp. 532-538.

Garrett R.G., Gawalko E., Wang N., Richter A., Warkentin T.D. (2013): *Macrorelationships between regional-scale field pea (*Pisum sativum*) selenium chemistry and environmental factors in western Canada*, «Canadian Journal of Plant Science», 93, pp. 1059-1071.

Garvin D.F., Welch R.M., Finley J.W. (2006): *Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm*, «Journal of the Science of Food and Agriculture», 86, pp. 2213-2220.

Graham R., Senadhira D., Beebe S., Iglesias C., Monasterio I. (1999): *Breeding for micronutrient density in edible portions of staple food crops: conventional approaches*, «Field Crops Research», 60, pp. 57-80.

Hart D.J., Fairweather-Tait S.J., Broadley M.R., Dickinson S.J., Foot I., Knott P., McGrath S.P., Mowat H., Norman K., Scott P.R., Stroud J.L., Tucker M., White P.J., Zhao F.J., Hurst R. (2011): *Selenium concentration and speciation in biofortified flour and bread: Retention of selenium during grain biofortification, processing and production of Se-enriched food*, «Food Chemistry», 126, pp. 1771-1778.

Ihnat M. (1989): *Plants and agricultural materials*, in *Occurrence and distribution of selenium*, edited by M. Ihnat, CRC Press, Boca Raton, Florida, USA, pp. 33-105.

Joy E.J.M., Broadley M.R., Young S.D., Black C.R., Chilimba A.D.C., Ander E.L., Barlow T.S., Watts M.J. (2015): *Soil type influences crop mineral composition in Malawi*, «Science of the Total Environment», 505, pp. 587-595.

Kumssa D.B., Joy E.J.M., Ander E.L., Watts M.J., Young S.D., Walker S, Broadley M.R. (2015): *Dietary calcium and zinc deficiency risks are decreasing but remain prevalent*, «Scientific Reports», 5, pp. 10974.

Kumssa D.B., Joy E.J.M., Young S.D., Odee D.W., Ander E.L., Broadley M.R. (2017): *Variation in the mineral element concentration of* Moringa oleifera *Lam. and* M. stenopetala *(Bak. f.) Cuf.: Role in human nutrition*, «PLoS ONE», 12 (4): e0175503.

Kumar V., Sinha A.K., Makkar H.P.S., Becker K. (2010): *Dietary roles of phytate and phytase in human nutrition: a review*, «Food Chemistry», 120, pp. 945-959.

Lazo-Vélez M.A., Chávez-Santoscoy A., Serna-Saldivar S.O. (2015): *Selenium-enriched breads and their benefits in human nutrition and health as affected by agronomic, milling, and baking factors,* «Cereal Chemistry Journal», 92, pp. 134-144.

Lee S., Woodward H.J., Doolittle J.J. (2011): *Selenium uptake response among selected wheat (*Triticum aestivum*) varieties and relationship with soil selenium fractions,* «Soil Science and Plant Nutrition», 57, pp. 823-832.

Li M., Wang S., Tian X., Zhao J., Li H., Guo C., Chen Y., Zhao A. (2015): *Zn distribution and bioavailability in whole grain and grain fractions of winter wheat as affected by applications of soil N and foliar Zn combined with N or P,* «Journal of Cereal Science», 61, pp. 26-32.

Liu H., Zhao P., Qin S., Nie Z. (2018): *Chemical fractions and availability of zinc in winter wheat soil in response to nitrogen and zinc combinations*, «Frontiers in Plant Science», 9, pp. 1489.

Liu J., Wu B., Singh R.P., Velu G. (2019): *QTL mapping for micronutrients concentration and yield component traits in a hexaploid wheat mapping population*, «Journal of Cereal Science», 88, pp. 57-64.

Lyons G.H., Genc Y., Stangoulis J.C.R., Palmer L.T., Graham R.D. (2005a): *Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content*, «Biological Trace Element Research», 103, pp. 155-168.

Lyons G., Ortiz-Monasterio I., Stangoulis J., Graham R. (2005b): *Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding?,* «Plant and Soil», 269, pp. 269-380.

Moore K.L., Schröder M., Lombi E., Zhao F.J., McGrath S.P., Hawkesford M.J., Shewry P.R., Grovenor C.R.M. (2010): *NanoSIMS analysis of arsenic and selenium in cereal grain*, «New Phytologist», 185, pp. 434-445.

Murphy K.M., Reeves P.G., Jones S.S. (2008): *Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars*, «Euphytica», 163, pp. 381-390.

Nelson A.G., Quideau S.A., Frick B., Hucl P.J., Thavarajah D., Clapperton M.J., Spaner D.M. (2011): *The soil microbial community and grain micronutrient concentration of historical and modern hard red spring wheat cultivars grown organically and conventionally in the black soil zone of the Canadian prairies*, «Sustainability», 3, pp. 500-517.

Pascoalino J.A.L., Thompson J.A., Wright G., Franco F.A., Scheeren P.L., Pauletti V., Moraes M.F., White P.J. (2018): *Grain zinc concentrations differ among Brazilian wheat genotypes and respond to zinc and nitrogen supply*, «PLoS ONE», 13 (7): e0199464.

Pellegrino E., Opik M., Bonari E., Ercoli L. (2015): *Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013*, «Soil Biology and Biochemistry», 84, pp. 210-217.

Prasad R., Shivay Y.S., Kumar D. (2014): *Agronomic biofortification of cereal grains with iron and zinc*, «Advances in Agronomy», 125, pp. 55-91.

Pu Z.E., Yu M., He Q.Y., Chen G.Y., Wang J.R., Liu Y.X., Jiang Q.T., Li W., Dai S.F., Wei Y.M., Zheng Y.L. (2014): *Quantitative trait loci associated with micronutrient concentrations in two recombinant inbred wheat lines*, «Journal of Integrative Agriculture», 13, pp. 2322-2329.

