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Biofortification in Tuscany: nutritional and nutraceutical aspects of old wheat genotypes and transferability to bread

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introduction

The World Health Organization (WHO) estimates that more than 2 billion people show deficiencies in key micronutrients, such as iron and zinc (Fe and Zn) (WHO, 2019). Iron deficiency is the most common and widespread nutritional disorder in the world, with major health consequences including poor pregnancy outcome, impaired physical and cognitive development, increased risk of morbidity in children, and reduced work productivity in adults (Hunt, 2005). Zinc deficiency represents the fifth major cause of diseases and mortality in developing countries, and its main consequences are losses of brain function, weakening of the immune system, and negative influences on physical growth (White and Broadley, 2009). Iron and Zn deficiency are caused by low dietary intake that is associated with a large consumption of foods based on cereals grown on Fe/Zn-deficient soil. Moreover, Fe and Zn deficiencies are projected to become more severe in the future with the predicted increase of carbon dioxide (CO_2) in the atmosphere (Myers et al., 2014).

The concentration of minerals in wheat flour is genetically determined by the cultivated genotype/variety and environmentally determined by soil, climate and management practices. Thus, effective strategies to enhance the uptake of micronutrients in grain rely on the selection of genotypes with high micronutrient use efficiency, field application of micronutrients as chemical fertilizers, and utilization of beneficial microorganisms.

selection of genotypes with high micronutrient use efficiency

Wheat shows a large variation in Fe and Zn efficiency, i.e. the ability of a

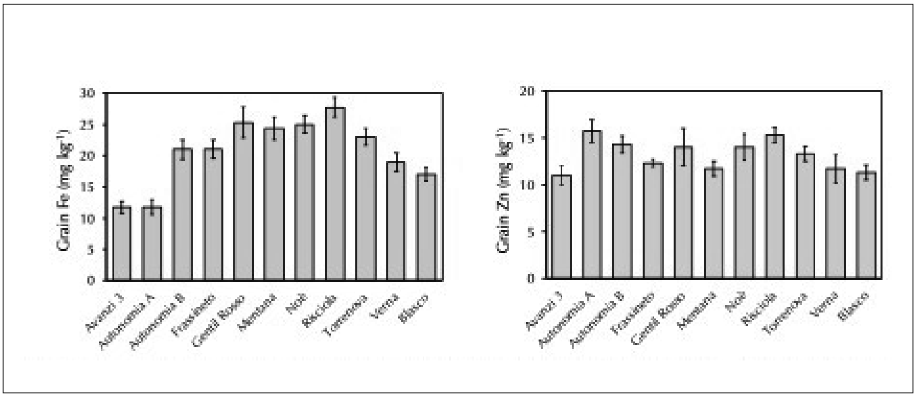

Fig. 1 *Grain Fe and Zn concentration of old and modern genotypes of bread wheat (Ercoli et al., 2016)*

genotype to grow and yield better under Fe- and Zn-deficient conditions in comparison with other genotypes. Monasterio and Graham (2000) reported a high variability in Fe and Zn concentration in grain (Fe: 20-99; Zn: 16-142 mg kg^{-1}) in over 3000 genotypes of bread wheat, durum wheat and triticale, including wild species, landraces and high-yielding modern varieties. Cakmak et al. (2000) in 58 lines of bread wheat found Fe and Zn concentrations in grain ranging from 24 to 51 mg Fe kg^{-1} and from 8 to 61 mg Zn kg^{-1}. The concentrations of the modern varieties were much lower and less variable than those of the wild ancestors.

Results from a field experiment carried out in a soil sufficient for Fe and deficient for Zn demonstrated that Fe and Zn concentration in wheat grain can be increased from 11 to 27.7 mg Fe kg^{-1} and from 11 to 15.7 mg Zn kg^{-1} by variety choice (fig. 1). Nutraceutical properties were also determined in grain and genotypes and showed similar variations. Indeed, total polyphenols and total flavonoids varied in the studied genotypes from 23 to 37 μmol GAE g^{-1} and from 1.1 to 1.9 μmol CE g^{-1}, respectively (fig. 2). Field experiments comparing wheat genotypes showed also that Fe and Zn concentrations in grain were highly unstable across locations and years, indicating that environmental conditions are substantial for the accumulation of grain Fe and Zn. Thus, wheat genotypes with ability to accumulate Fe and Zn into grain still depend on readily available and steady resupplies of minerals from the soil matrix that can only be improved by adequate agronomic soil fertility management practices (Frossard et al., 2009; White, 2016).

