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Research article

Shoot ionome to predict the synergism and antagonism between nutrients as affected by substrate and physiological status

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ABSTRACT

The elemental composition of a tissue or organism is defined as ionome. However, the combined effects on the shoot ionome determined by the taxonomic character, the nutrient status and different substrates have not been investigated. This study tests the hypothesis that phylogenetic variation of monocots and dicots grown in iron deficiency can be distinguished by the shoot ionome.

We analyzed 18 elements in barley, cucumber and tomato and in two substrates (hydroponic vs soil) with different nutritional regimes. Multivariate analysis evidenced a clear separation between the species. In hydroponic conditions the main drivers separating the species are non essential-nutrients as Ti, Al, Na and Li, which were positively correlated with macro- (P, K) and micro-nutrients (Fe, Zn, Mo, B). The separation between species is confirmed when plants are grown on soil, but the distribution is determined especially by macronutrients (S, P, K, Ca, Mg) and micro-nutrients (B).

A number of macro (Mg, Ca, S, P, K) and micronutrients (Fe, Mn, Zn, Cu, Mo, B) contribute to plant growth and several other important physiological and metabolic plant activities. The results reported here confirmed that the synergism and antagonism between them and other non-essential elements (Ti, Al, Si, Na) define the plant taxonomic character.

The ionome profile might thus be exploited as a tool for the diagnosis of plants physiological/nutritional status but also in defining biofortification strategies to optimize both mineral enrichment of staple food crops and the nutrient input as fertilizers.

1. Introduction

Inorganic elements in plant tissues, classified according to Marschner (2011) as macronutrients (N, S, P, Ca, Mg, K), micro-nutrients (Ni, Mo, Cu, Zn, Mn, B, Fe, Cl) and beneficial elements (e.g. Na, Co, Al, Se, Si), are acquired through the root system as ions from the rhizosphere soil solution. A balanced supply of these elements is needed for an optimal plant growth and development, and therefore plants possess finely regulated mechanisms that govern ions accumulation and homeostasis (Williams and Salt, 2009). Mineral elements are essential to cells life and are required for almost every process in an organism. In this vision, the unraveling of elements functions and dynamics within living cells is of paramount importance to assess how plants control and regulate their nutritional status (Baxter, 2010). The ionome, i.e. 'the mineral nutrient and trace element composition of an organism, representing the inorganic component of cellular and organismal systems' (Salt et al., 2008), together with transcriptome and metabolome, defines a dynamic network that determines the physiology and biochemistry of the plant, which are ultimately controlled by the genome, in response to both internal and external stimuli.

The availability and consequently plant uptake, translocation and allocation of nutrients are affected not exclusively by the type of nutrient source (e.g. Fe, Tomasi et al., 2009) but also by several physical, chemical and biological soil characteristics, as for instance pH, redox potential and microbial activity (Mimmo et al., 2014; Pii et al., 2015a). Therefore, plants often face biotic and/or abiotic stresses (e.g. nutrients deficiencies and/or toxicities) that alter the plant physiology, by inducing for example modification in the root architecture and in the nutrients uptake efficiency (Marschner, 2011). Most likely, such stresses can also alter the homeostasis and the distribution of elements within plants tissues (Baxter et al.,

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2012). For instance, it has been recently demonstrated that the plant ionome (*i.e. Arabidopsis thaliana* L.) could be exploited as a tool for an unsupervised prediction and detection of specific physiological responses to environmental or genetic perturbations as well as of nutritional statuses and asymptomatic deficiencies (Baxter et al., 2008).

Iron is an essential nutrient required for many biochemical processes in plants, including photosynthesis and respiration, where it participates in electron transport processes. However, the solubility of Fe in soils is very often limited, particularly in calcareous soils, causing in plant species the onset of the typical symptoms of Fe-deficiency. In addition, Fe starvation also induces plants responses influencing both the rhizosphere bioavailability of the nutrient and its transport across the plasma membrane of root cell (Kim and Guerinot, 2007). With respect to Fe uptake, monocots and dicots adopt different mechanisms (Kobayashi and Nishizawa, 2012). Dicots (*Strategy I* plants, Marschner and Römheld, 1994) acquire Fe via a mechanism that involves the reduction of Fe^{III} to Fe^{II} and the uptake of Fe^{II} thanks to the transmembrane electrochemical gradient guaranteed by the activity of plasma membrane H^+-ATPase. Differently, grasses (*Strategy II* plants) base their capacity to take up Fe on the biosynthesis and exudation of phytosiderophores (PSs) with a strong chelation affinity for Fe^{III} (Schaaf et al., 2004). The resulting Fe-PS complex is then transported into root cells through a high affinity uptake system (Curie et al., 2001; Inoue et al., 2009).

In plants experiencing Fe deficiency, an unbalanced cation/anion uptake rate in favor to cations has been well described, also recently (Astolfi et al., 2014; Tomasi et al., 2014), provoking an unusual accumulation of several cationic nutrients at the shoot level. This effect has been ascribed to the activity of the Fe-deficiency-induced plasma membrane-Fe^{III}-chelate reductase (Welch et al., 1993; Wulandari et al., 2013) and to the activity of Fe transporters which are able to transport also other metals, such as Cd, Cu, Mn, and Zn (Eide et al., 1996; Rogers et al., 2000). An ionomic approach has thus the ability to capture information about the functional state of living organisms driven by nutrient shortages. Baxter et al. (2008) for instance showed how a multivariable ionomic signature of *Arabidospis* shoots was able to predict the plants nutritional status of the plants.