Pu Z., Pei Y., Yang J., Ma J., Li W., Liu D., Wang J., Wei J., Zheng Y. (2018): *A QTL located on chromosome 3D enhances the selenium concentration of wheat grain by improving phytoavailability and root structure*, «Plant and Soil», 425, pp. 287-296.

Raboy V. (2009): *Approaches and challenges to engineering seed phytate and total phosphorus*, «Plant Science», 177, pp. 281-296.

Rodríguez L.H., Morales D.A., Rodríguez E.R., Romero C.D. (2011): *Minerals and trace elements in a collection of wheat landraces from the Canary Islands*, «Journal of Food Composition and Analysis», 24, pp. 1081-1090.

Ros G., van Rotterdam A., Bussink D., Bindraban P.S. (2016): *Selenium fertilization strategies for bio-fortification of food: an agro-ecosystem approach*, «Plant and Soil», 404, pp. 99-112.

Shi R., Zhang Y., Chen X., Sun Q., Zhang F., Römheld V., Zou C. (2010): *Influence of long-term nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum *L.)*, «Journal of Cereal Science», 51, pp. 165-170.

Stein A.J. (2010): *Global impacts of human mineral malnutrition*, «Plant and Soil», 335, pp. 133-154.

Tabbita F., Pearce S., Barneix A.J. (2017): *Breeding for increased grain protein and micronutrient content in wheat: Ten years of the GPC-B1 gene*, «Journal of Cereal Science», 73, pp. 183-191.

Velu G., Ortiz-Monasterio I., Cakmak I., Hao Y., Singh R.P. (2014): *Biofortification strategies to increase grain zinc and iron concentrations in wheat*, «Journal of Cereal Science», 59, pp. 365-372.

Velu G., Tutus Y., Gomez-Becerra H.F., Hao Y., Demir L., Kara R., Crespo-Herrera L.A., Orhan S., Yazici A., Singh R.V., Cakmak I. (2017): *QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations*, «Plant and Soil», 411, pp. 81-99.

Wang P., Wang H., Liu Q., Tian X., Shi Y., Zhang X. (2017): *QTL mapping of selenium content using a RIL population in wheat*, «PLoS ONE», 12(9): e0184351.

White P.J. (2016a): *Biofortification of edible crops*, in eLS, John Wiley & Sons, Chichester UK. (doi 10.1002/9780470015902.a0023743).

White P.J. (2016b): *Selenium accumulation by plants*, «Annals of Botany», 117, pp. 217-235.

White P.J., Broadley M.R. (2005): *Biofortifying crops with essential mineral elements*, «Trends in Plant Science», 10, pp. 586-593.

White P.J., Broadley M.R. (2009): *Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine*, «New Phytologist», 182, pp. 49-84.

White P.J., Broadley M.R. (2011): *Physiological limits to zinc biofortification of edible crops*, «Frontiers in Plant Science», 2, pp. 80.

White P.J., George T.S., Dupuy L.X., Karley A.J., Valentine T.A., Wiesel L., Wishart J. (2013a): *Root traits for infertile soils*, «Frontiers in Plant Science», 4, pp. 193.

White P.J., George T.S., Gregory P.J., Bengough A.G., Hallett P.D., McKenzie B.M. (2013b): *Matching roots to their environment*, «Annals of Botany», 112, pp. 207-222.

Xue Y.F., Yue S.C., Zhang Y.Q., Cui Z.L., Chen X.P., Yang F.C., Cakmak I., McGrath S.P., Zhang F.S., Zou C.Q. (2012): *Grain and shoot zinc accumulation in winter wheat affected by nitrogen management*, «Plant and Soil», 361, pp. 153-163.

Xue Y.F., Zhang W., Liu D.Y., Xia H.Y., Zou C.Q. (2016): *Nutritional composition of iron, zinc, calcium and phosphorus in wheat grain milling fractions as affected by nitrogen supply*, «Cereal Chemistry», 93, pp. 543-549.

Zhang Y.Q., Deng Y., Chen R.Y., Cui Z.L., Chen X.P., Yost R., Zhang F.S., Zou C.Q. (2012a): *The reduction in zinc concentration of wheat grain upon increased phosphorus-fertilization and its mitigation by foliar zinc application*, «Plant and Soil», 361, pp. 143-152.

Zhang Y.Q., Sun Y.X., Ye Y.L., Karim M.R., Xue Y.F., Yan P., Meng Q.F., Cui Z.L., Cakmak I., Zhang F.S., Zou C.Q. (2012b): *Zinc biofortification of wheat through fertilizer applications in different locations of China*, «Field Crop Research», 125, pp. 1-7.

Zhao F.-J., Lopez-Bellido F.J., Gray C.W., Whalley W.R., Clark L.J., McGrath

S.P. (2007): *Effects of soil compaction and irrigation on the concentrations of selenium and arsenic in wheat grains*, «Science of the Total Environment», 372, pp. 433-439.

Zhao F.J., Su Y.H., Dunham S.J., Rakszegi M., Bedo Z., McGrath S.P., Shewry P.R. (2009): *Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin*, «Journal of Cereal Science», 49, pp. 290-295.

Zou C.Q., Zhang Y.Q., Rashid A., Ram H., Savasli E., Arisoy R.Z., Ortiz-Monasterio I., Simunji S., Wang Z.H., Sohu V., Hassan M., Kaya Y., Onder O., Lungu O., Yaqub Mujahid M., Joshi A.K., Zelenskiy Y., Zhang F.S., Cakmak I. (2012): *Biofortification of wheat with zinc through zinc fertilization in seven countries*, «Plant and Soil», 361, pp. 119-130.