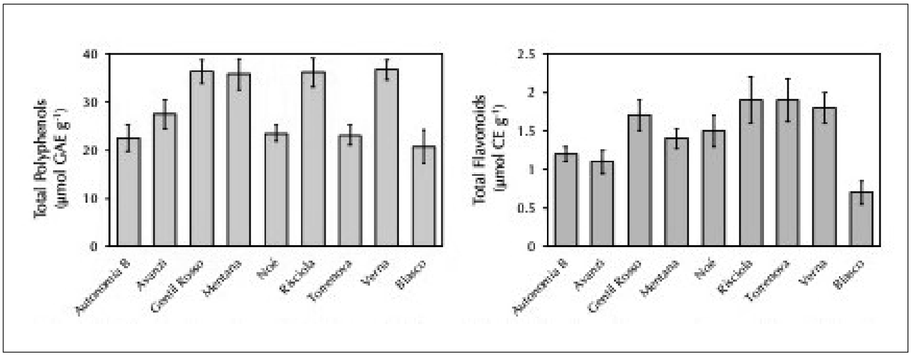

Fig. 2 *Total polyphenols and total flavonoids in grain of old and modern genotypes of bread wheat (Ercoli et al., 2016)*

FIELD APPLICATION OF MICRONUTRIENTS AS CHEMICAL FERTILIZERS

Agronomic biofortification aims to improve the micronutrient content of the edible parts of the crops by micronutrient fertilizer application to the soil and/or foliar application. The efficacy of soil and foliar application is influenced by many soil factors (i.e. pH, organic matter content, soil aeration and moisture and interactions with other elements) and by the structure and functioning of the roots of the crop variety (Cakmak, 2008; Zhang et al., 2010; Acisok et al., 2011).

Field foliar Fe and Zn biofortification differently increased concentrations of Fe and Zn in the wholemeal flour of five bread wheat genotypes. Increases ranged from 15% of Gentil Rosso to 71% of Blasco for Fe and from 13% of Frassineto to 80% of Blasco for Zn (fig. 3).

The variable response of wheat varieties to biofortification was confirmed in a following experiment, comparing an old variety (Gentil Rosso) with a modern one (Blasco) under similar pedoclimatic conditions (Ciccolini et al., 2017). Foliar biofortification with Fe and Zn greatly increased concentration and bioavailability of Zn only in the flour of the old variety of wheat, whereas it was ineffective on Fe concentration in both varieties. However, the old variety had higher concentration and bioavailability of Fe than the modern one. Moreover, wholemeal flour had higher Fe, Zn and health-promoting compounds compared to white flour, as minerals, antioxidant compounds and lipoic acid are mainly concentrated in the outer layers of the caryopsis, i.e., bran, and are lost by the refining processes during milling (fig. 4). Bread making slightly changed Fe and Zn concentration, but increased their bioavailability by over 70%, due to the reduction of phytate (data not shown).

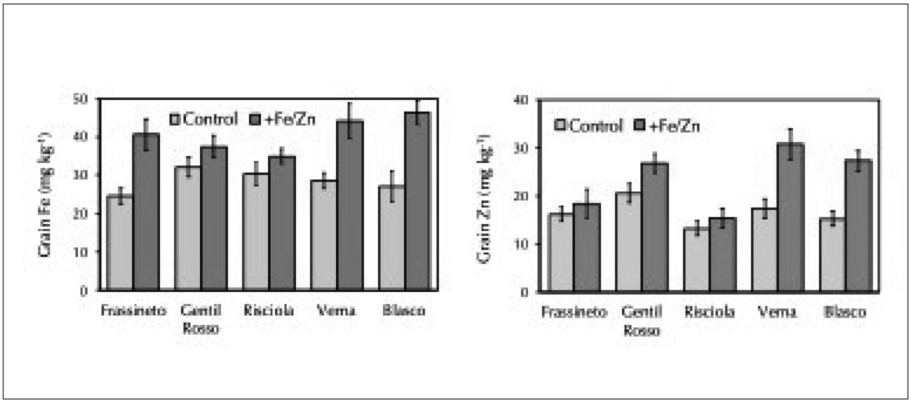

Fig. 3 *Grain Fe and Zn concentration of old and modern genotypes of bread wheat (unpublished results)*

