Wheat (*Triticum aestivum* L.) response to boron in contrasting soil acidity conditions

Julierme Zimmer Barbosa[1], Antonio Carlos Vargas Motta[1], Rangel Consalter[1], Volnei Pauletti[1]

[1] Universidade Federal do Paraná, Setor de Ciências Agrárias, Departamento de Solos e Engenharia Agrícola, Rua dos Funcionários, 1540, Juvevê, CEP 80035-050, Curitiba-PR, Brasil. E-mail: barbosajz@yahoo.com.br; mottaacv@ufpr.br; rangelconsalter@hotmail.com.br; vpauletti@ufpr.br

ABSTRACT

Wheat cultivation in acid soils may be simultaneously limited by metal toxicity, deficiency or Ca and Mg imbalance, and B deficiency. The aim of the present study is to assess whether the wheat response to B increases, as the crop condition in the acid soil becomes more stressful. The study was conducted in a greenhouse, and an Oxisol sample was used as the substrate. It assessed the interaction between three soil acidity treatments (low [pH-CaCl$_2$ 5.6], very high [pH-CaCl$_2$ 4.2] and very high acidity with gypsum [pH-CaCl$_2$ 4.3]) and five B treatments (0, 0.15, 0.40; 1.25 and 3.50 mg dm^{-3}). The very high addition of gypsum into a very acid soil caused a nutritional imbalance in wheat (mainly to Mg) and led to lower dry matter accumulation values, a condition in which B presented stress alleviation. Such condition resulted in significant changes in root growth, transpiration and the availability of Mg and Ca/Mg ratio in the rhizosphere soil. The concentration of K, Ca, Mg, P, Fe, Mn, Zn, Cu and Al was little affected by B, since the K:Mg ratio was more important. The treatment with very high acidity, with gypsum, also showed higher B toxicity. Thus, wheat has shown higher B response in acid soil under the most stressful condition.

Key words: metal toxicity; nutrient imbalance; root traits; rhizosphere; transpiration

Resposta do trigo (Triticum aestivum L.) ao boro em condições contrastantes de acidez do solo

RESUMO

O cultivo do trigo em solos ácidos pode ser limitado, simultaneamente, pela toxidez por metais, por deficiência ou desbalanço da relação Ca e Mg, e por deficiência de B. O presente estudo tem o objetivo de verificar se a resposta do trigo ao B aumenta, na medida em que a condição de cultivo em solo ácido se torna mais estressante. O estudo foi conduzido em casa-de-vegetação usando uma amostra de um Latossolo Vermelho-Amarelo como substrato. A interação entre três tratamentos de acidez do solo (baixa [pH-CaCl$_2$ 5,6], muito alta [pH-CaCl$_2$ 4,2], acidez muito alta com gesso [pH-CaCl$_2$ 4,3]) e cinco de B (0; 0,15; 0,40; 1,25 e 3,50 mg dm^{-3}) foi avaliada. A alta adição de gesso em solo com elevada acidez, causou desbalanço nutricional (principalmente para Mg) no trigo, e levou à menor acumulação de matéria seca, sendo esta a condição em que o B apresentou amenização do estresse. Houve significativas alterações sob tal condição: no crescimento radicular, na transpiração das plantas e na disponibilidade de Mg, e na relação Ca/Mg no solo da rizosfera. Em contraste, a concentração de K, Ca, Mg, P, Fe, Mn, Zn, Cu e Al foi pouco afetada pelo B, uma vez que a relação K:Mg foi mais importante. Também ocorreu maior toxidez de B no tratamento com alta acidez e gesso. Assim, o trigo apresentou maior resposta ao B no solo ácido sob a condição mais estressante.

Palavras-chave: toxidez por metais; desbalanço nutricional; características das raízes; rizosfera; transpiração

Introduction

One of the main factors associated with the low production of crops cultivated in acid soils is the excess of Al. Also, Ca, Mg and B deficiency and Mn excess may simultaneously occur with Al toxicity (Bell, 1997; Millaleo et al., 2010; Kochian et al., 2015). Aluminum toxicity and B, Ca and Mg deficiency symptoms affect cell wall functions by reducing growth and water, as well as the plant's nutrient acquisition capacity (Horst et al., 2010; Broadley et al., 2012).

Studies have been conducted in order to assess the B/Al interaction in acidic cultivation media, and Al toxicity mitigation by the action of B, mainly in non-gramineous species (LeNoble et al., 1996; Favaretto et al., 2007; Heidarabadi et al., 2011; Zhou et al. 2015). Hossain et al. (2005) found that the wheat response to B depends on Ca availability. However, a high Ca availability can be harmful when Mg availability is low, mainly in acid soils, the fact that leads to an imbalance in the Ca/Mg ratio (Osemwota et al., 2007).