To date the majorities of the ionomic studies are based on experiments carried out either in hydroponic or soil conditions and were aimed at identifying and characterizing gene networks in mutant plants. Yet, recent studies highlighted the importance of studying processes and mechanisms governing nutrient availability in soil conditions rather than in solution cultures (Mimmo et al., 2013; Oburger et al., 2014). The bioavailability of nutrients is in fact closely related to rhizosphere processes at the soil-root interface and to the environmental growth conditions, such as soil composition, hydrology and biotic component of the rhizosphere (Pii et al., 2015a; Ziegler et al., 2013).

The effects of the Fe deprivation on the ionome of agricultural crops have not been investigated yet, whilst it has been studied in the model plant *A. thaliana* (Baxter et al., 2008). On these premises, this study aimed at assessing the ionomic profiles of three different plant species (barley, tomato and cucumber) grown in either Fe sufficiency or Fe deficiency in hydroponic and soil conditions. This growth system was adopted in order to obtain two different rhizosphere effects for the mobilization of nutrients, as already demonstrated (Pii et al., 2015b). The hypothesis of this work is that this dataset would help understanding the possible synergistic and antagonistic interactions between elements that might exist regardless of the tissue, species or environment under study.

2. Materials and methods

2.1. Plant growth

Seeds of barley (*Hordeum vulgare* L. cv. Europa), tomato (*Solanum lycopersicum* L. cv. Marmande) and cucumber (*Cucumis sativus* L. cv. Chinese Long) were soaked for 24 h in an aerated 0.5 mM $CaSO_4$ solution and germinated for 4–5 days in the dark at 22 °C between two layers of filter paper moistened with 0.5 mM $CaSO_4$. Homogenous seedlings were then transferred to plant pots in 5 L tanks containing a full nutrient solution using the RHIZOTest system (ISO/CD 16198: 2011, Cirad, France) (Bravin et al., 2010), and were grown hydroponically under controlled conditions in a climatic chamber 14/10 h light/dark, 24/19 °C, 70% Relative Humidity and 250 μmol m^{-2} s^{-1} light intensity. Plants were cultured in either Fe-free or Fe-supplemented nutrient solution (NS). The composition of the NS is as follows: 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.7 mM K_2SO_4, 0.1 mM KCl, 0.1 mM KH_2PO_4, 1 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, 100 μM Fe(III)-EDTA. The NS was continuously aerated, changed three times a week and the pH was adjusted to 6 with KOH 1 N. In order to limit photo-chemical reduction phenomena of the micronutrients in the NS (Zancan et al., 2006), tanks were covered with black plastic foil during the entire experiment.

After 14 days of hydroponic culture, when the first symptoms of Fe deficiency were visible, a set of Fe-deficient and Fe-sufficient plants were transferred to an agricultural calcareous soils for 6 days using the plant-soil RHIZOtest system (Bravin et al., 2010). A polyamide membrane with a 30-μm mesh enabled the physical separation between the root mat and the soil. The soil used was a silt loam with the following characteristics: pH_{H2O} 7.72, C_{org} 0.86% w/w, N_{tot} 1.16 g/kg, $CaCO_3$ 61.20% w/w, CEC 20.40 $cmol_+$/kg, Fe_{tot} 1.60% w/w.

2.2. Plants biometric parameters

Light transmittance of fully expanded leaves was determined using a portable chlorophyll meter SPAD-502 (Minolta, Osaka, Japan) and presented as SPAD index values. At least, five SPAD measurements were taken per leaf and averaged.

At harvest, shoots and roots were separated and dried at 45 °C until constant weight was reached. Plant dry weight of roots and shoots were recorded.

2.3. ICP-OES analysis

Representative samples of shoot tissue were digested with concentrated HNO_3 (65% (v/v), Carlo Erba) using a single reaction chamber (SRC, UltraWAVE, Milestone Inc, Shelton, CT, USA). The elements concentration was subsequently determined by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES, Spectro CirosCCD, Spectro, Germany). Elements quantifications were carried out using certified multi-element standards (CPI International, https://cpiinternational.com). The limits of detection for each element are reported as follow: Al 6.7 μg L^{-1}, B 1.8 μg L^{-1}, Ba 0.1 μg L^{-1}, Ca 2.0 μg L^{-1}, Cu 3.0 μg L^{-1}, Fe 0.4 μg L^{-1}, K 2.0 μg L^{-1}, Li 0.1 μg L^{-1}, Mg 3.0 μg L^{-1}, Mn 0.2 μg L^{-1}, Mo 6.0 μg L^{-1}, Na 1.0 μg L^{-1}, P 4.0 μg L^{-1}, S 4.0 μg L^{-1}, Si 12.0 μg L^{-1}, Sr 0.1 μg L^{-1}, Ti 1.3 μg L^{-1}, Zn 0.2 μg L^{-1}. Tomato leaves (SRM 1573a) and spinach leaves (SRM 1547) have been used as external certified reference material.

