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Does Fe accumulation in durum wheat seeds benefit from improved whole-plant sulfur nutrition?

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ABSTRACT

Sulfur and iron balanced supply is of paramount importance for plants, since Fe homoeostasis in plants has been shown to be strongly dependent on sulfate availability; *vice versa* the adaptation to Fe deficiency requires the adjustment of S uptake and assimilation rate. Interestingly, it has been demonstrated that providing S above adequate concentrations may enhance Fe use efficiency in wheat and this effect seems to be especially advantageous for plants grown under severe Fe shortage. Therefore, the investigation of sulfate effect on Fe uptake and allocation in crop could be of great significance.

Aim of this study was to clarify in wheat at both leaf and seed level whether and to what extent the changes in S and Fe supply affect concentration and distribution of sulfate and also how different availability of S changes the mineral concentration and distribution in wheat adequately or poorly fed with Fe.

Obtained results showed how plants recovered from Fe deficiency stress by means of a tuned S fertilization, without additional input of Fe fertilizers. Also, with decreasing Fe availability the Zn concentration of grains significantly increased, suggesting that a balanced crop Fe nutrition could allow a successful biofortification of wheat grains with Zn.

1. Introduction

Iron (Fe) is one of the most critical nutrients, being not only one of the main causes of yield limitation of crops in the World but also one of the most widespread human nutritional disorders affecting over 30% of the World's population (Hind and Guerinot, 2012). Cereals are the primary food source for humans, particularly in developing countries; thus, the nutritional level of the grain (as well as the nutritional state of plants) is of central importance to human health (Grusak and Dellapenna, 1999). Both plants and humans need an adequate supply of minerals for their nutrition; in this regard, the acquisition of Fe from soil can be often problematic for plants.

Iron is sparingly soluble under aerobic conditions, especially in high pH and calcareous soils, representing a serious problem for more that 30% of the World's cultivated soils (Guerinot and Yi, 1994). To cope with this nutritional disorder and to favour the micronutrient acquisition, higher plants have developed specific strategies (Marschner et al., 1986). In particular, graminaceous species cope with Fe deficiency stress by enhancing the exudation of phytosiderophores (PS) into the rhizosphere. These non-proteinogenic amino acids belonging to the mugineic acid family, form stable complexes with Fe^{3+} and are taken up by roots as intact Fe^{3+}–PS complexes *via* the Yellow Stripe 1 (YS1) transporter (Murata et al., 2006). Iron metabolism in plants is closely linked to sulfur (S) since the sulfur-containing amino acid methionine (Met) is the sole precursor of the mugineic acid family of PS (Mori and Nishizawa, 1987). In fact, it has been clearly demonstrated that plant capability to take up and accumulate Fe is strongly dependent on S availability in the growth medium in cereal plants (Bouranis et al., 2003; Astolfi et al., 2006; Zuchi et al., 2012). On the other hand, the modulation of S uptake and assimilation rate play a significant role in the plant adaptation to the changes of Fe availability (Ciaffi et al., 2013; Celletti et al., 2016a). For instance, it has been shown that a super-optimal S feeding (2.4 mM vs 1.2 mM which is considered as optimal) favours an accumulation of Fe in shoots of durum wheat (Zuchi et al., 2012). Recently, it has been observed a positive correlation between changes in S accumulation and plant capability to release PS (and correspondingly to accumulate Fe), indicating that a super-optimal S fertilization of plants can increase the Fe use efficiency of roots. Since

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this phenomenon is specifically observed in wheat plants, this plant species could represent a potentially useful model system to study S/Fe interactions (Celletti et al., 2016b). These findings open a significant outlook on exploring potential and sustainable use of S nutrition in improving Fe distribution within the plant and its accumulation into grains of durum wheat. The Fe-S interplay might be exploited from both a scientific and an applicative point of view identifying both the response mechanisms associated with multiple deficiency and developing agronomic practices aimed at increasing Fe acquisition from soil (i.e. more sustainable agriculture) and at obtaining biofortified agricultural products (Welch and Graham, 2004). In fact, one of the most important challenges to be met in the next future is also to increase the nutritional value of the agricultural products, like the content of Fe. However, in this respect, the evidence concerning the possible contribution of an over availability of S in the accumulation of Fe at the seed level of cereals is still missing.

Starting from these premises, this study focused on the potential effects of S application rates and concentrations on plant capability to accumulate Fe in grains especially in Fe-deficient conditions. To this aim, durum wheat plants were grown on sand/perlite mixture under two different S and Fe supplies throughout the growing season. At harvest, plants samples and seeds were collected and analysed for their nutrients content by inductively coupled plasma-optical emission spectroscopy (ICP-OES). The distribution of the nutrients, in particular of S and Fe, in whole seeds and seed sections was assessed by micro-focused X-ray fluorescence (μ−XRF) imaging. Recently Lemmens et al. (2018) showed the high relevance of μ−XRF imaging data in studying element distribution in wheat grains. Data of plant and seed analysis are discussed in relation to the S and Fe availability levels in the growth medium.