Besides the nutritional aspects, the B/Al interaction under acid conditions may, however, be related to variations in the used species, since gramineous and non-gramineous species have different mechanisms to avoid metal toxicity, such as metal transportation to aerial organs, rhizosphere alkalinization, and root cation exchange capacity (CEC) (Guigues et al., 2014; Kochian et al., 2015).

Wheat is a gramineous species cultivated worldwide and one of the most produced cereals in several countries. There is the natural prevalence of very acidic soils in many wheat cultivation regions (Tang et al., 2011; Baquy et al., 2016). Nevertheless, there are few data on the effect of B on wheat plants grown in acid soils, mainly in soils presenting more than one stress factor.

Thus, the aim of the present study is to assess whether the wheat response to B increases as the crop condition in acid soils becomes more stressful.

Materials and Methods

A sample of Red-Yellow Latosol (Oxisol) was collected 0-20 cm from ground level to be used as the substrate. The sample presented 800, 15 and 185 g kg^{-1} of sand, silt, and clay, respectively. The substrate was sieved through 4 mm mesh and separated into three parts, which were incubated for 60 days with the following treatments: (I) low acidity, 2 g kg^{-1} CaO/MgO (1:1 ratio); (II) very high acidity, 0.2 g kg^{-1} CaO/MgO (1:1 ratio); (III) very high acidity with gypsum, 0.2 g kg^{-1} CaO/MgO (1:1 ratio), and 3.5 g kg^{-1} CaSO$_4$.H$_2$O. The soil acidity classification in each treatment was based on the pH values

by Raij et al. (1997). The corrective agent was applied to the treatment I to raise the V rate to 70%. The idea, in treatment II, was to increase V to 20%; and in treatment III, was to slightly raise the pH and apply a dose of CaSO$_4$.H$_2$O equivalent to 7 t ha^{-1} to the soil. Each substrate fraction was sampled and chemically analyzed after the incubation period (Table 1).

The soil in each treatment was added with 120 mg P kg^{-1} (KH$_2$PO$_4$) and 180 mg K kg^{-1} (KNO$_3$). Each treatment was then separated into five parts, which were individually added with 0 (B1); 0.15 (B2); 0.40 (B3); 1.25 (B4) and 3.50 (B5) mg B dm^{-3} as boric acid. It was applied to B2 and B3 in order to deliver the nutrient in an overall recommended range; whereas in B4 and B5, it was overall used in toxic amounts.

Nine kilograms (9 kg) of soil from each of the treatments above were placed in 10-L plastic pots (diameter: 21 cm; height: 30 cm). Soil moisture was kept close to 60 % of water retention; therefore, the pots were periodically weighed (until the end of the wheat cultivation period) to adjust moisture through the addition of deionized water. Moreover, two pots were kept with no plants in them. These containers were used to measure soil evaporation.

Seeds of CD 109 (moderately sensitive to Al) wheat cultivar were sown in each pot, under protected environment conditions. Eight plants per pot were maintained after germination and seedling establishment. Approximately 15 days after emergence (DAE), 70 mg N kg^{-1} (urea source) were applied to the soil. The plants were grown until the total inflorescence emission (50 DAE).

The roots were separated from the soil through joint soil/root declodding, which was conducted in a 4 mm mesh sieve. The soil adhered to the roots (rhizosphere soil) and that not associated with the roots (bulk soil) were collected. The rhizosphere soil was collected by shaking the roots with adhered soil within a plastic bag. The soil residue found on the roots was removed under tap water, and the root systems from three plants were stored in 60 % ethanol (v/v). The remaining roots were shaken in a sequence of deionized water, 0.01 mol L^{-1} HCl and deionized water (~1 minute by step), and then placed in paper bags and stored for 72 h at 65 °C. After this period, the dry plant material mass was quantified on a digital scale (0.001 g). The wheat aerial part was rinsed in deionized water, separated into two fractions (stem + leaves, and inflorescence) and, subsequently, packaged in paper bags, subjected to the drying process and dry matter quantification, as recommended for roots.

The roots stored in ethanol were scanned in the WinRhizo software to find the length, surface area, volume, and forks. The dried root tissue and both shoot fractions (stem + leaf and inflorescence) were ground in Wiley mill and subjected

Table 1. Chemical analysis of soil after incubation in different inorganic materials[1].

Treatment	pH-CaCl$_2$	Al^{3+}	H + Al^{3+}	Ca^{2+}	Mg^{2+}	K$^+$	CEC	V	m	Ca/Mg	P	B
		cmol$_c$ dm^{-3}						%			mg dm^{-3}	
LA	5.6	0.0	4.8	3.1	1.1	0.2	9.2	47.5	0.0	2.8	2.0	0.32
VHA	4.2	0.8	8.6	0.9	0.6	0.2	11.1	15.1	32.0	1.5	6.2	0.40
VHAG	4.3	0.7	7.9	5.5	0.8	0.2	15.1	42.9	9.7	6.8	5.9	0.38