2.4. Statistical analysis

Data are expressed as mean values ± SE. Statistical analyses as

Student's t-test, ANOVA and Pearson's correlation were carried out using GraphPad Prism version 6.00 for Mac OS X, GraphPad Software, San Diego California USA. Multivariate analyses were carried out by using STAT Graphic Centurion XV, version 15.1.02. The validity of the PCA models were assessed by the cross-validation approach previously described (Bro et al., 2008).

3. Results

3.1. Plants biometric parameters

Plants were grown in hydroponic solution, either complete or Fe-free, for 14 days until the appearance of the characteristic symptoms of Fe starvation. Fe-deficient plants, irrespectively from the species, showed a significant reduction in the total chlorophyll content, measured as SPAD index, of about ten units as compared to Fe sufficient plants (Table 1). In barley, belonging to the *Strategy II* plants, Fe starvation did not induce any alteration to the root system but lead to a decrease of the shoot growth respect to Fe sufficient plants (Table 1). Conversely, in the plants belonging to the *Strategy I* (*i.e.* cucumber and tomato) a significant increase was recorded in the root dry biomass of Fe deficient plants whilst no variations were observed in the growth of the aerial parts (Table 1). Despite these, the root/shoot ratios of Fe starved plants were greater than those of Fe sufficient plants, irrespectively from the plant species (Table 1).

Biomass data and SPAD indexes were recorded also for soil-grown Fe-deficient or Fe-sufficient plants. These plants were previously cultured in a hydroponic condition and then transferred on soil for 6 days using the RHIZOtest system (Bravin et al., 2010; Pii et al., 2015b). As reported in Table 1, Fe deficient plants increased their SPAD index by about eight units whilst the plants biomass did not show any significant difference, except for Fe-sufficient cucumber plants whose root dry biomass was almost doubled as compared to Fe starved cucumber plants as well as the root/shoot ratio (Table 1).

3.2. Multiple elements concentration

The elemental analyses of the shoot tissues of the plants considered in this study highlighted that the majority of the elements (about 13 elements out of 18 analyzed) varied significantly according to the plant species (Fig. 1 and Supplementary Fig. S1).

When plants were grown in a complete hydroponic nutrient solution, cucumber and tomato plants accumulate at two and four times higher concentrations of calcium (Ca) with respect to barley. Magnesium (Mg) concentration was twice as high in cucumber and tomato compared to barley (Fig. 1). On the other hand, barley and cucumber displayed a higher concentration of sulphur (S), phosphorus (P) and potassium (K) as compared to tomato plants (Fig. 1A). In the case of micronutrients, barley and cucumber showed similar concentration of boron (B), copper (Cu), zinc (Zn) and molybdenum (Mo), whilst iron (Fe) was more concentrated in the tissues of dicots plants (Fig. 1A).

Iron-deficient conditions (Fig. 1B) induced a significant increase of Mg and manganese (Mn) only in dicots, whilst P and S decreased only in cucumber, remaining constant in barley and tomato as compared to control. The concentration of Zn increased an all the three species with respect to Fe-sufficient plants (Fig. 1B).

When Fe-sufficient plants were cultivated for 6 days on calcareous soil, they showed a significantly modified ionomic profile (Table 2, Fig. 1). Barley and cucumber plants showed a decreased (approximately −50%) concentration of K, whilst it increased in tomato plants. Barley still showed the highest concentration of P and S, as for hydroponically grown plants, whereas dicots showed similar values. Concerning Fe, barley showed an enhanced concentration with respect to hydroponically grown plants, however lower than dicots that did not display any variation. Soil-grown Fe-deficient plants displayed a general enhancement in the concentration of Ca, Mg, Mn, S and Fe, whilst B increased only in barley and decreased in only cucumber (Fig. 1D).

3.3. Principal component analysis (PCA)

Pattern recognition techniques were used to reveal differences and similarities between the plant species (i.e. barley, tomato and cucumber), the treatments (Fe-deficiency and Fe-sufficiency) and the growth substrate (hydroponic solution and soil), and to identify the variables that explain the behavior of the samples.

The principal component analysis (PCA) carried out on the data set of hydroponic grown plants (Fe-deficient and Fe-sufficient) extracted four factors with eigenvalues of 9.39, 4.33, 2.30 and 1.01, respectively, and accounted for the 94.45% of total variance (Supplementary Table S1). The first two components described 76.2% of the total variance and both the corresponding scatter plot and biplot are displayed in Fig. 2A and Fig. 2B, respectively. The first component, accounting for the 52.14% of the total variance, separated the samples in three clusters in function of the plant species

Table 1
Biometric parameters of plants grown either in hydroponic solution or in soil.