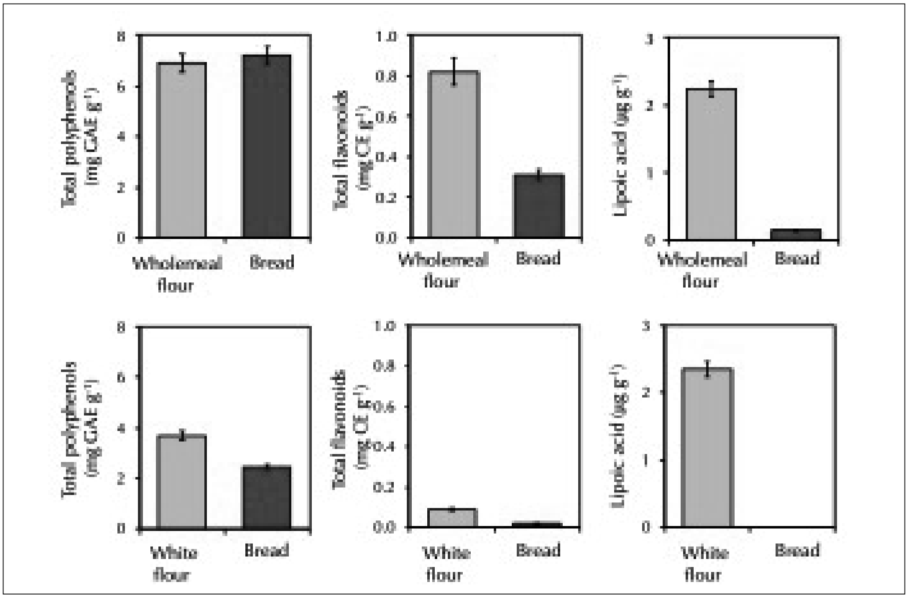

Fig. 4 *Total polyphenols, total flavonoids and lipoic acid in wholemeal and white flour and in wholemeal and white bread (Ciccolini et al., 2017)*

UTILIZATION OF BENEFICIAL MICROORGANISMS

Inoculation with arbuscular mycorrhizal fungi (AMF) promotes crop growth and yield by increasing mineral nutrient uptake, disease resistance and drought tolerance. The fungal network acts as an extension of the root system, increa-

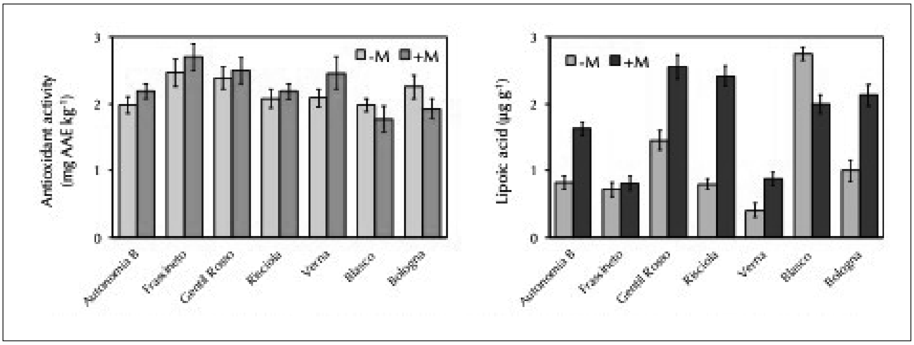

Fig. 5 *Antioxidant activity and lipoic acid in grain of old and modern genotypes of bread wheat (unpublished results)*

sing the volume of soil explored for nutrient uptake and the uptake of nutrients that are sparingly soluble in soil, such as Fe and Zn (Smith and Read, 1997). A meta-analysis studying the responses of wheat to field inoculation with AMF indicated increases in yield (20%), N content (31%) and Zn concentration (12.8%) in grain and a positive correlation between AMF root colonization rate and grain yield and Zn concentration (Pellegrino et al., 2015). These results were confirmed by a field experiment on durum wheat, showing increases due to inoculation by 42-63% for Fe and by 78-101% for Zn (Ercoli et al., 2017). Modern and old varieties distinctly responded to AMF inoculation, and the modern variety showed higher responsiveness in term of root length, AMF root colonization and grain macro- and micro-nutrient content.

AMF inoculation also affected health-promoting compounds in grain, but the effect varied according to the bread wheat genotype (fig. 5). Antioxidant activity was increased (4.2-19%) in five genotypes and decreased in two genotypes (10-17%), whereas lipoic acid was increased (14-203%) in six genotypes and decreased (28%) in one genotype.

CONCLUSIONS

The results presented here demonstrate that wheat micronutrient and nutraceutical content in grain can be effectively promoted by combined reliance on efficient crop genotypes, agronomic biofortification, and targeted utilization of beneficial microorganisms.

Moreover, it was shown that old wheat varieties are a good source of genes to enhance micronutrient and nutraceutical content of grain. The increase of

Fe and Zn concentration in grain depends on wheat genotypes carrying genes encoding for efficient mineral uptake and translocation or to high compatibility between plant and AMF.

However, the variable responses of wheat genotypes should be taken into consideration for the introduction of the agronomic biofortification in the ordinary management techniques of cereal farms and for planning breeding strategies aiming to improve mineral and nutraceutical content in grain.