2. Materials and methods

2.1. Growth conditions

Seeds of durum wheat (*Triticum durum* L. cv. Svevo) were germinated on moistened paper in the dark at 20 °C for 4 d. Seedlings were then transferred in 20 cm diameter plastic pots (three seedlings in each pot) filled with 3 L of 50% (v/v) sand/perlite mixture as substrate and were grown in the greenhouse (a low-technology model in which the active environmental control was limited to a natural ventilation system through wall and roof windows and the photoperiod was provided by natural sunlight). Plants were watered with 1 L pot^{-1} of nutrient solution (1 L pot^{-1}) (NS) (Zhang et al., 1991) was applied from above every other day (three times per week, Monday, Wednesday and Friday) and with 1 L pot^{-1} of demineralised water on the other days (three times per week, Tuesday, Thursday and Saturday). Pots were allowed to drain freely to prevent any accumulation of nutrients in pots.

The experiment examined the effect of two target sulfate concentrations (*i.e.*1.2 and 2.4 mM) on Fe accumulation in durum wheat plants and grains. Sulfate concentrations in the NS were selected and applied according to our previous report (Zuchi et al., 2012; Celletti et al., 2016b). The highest concentration was considered as extra sulfate supply condition and labelled with E, whereas the lowest concentration was considered as sufficient condition and labelled with C. Furthermore, NS was supplemented with two different concentrations of Fe (III)-EDTA (10 and 80 μM, deficiency and sufficiency condition, respectively).

Thus, the treatments were consisted on two factors, sulfate and iron, and two levels of each factor were taken, determining four different conditions, listed as follows: C = control (1.2 mM sulfate and 80 μM Fe^{III}-EDTA), F = Fe deficiency (1.2 mM sulfate and 10 μM Fe^{III}-EDTA), E = excess S supply (2.4 mM sulfate and 80 μM Fe^{III}-EDTA) and EF = excess S supply and Fe deficiency (2.4 mM sulfate and 10 μM Fe^{III}-EDTA).

Nutrient solution, containing both sulfate and Fe^{III}-EDTA, was supplied to the plants until maturity (about 56 days post anthesis); after this point the plants were left without water to allow them to senesce prior to harvest at 170 days after sowing. At harvest, whole plants were collected by cutting them at the stem base, separated into shoots (stems + leaves) and ears. To estimate grain yield, ears were hand-harvested and counted (number of ears per plant) and grains were weighed (g per plant) to obtain mean grain yield.

The experiment was arranged as a completely random design with three replications (pots) and the pots were randomly moved daily to minimize position effects.

2.2. Chlorophyll content

The chlorophyll content per unit area was estimated in attached leaves by a portable apparatus (SPAD-meter, Minolta Co., Osaka, Japan) using the first fully expanded leaf from the top of the plant. Recordings were conducted approximately every week during the whole experimental period.

2.3. Analysis of micro- and macronutrient concentrations

Shoot tissues and grains were dried to a constant weight at 80 °C, weighed and acid digested with concentrated ultrapure HNO_3 (65% v/v, Carlo Erba, Milano, Italy), using a Single Reaction Chamber (SRC) microwave digestion system (UltraWAVE, Milestone, Shelton, CT, USA). The elements concentration was subsequently analyzed by ICP-OES (Spectro Arcos, Spectro, Germany). Elements quantifications were carried out using certified multi-element standards (CPI International, https://cpiinternational.com). Tomato leaves (SRM 1573a) and spinach leaves (SRM 1547) have been used as external certified reference material.

To determine total S concentration, shoot and root tissues and grains were homogenized and one g of each sample was dried at 80 °C and then ashed in a muffle furnace at 500 °C. The ashes were dissolved in 10 mL of 3 N HCl and filtered through Whatman No. 42 paper. In contact with $BaCl_2$, a $BaSO_4$ precipitate is formed which is determined turbidimetrically (Bardsley and Lancaster, 1960).

2.4. Micro-focused X-ray fluorescence (μ−XRF) imaging

Micro X-ray fluorescence maps were collected by a laboratory benchtop μ-XRF spectrometer (M4 Tornado, Bruker Nano GmbH, Berlin, Germany). This instrument is equipped with a micro-focus Rh X-ray source (50 kV, 600 μA), a polycapillary X-ray optics with a spotsize of 25 μm and two XFlash™ energy dispersive silicon drift detectors with 30 mm^2 sensitive area and an energy resolution of 140 eV @ Mn K_α. The two detectors, placed at opposite sites compared to the X-ray optics, allow to reduce shadowing effects in the elemental maps. Wheat grains were impregnated in epoxy resin and sectioned both longitudinally (along the crease tissue) and transversely (at the middle of the seed). The cut seeds where then glued on glass slides with an epoxy resin and trimmed to 200 μm thickness. All the analyses were performed under reduced pressure (20 mbar) by acquiring one spectrum every 20 μm step, with an acquisition time of 20 ms per step. In order to increase the signal-to-noise ratio, each sample was scanned 30 times and the spectra averaged. XRF hyperspectral data and images were processed with the ESPRIT™ built-in software from the M4 Tornado. All the maps were collected with the same analytical conditions and the same scale was used for the same element in all the maps. Therefore the elemental maps can be compared for the same element and brighter colors mean a higher concentration of the element. Three sections (both longitudinal and transverse) from three different seeds were prepared and analysed for each treatment. Results were similar within the same treatment, therefore only the images from one section for each treatment are shown as representative of the treatment. Correlation maps were obtained by using the software Datamuncher (Alfeld and Janssens, 2015),