	Hydroponics		Soil		p
	+Fe	-Fe	+Fe	-Fe	
Barley					
SPAD	25.75 ± 0.57^{a}	15.02 ± 0.45^{b}	29.92 ± 1.16^{c}	23.36 ± 1.00^{a}	***
Shoot DW (g)	0.052 ± 0.007 ^{ab}	0.037 ± 0.004^{b}	0.068 ± 0.010^{c}	0.053 ± 0.005 ^{ab}	*
Root DW (g)	0.012 ± 0.002^{a}	0.12 ± 0.001^{a}	0.019 ± 0.003 ^{ab}	0.020 ± 0.003^{b}	*
Root/Shoot	0.231 ± 0.007^{a}	0.324 ± 0.004^{b}	0.280 ± 0.010^{c}	0.377 ± 0.006^{d}	***
Tomato					
SPAD	33.73 ± 1.02^{a}	17.96 ± 0.66^{b}	33.36 ± 1.26^{a}	25.53 ± 2.51^{b}	***
Shoot DW (g)	0.019 ± 0.001^{a}	0.017 ± 0.002^{a}	0.024 ± 0.001^{b}	0.027 ± 0.002^{b}	**
Root DW (g)	0.008 ± 0.001^{a}	0.013 ± 0.002^{a}	0.025 ± 0.001^{b}	0.030 ± 0.003^{b}	***
Root/Shoot	0.421 ± 0.008^{a}	0.765 ± 0.003^{b}	1.042 ± 0.001^{c}	1.111 ± 0.003^{d}	***
Cucumber					
SPAD	36.80 ± 0.30^{a}	25.63 ± 0.52^{b}	44.71 ± 1.15^{c}	33.38 ± 1.12^{d}	***
Shoot DW (g)	0.069 ± 0.002 ^{ab}	0.059 ± 0.001^{a}	0.076 ± 0.007 ^{bc}	0.073 ± 0.003 ^{ab}	*
Root DW (g)	0.008 ± 0.001^{a}	0.011 ± 0.001^{b}	0.025 ± 0.002^{c}	0.012 ± 0.001^{b}	***
Root/Shoot	0.116 ± 0.002^{a}	0.186 ± 0.001^{b}	0.328 ± 0.007^{c}	0.164 ± 0.003^{d}	***

Data are reported as means ± SE, n = at least 3 biological replicates. The statistical significance was tested by means of ANOVA with Tukey post-test. Different letters indicates statistically different values (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

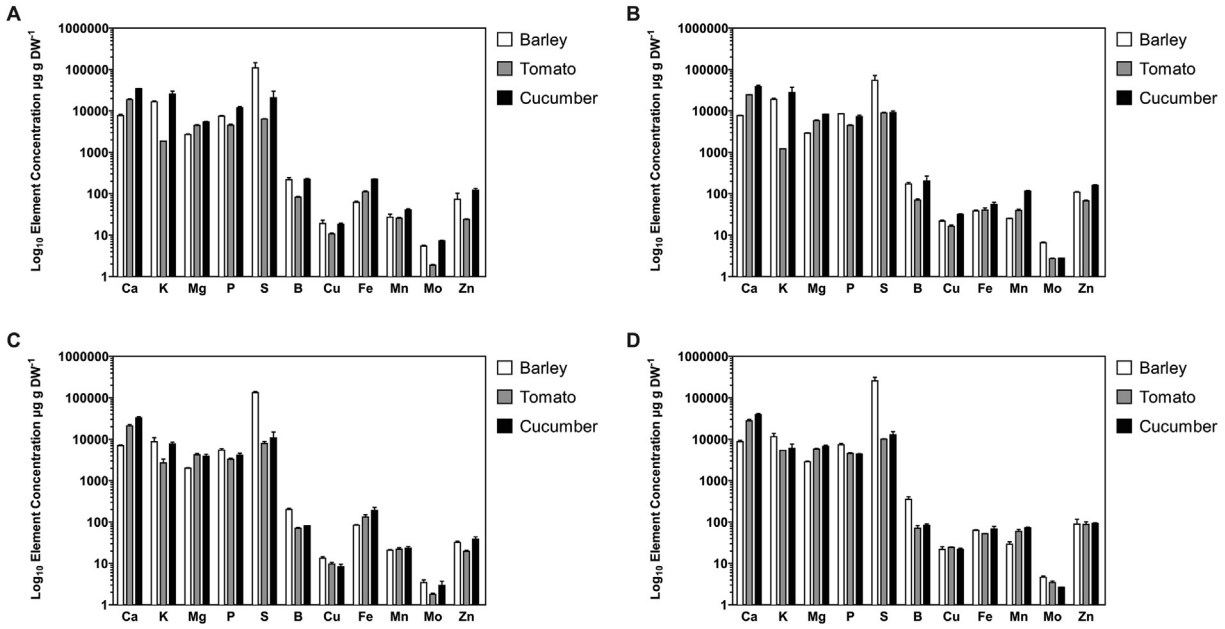

Fig. 1. Concentration of mineral macro- and micronutrients in the shoots of barley, tomato and cucumber plants. (A) Concentration of mineral macro- and micronutrients in plants grown in a full hydroponic nutrient solution. The statistical significance of data was tested by two-way ANOVA (Species, $p = 0.0021$; Elements, $p < 0.0001$; Interaction between variables, $p < 0.0001$). (B) Concentration of mineral macro- and micronutrients in plants grown in a Fe-free hydroponic nutrient solution. The statistical significance of data was tested by two-way ANOVA (Species, $p = 0.0083$; Elements, $p < 0.0001$; Interaction between variables, $p < 0.0001$). (C). Concentration of mineral macro- and micronutrients in plants grown on calcareous soils. The statistical significance of data was tested by two-way ANOVA (Species, $p < 0.0001$; Elements, $p < 0.0001$; Interaction between variables, $p < 0.0001$). (D) Concentration of mineral macro- and micronutrients in Fe-deficient plants grown on calcareous soils. The statistical significance of data was tested by two-way ANOVA (Species, $p < 0.0001$; Elements, $p < 0.0001$; Interaction between variables, $p < 0.0001$). The concentration of nutrients (mg g^{-1} DW), reported in the plot as $\log_{10}$, is expressed as means ± SE (n = at least 3).