All these results are of great support for developing a production chain of bread enriched with health-promoting compounds and bioavailable minerals and with potential protective role against chronic diseases.

RIASSUNTO

Vengono presentate e discusse le potenziali strategie di biofortificazione per migliorare la concentrazione di micronutrienti e nutraceutici nei cereali. Queste strategie includono la selezione di genotipi con elevata efficienza d'uso dei micronutrienti, l'applicazione in campo di microelementi come fertilizzanti chimici el' utilizzo di microrganismi rizosferici.

I risultati di esperimenti sul campo hanno dimostrato che la concentrazione di Fe e Zn nella granella di frumento tenero può essere aumentata da 11 a 27,7 mg di Fe kg^{-1} e da 11 a 15,7 mg di Zn kg^{-1} attraverso la scelta varietale. Analogamente, i polifenoli totali e i flavonoidi totali possono essere aumentati rispettivamente da 23 a 37 µmol di GAE g^{-1} e da 1,1 a 1,9 µmol di CE g^{-1}.

La biofortificazione fogliare con Fe e Zn durante la coltivazione ha aumentato la concentrazione e la biodisponibilità di Fe e Zn nella farina integrale di frumento tenero. Gli aumenti nelle varietà testate variavano dal 15 al 71% per il Fe e dal 13 all'80% per lo Zn. La farina integrale aveva una concentrazione più elevata di Fe, Zn e composti nutraceutici rispetto alla farina bianca. La trasformazione in pane ha modificato leggermente la concentrazione di Fe e Zn ma ha aumentato di oltre il 70% la loro biodisponibilità in conseguenza della riduzione dei fitati.

Una meta-analisi condotta per sintetizzare le risposte dell'inoculo con AMF al frumento ha indicato aumenti della produzione di granella (20%), del contenuto di N (31%) e della concentrazione di Zn (13%) nella granella e una correlazione positiva tra il tasso di colonizzazione delle radici da parte di AMF e la produzione e concentrazione di Zn nella granella. Questi risultati sono stati confermati da esperimenti in campo che hanno mostrato aumenti dovuti all'inoculazione che vanno dal 12% al 119% per Fe ed effetti che vanno da -20% a + 122% per Zn in dipendenza della varietà di frumento tenero utilizzata. L'inoculazione con AMF ha influito anche sui composti che promuovono la salute nei cereali, con effetti variabili in relazione alla varietà. L'attività antiossidante è aumentata (4-19%) in cinque varietà e diminuita in due varietà (10-17%), mentre l'acido lipoico è aumentato (14-203%) in sei varietà e diminuito (28%) in una varietà. Questi risultati sono di grande supporto per lo sviluppo di una catena produttiva di pane arricchita con composti che promuovono la salute e minerali biodisponibili e con potenziale ruolo protettivo contro le malattie croniche.

ABSTRACT

Potential biofortification strategies to enhance the concentration of micronutrients and nutraceuticals in cereals are presented and discussed. These strategies rely on the selection of genotypes with high micronutrient use efficiency, field application of micronutrients as chemical fertilizers, and utilization of rhizospheric microorganisms.

Results from field experiments demonstrated that Fe and Zn concentration in wheat grain can be increased from 11 to 27.7 mg Fe kg^{-1} and from 11 to 15.7 mg Zn kg^{-1} by variety choice. Similarly, total polyphenols and total flavonoids can be increased from 23 to 37 µmol GAE g^{-1} and from 1.1 to 1.9 µmol CE g^{-1}, respectively.

Field foliar Fe and Zn biofortification increased concentration and bioavailability of Fe and Zn in the wholemeal flour of bread wheat. Increases ranged from 15 to 71% for Fe and from 13 to 80% for Zn in the tested varieties. Wholemeal flour had higher Fe, Zn concentration and health-promoting compounds compared to white flour. Bread making slightly changed Fe and Zn concentration, but increased their bioavailability by over 70%, due to the reduction of phytate.

A meta-analysis studying the responses of wheat to AMF field inoculation indicated increases in yield (20%), N content (31%) and Zn concentration (12.8%) in grain and a positive correlation between AMF root colonization rate and grain yield and Zn concentration. These results were confirmed by field experiments showing increases due to inoculation ranging from 12% to 119% for Fe and effects ranging from -20% to +122% for Zn according to bread wheat variety. AMF inoculation also affected health-promoting compounds in grain but the effect varied according to the variety. Antioxidant activity was increased (4.2-19%) in five varieties and decreased in two varieties (10-17%), whereas lipoic acid was increased (14-203%) in six varieties and decreased (28%) in one variety.

All these results are of great support for developing a production chain of bread enriched with health-promoting compounds and bioavailable minerals and with potential protective role against chronic diseases.

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