(Fig. 2A). Cucumber, despite being a dicot, showed that a more similar behavior to barley instead to tomato. According to the biplot, strontium (Sr), titanium (Ti), sodium (Na), aluminum (Al), barium (Ba), B and K were the main elements contributing to this clustering (Fig. 2B). The second component, describing 24.06% of the total variance, discriminates the nutritional regime imposed to the plants (i.e. Fe-sufficiency and Fe-deficiency) within each cluster (Fig. 2A). In this case, the highest positive influence on the principal component 2 is given by divalent cations, such as Mg, Ca and Mn (Fig. 2B).

The PCA analysis of the data set of soil-grown plants extracted three factors with eigenvalues of 8.94, 4.95 and 1.93 respectively, and accounting for the 87.90% of total variance (Supplemental Table 2). The first two principal components described 77.19% of the total variance and the resulting scatterplot showed that samples were subdivided in two distinct groups (Fig. 3A), one containing the *strategy II* barley plants and the other encompassing the *strategy I* tomato and cucumber plants, irrespectively from the nutritional status. Along the first component (49.68% of the total variance) samples are clustered according to the Fe acquisition strategy (Fig. 3A); the highest positive contributions to this separation were given by Al, B, S and Si, and they were contrasted by the concentration of divalent cations, as Ca, Mg, and Sr (Fig. 3B). As observed before (Fig. 2), the second principal component (27.51% of the total variance) distinguished the sample in function of the plant nutritional status and, as shown in the biplot, highest positive contributions to this separation were given by cations, such as copper (Cu), Zn and Mn, whilst they were contrasted essentially by the concentration of Fe (Fig. 3B).

Table 2
Analysis of variance (ANOVA) of the data set of the 18 element concentrations of the shoots of barley, tomato and cucumber grown either hydroponically or on soil.

	Barley	Tomato	Cucumber
+Fe			
Growth Substrate	ns	ns	0.008
Elements	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Interaction	ns	0.0217	0.001
-Fe			
Growth Substrate	0.0013	$P < 0.0001$	0.0008
Elements	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Interaction	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

3.4. Analyses of the relationships between elements

The relationships between the element concentrations that generated the PCA models were further investigated through the determination of the Pearson product–moment correlation coefficient for each growing conditions (hydroponic vs soil). The correlation matrixes (Table 3) highlighted that certain elements display correlations that are independent from the nutritional status and specific to the cultivation system, whilst other correlation are peculiar for the substrate.

Independently from the plant species, in hydroponic conditions Cu, Li and Zn showed the highest number of correlations (Table 3). Copper had the strongest positive correlations with divalent cations, namely Ba, Mn and Zn, whilst Li resulted to be strongly, positively correlated with Al and B (Table 3). Zinc, beside the correlation with Cu, was positively correlated to K and Na.

On the other hand, the interactions that mostly characterized soil conditions were Ca and Mg. Both showed positive interactions with other divalent cations, namely Ba, Mn and Sr, whereas they had negative correlations with Al, B, S, Si and Ti (Table 3). In addition, the relationships of Sr with Al, B, S and Si, changed as a function of the plant cultivation system, being strongly positive in hydroponics and negative in soil (Table 3).

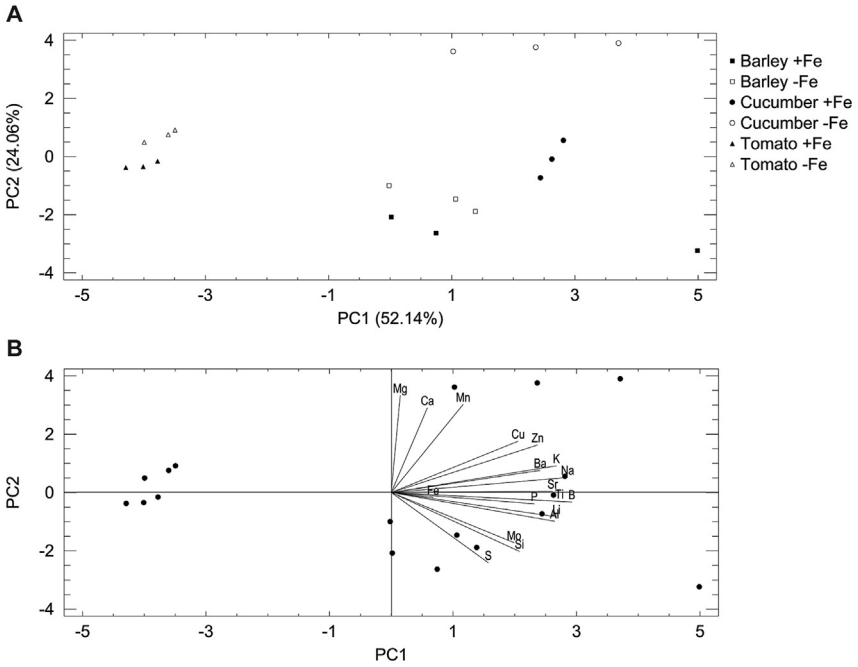

Fig. 2. Principal components analysis of the shoot ionome of hydroponically-grown plants. (A) Scatterplot representing the modification of the shoot ionome as a function of the plant species and the nutritional regime (i.e. Fe-deficiency and Fe-sufficiency). (B) Biplot graphs from the ionome of barley, tomato and cucumber plants. The length of each eigenvector is proportional to the variance in the data for that element. The angle between the eigenvectors represents the correlations among the different elements.

4. Discussion

It is well known that the mechanisms underlying the homeostatic control of ions in an organism are strongly interrelated (Eide et al., 2005). The ionomic approaches, applied on several genetic lines of a single species or on inbred plant population deriving from few parental lines, revealed that the correlation between elements are highly species- and environment-specific (Prinzenberg et al., 2010; Buescher et al., 2010; Ding et al., 2010; Ghandilyan et al., 2009a, 2009b; Klein and Grusak, 2009; Liu et al., 2009; Vreugdenhil et al., 2004; Waters and Grusak, 2008; Baxter et al., 2012). In the present work, we analyzed 18 elements in three plant species (barley, tomato and cucumber), in two growth-conditions (hydroponics or soil), with two different nutritional

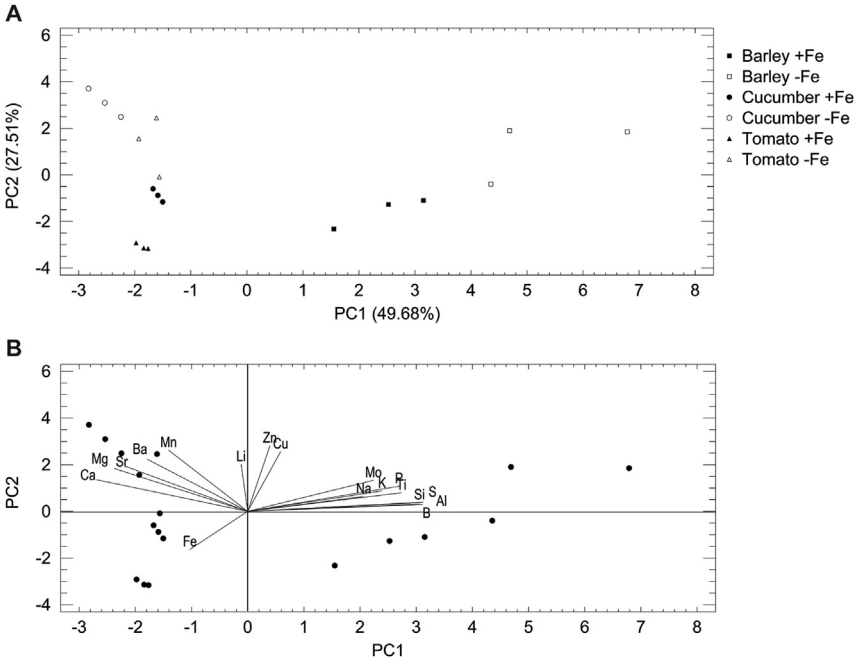

Fig. 3. Principal components analysis of the shoot ionome of soil-grown plants. A) Scatterplot representing the modification of the shoot ionome as a function of the plant species and the nutritional regime (i.e. Fe-deficiency and Fe-sufficiency), imposed prior soil contact period. (B) Biplot graphs from the ionome of barley, tomato and cucumber plants. The length of each eigenvector is proportional to the variance in the data for that element. The angle between the eigenvectors represents the correlations among the different elements.

Table 3
Pearson's correlation matrix for the element concentration in the shoot tissue of plants grown either hydroponically or on soil. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

	B	Ba	Ca	Cu	Fe	K	Li	Mg	Mn	Mo	Na	P	S	Si	Sr	Ti	Zn
Hydroponics																	
Al	0.92***	0.58**	0.05	0.36	0.36	0.60**	0.80***	−0.16	0.10	0.63	0.78***	0.62**	0.70***	0.88***	0.85***	0.77***	0.32**
B	–	0.79***	0.15	0.52*	0.30	0.85***	0.90***	−0.03	0.29	0.64**	0.94***	0.72***	0.54*	0.71***	0.79***	0.86***	0.62**
Ba	–	–	0.16	0.74***	−0.20	0.85***	0.79***	0.18	0.57*	0.29	0.80***	0.36	0.32	0.42	0.63**	0.54*	0.69**
Ca	–	–	–	0.36	0.43	0.35	−0.15	0.92***	0.74***	−0.23	0.31	0.19	−0.59*	−0.36	0.21	0.20	0.50
Cu	–	–	–	–	−0.30	0.68**	0.53*	0.48*	0.80***	0.14	0.65**	0.31	0.14	0.24	0.68**	0.45	0.92**
Fe	–	–	–	–	–	0.18	−0.03	0.06	−0.15	0.39	0.28	0.59**	−0.16	0.07	0.10	0.51*	0.01
K	–	–	–	–	–	–	0.78***	0.24	0.54*	0.52*	0.93***	0.73***	0.16	0.30	0.59**	0.81***	0.77**
Li	–	–	–	–	–	–	–	−0.22	0.19	0.59*	0.83***	0.56*	0.71**	0.74***	0.74***	0.71**	0.51*
Mg	–	–	–	–	–	–	–	–	0.87***	−0.48*	0.17	−0.09	−0.62**	−0.48*	0.13	−0.04	0.48*
Mn	–	–	–	–	–	–	–	–	–	−0.32	0.50*	0.04	−0.30	−0.18	0.41	0.17	0.71**
Mo	–	–	–	–	–	–	–	–	–	–	0.50*	0.87***	0.47*	0.57**	0.40	0.83***	0.36
Na	–	–	–	–	–	–	–	–	–	–	–	0.71***	0.34	0.51*	0.74***	0.84***	0.73***
P	–	–	–	–	–	–	–	–	–	–	–	–	0.21	0.39	0.49*	0.96***	0.58*
S	–	–	–	–	–	–	–	–	–	–	–	–	–	0.94***	0.63**	0.36	0.11
Si	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.78***	0.55*	0.27
Sr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.68**	0.73***
Ti	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.68**
Soil																	
Al	0.98***	−0.39	−0.69***	0.18	−0.3	0.76***	0.14	−0.62**	−0.36	0.60	0.68***	0.77***	0.98***	0.99***	−0.51*	0.87***	0.16
B	–	−0.4	−0.71**	0.22	−0.34	0.77***	0.08	−0.59**	−0.33	0.65**	0.74***	0.81***	0.99***	0.99***	−0.54*	0.82***	0.19
Ba	–	–	0.88***	0.30	0.03	−0.11	0.73***	0.80***	0.77***	−0.12	−0.05	−0.25	−0.43	−0.39	0.98***	−0.22	0.48*
Ca	–	–	–	0.07	0.26	−0.39	0.47*	0.88***	0.66**	−0.41	−0.37	−0.58*	−0.73***	−0.71***	0.95***	−0.56*	0.25
Cu	–	–	–	–	−0.80***	0.22	0.18	0.42	0.74***	0.52*	0.17	0.48*	0.22	0.22	0.20	0.27	0.91***
Fe	–	–	–	–	–	−0.15	0.03	−0.15	−0.52*	−0.33	−0.20	−0.44	−0.35	−0.33	0.14	−0.28	−0.58*
K	–	–	–	–	–	–	0.39	−0.44	−0.19	0.73***	0.55*	0.58*	0.76***	0.78***	−0.20	0.69**	0.27
Li	–	–	–	–	–	–	–	0.27	0.41	0.25	0.09	0.14	0.03	0.11	0.68**	0.34	0.40
Mg	–	–	–	–	–	–	–	–	0.87***	−0.31	−0.24	−0.45	−0.61**	−0.63**	0.82***	−0.57*	0.47
Mn	–	–	–	–	–	–	–	–	–	0.03	−0.13	−0.06	−0.34	−0.34	0.73***	−0.22	0.75***
Mo	–	–	–	–	–	–	–	–	–	–	0.45	0.80***	0.63**	0.65**	−0.23	0.66**	0.52*
Na	–	–	–	–	–	–	–	–	–	–	–	0.50*	0.71***	0.71***	−0.21	0.48*	0.14
P	–	–	–	–	–	–	–	–	–	–	–	–	0.80***	0.83***	−0.39	0.85***	0.49*
S	–	–	–	–	–	–	–	–	–	–	–	–	–	0.99***	−0.57*	0.82***	0.17
Si	–	–	–	–	–	–	–	–	–	–	–	–	–	–	−0.53*	0.88***	0.20
Sr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	−0.34	0.39
Ti	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.35

regimes (Fe deficiency and Fe sufficiency). In both growing conditions, the principal component analysis (PCA) carried out on the data set representing the shoot ionome evidenced a clear separation between plant species; even if many authors suggested that the ionomic signature is tightly bound to the plant species (Broadley et al., 2003, 1999, 2001; Hodson et al., 2005; White et al., 2004; Willey and Wilkins, 2006), a large body of evidence also highlight the fact that the ability of plants to take up and allocate nutrient from the substrate depends on the substrate itself. In fact, it has been demonstrated that the interaction between plants and the growth media can determine the quality and the magnitude of the rhizosphere activity aiming at increasing nutrients bioavailability and their uptake (Mimmo et al., 2013). On these premises, to our knowledge, this is the first experience where different crop plants are compared in the very same growing conditions, with both a hydroponic-based and soil-based cultivation system. In hydroponic conditions, the main drivers separating the species were non essential-nutrients as Ti, Al, Na and Li (Fig. 2), which were positively correlated with macro- (P, K) and micronutrients (Fe, Zn, Mo, B) (Table 3). This might indicate that non-essential elements are closely linked to genetic traits rather than environmental parameters as light, substrate, air temperature, water, humidity and carbon dioxide (Quadir et al., 2011). In this context, despite cucumber is a dicot, this plant species displayed an ionomic signature more similar to that of barley, a monocot, instead of that observed in tomato plants (Fig. 2). The separation between the three plants species was also confirmed when plants are grown on soil; in this case, the distribution was especially dependent on macronutrients (S, P, K, Ca, Mg) and micronutrients (B), (Fig. 3). Except for hydroponically-grown barley plants, a positive correlation between Ca and Mg was observed, as also described in other plant species (Baxter et al., 2012, 2009). This correlation has been reported as quite robust, even though these two cations use different pathways for uptake and translocation (Baxter et al., 2012). In addition to Mg, Ca was further correlated with Mn and this is supported by functional studies carried out on plant genes, as Ca exchange 2 (CAX2, codifying for a Ca^{2+}/H^{+} antiporter of the tonoplast) and endoplasmic reticulum type Ca-ATPase 1 (ECA1) (Hirschi et al., 2000; Wu et al., 2003). In this respect, it has been shown that the overexpression of CAX2 in heterologous species as tobacco enhances Mn tolerance via its accumulation within plant tissues (Hirschi et al., 2000). Furthermore, Punshon et al. (2012) have demonstrated the capability of CAX2 to transport also other bivalent cations like Cd. It should be highlighted, however, that in this phenomenon the chemical similarity between ions (e.g. charge, radius, etc.) is not necessarily the only parameter triggering the interactions between elements (i.e. favoring or hindering their absorption). For instance, it is well documented that the monovalent cation K^{+} can compete with the absorption of the divalent Mg^{2+}, thus causing Mg deficiency symptoms in both herbaceous and tree plants (Heenan and Campbell, 1981; Sun and Payn, 1999).

Element uptake is tightly controlled by biochemical activities of plants, which in turn depend on the nutritional and physiological status. Enzyme activities at the root plasma membrane are involved in the element uptake mechanisms determining the cation/anion uptake rates (Tomasi et al., 2014). For instance, Fe^{2+} is absorbed at the root level via the IRT1-like protein (Connolly et al., 2003) which is also able to transport Mn, Zn, Cu and Cd (Korshunova et al., 1999). In the present study, Fe was negatively correlated with these micronutrients indicating that Fe deficiency, which is known to induce the overexpression of IRT1 genes in both cucumber and tomato (Borlotti et al., 2012; Zamboni et al., 2012), enhances their accumulation. Thanks to the antagonism previously described (Fe vs Mn, Zn, Cu and Cd), the PCA analysis highlighted the separation in function of the nutritional status within each plant species (Figs. 2 and 3). This phenomenon was more evident in soil-grown plants most likely due to a sink-source effect induced by plant-soil interactions.

Furthermore, Fe was correlated to K and P (Figs. 2 and 3). Such relationship might be due to fact that a molecular cross talk has been suggested for many genes responding to K, P and Fe deficiencies in tomato plants (Wang et al., 2002). As observed in Fig. 1 and Fig. 3B, barley was strongly characterized by high S concentrations. Previous studies conducted with durum wheat showed that Fe deprivation causes an increase in S concentration in shoots closely linked to the thiol concentration (Ciaffi et al., 2013). Durum wheat and barley belong to *Strategy II* plants (Marschner and Römheld, 1994), which cope with Fe starvation with the huge release of phytosiderophores (PS) and, then, the uptake of Fe-PS complexes (Kobayashi et al., 2010). Phytosiderophores are non proteinogenic amino acids deriving from nicotianamine, whose percursors are methionine and S-adenosylmethionine (SAM); in this context, the methionine cycle is fundamental in order to supply SAM efficiently for PS synthesis (Kobayashi et al., 2010; Kobayashi and Nishizawa, 2012). Besides S, barley is characterized by the positive correlation with each other of B, Mo and P, both in hydroponic and soil conditions (Table 3, Figs. 2 and 3).

A relevant number of macro- (Mg, Ca, S, P, K) and micronutrients (Fe, Mn, Zn, Cu, Mo, B) contribute to plant growth and to several other important physiological and metabolic plant activities (Marschner, 2011). Results here presented confirmed that the synergism and antagonism between these nutrients and other non-essential elements (Ti, Al, Si, Na) define the plant behavior with respect to the root acquisition of the elements and their allocation in the different plant tissues. Thus, the present ionomic approach applied at the shoot level allows distinguishing plant species behavior even when they are grown in contrasting environment and under abiotic stress, based on their taxonomic character. The ionome profile might thus be exploited for the identification of a set of marker elements for the development of predictive tools aiming at the diagnosis of plants physiological/nutritional status. The dissection of the mobilization, uptake and redistribution of elements within the plant tissues is also a prerequisite in defining biofortification strategies to optimize both mineral enrichments of staple food crops and the nutrient input as fertilizers.

Authors' contributions

Designing and performing the experiments: YP, TM.
Critical discussion of the data: YP, TM, SC.
Paper preparation: YP, TM, SC.
Research coordination: TM.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2015.05.002.

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