[1] The soil acidity classification in each treatment was based on the pH values by Raij et al. (1997); LA, low acidity; VHA, very high acidity; VHAG, very high acidity with gypsum; pH (CaCl$_2$ 0.01 mol L^{-1}); K$^+$ and P (Mehlich-1 extraction); Ca^{2+}, Mg^{2+}, Al^{3+} (extracted through the use of KCl 1mol L^{-1}); H + Al^{3+} (calcium acetate 0.5 mol L^{-1} extraction); B (extracted through the use of hot water); cation exchange capacity = CEC = Al^{3+} + (H + Al^{3+}) + Ca^{2+} + Mg^{2+} + K$^+$; Bases saturation = V = [(Ca^{2+} + Mg^{2+} + K$^+$) * 100]/CEC; Al saturation = m = [(Al * 100)/(Al^{3+} + Ca^{2+} + Mg^{2+} + K$^+$)]

to elemental analysis. Approximately 1 g of ground plant material was ashed in porcelain crucibles, in a muffle, at 500 °C for 4 hours. After the crucibles had cooled off, drops of 3 mol L^{-1} HCl were added to the ashes, and a new crucible was placed in the muffle at 500 ° C for 4 hours. Then, 10 ml of 3 mol L^{-1} HCl were added to the crucible, which remained on a hot plate, at 70 °C, for 10 minutes. After this period was elapsed, the digestion solutions were filtered through filter paper (pore diameter 8 μm), and the extracts were collected in 50-ml volumetric flasks. Deionized water was used to gauge the volumetric flasks. The elements Al, K, Ca, Mg, Fe, Mn, Cu and Zn were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian 720-ES) in extracts obtained after digestion; as well as and B (azomethine-H) and P (yellow vanadate), through UV-VIS spectrometry (Bel Photonics, SP2000). A small fraction of the roots was ground in a porcelain mortar and analyzed for CEC through the titration method proposed by Crooke (1964).

The rhizosphere and bulk soils were subjected to chemical analysis and determination in a digital pH-meter by using soil: solution ratio 1:2.5, at 30 minutes agitation and 30 minutes rest for pH-CaCl$_2$. The elements Ca and Mg were extracted through the use of KCl 1mol L^{-1} (soil: solution ratio 1:10) and determined in atomic absorption spectrometer (Varian, AA240FS). The B was extracted through BaCl$_2$ in hot water bath (5 minutes at 70°C) and determined through UV-VIS spectrometry (Bel Photonics, SP2000) by using azomethine-H.

Data were subjected to analysis of variance (ANOVA) according to an entirely randomized design, with the factorial arrangement (three soil acidity treatments x five B treatments) and four replications. The Tukey test at 5% probability was used for data analysis.

Results and Discussion

Boron application has affected the total, stem+leaves, inflorescence and root DM accumulation in plants cultivated in very high acidity soil, with gypsum (Table 2). Only the

Table 2. Wheat total, stem + leaves, inflorescence and root dry matter according to soil acidity and B application.

Soil acidity treatment[1]	B application (mg dm^{-3})[2]				
	0	0.15	0.40	1.25	3.50
Total (g plant^{-1})					
LA	2.86 Aa	2.82 Aa	3.06 Aa	2.89 Aa	2.45 Aa
VHA	1.99 Ba	2.05 Ba	2.03 Ba	1.78 Ba	1.65 Ba
VHAG	1.49 Cab	1.89 Ba	1.81 Ba	1.44 Bab	1.10 Cb
Stem+leaves (g plant^{-1})					
LA	1.82 Aa	1.76 Aa	1.90 Aa	1.84 Aa	1.54 Aa
VHA	1.29 Ba	1.24 Ba	1.25 Ba	1.10 Ba	1.03 Ba
VHAG	0.97 Bab	1.19 Ba	1.14 Ba	0.94 Bab	0.68 Cb
Inflorescence (g plant^{-1})					
LA	0.56 Aab	0.55 Aab	0.61 Aa	0.55 Aab	0.41 Ab
VHA	0.38 Ba	0.43 Ba	0.40 Ba	0.33 Ba	0.29 Ba
VHAG	0.29 Bab	0.39 Ba	0.37 Bab	0.28 Bab	0.23 Bb
Root (g plant^{-1})					
LA	0.48 Aa	0.51 Aa	0.55 Aa	0.50 Aa	0.50 Aa
VHA	0.32 Ba	0.38 Ba	0.38 Ba	0.35 Ba	0.33 Ba
VHAG	0.23 Bab	0.31 Ba	0.29 Bab	0.22 Cab	0.19 Cb

[1]LA, low acidity; VHA, very high acidity; VHAG, very high acidity with gypsum. [2]Different values, lowercase (B application) and uppercase letters (soil acidity) indicate significant difference through the Tukey test (p < 0.05)

inflorescence dry matter fraction has increased due to the addition of B in B3 in the low soil acidity treatment. The plants have shown greater DM accumulation in the low soil acidity treatment.

The decreased DM in response to the higher Ca/Mg rate was previously observed by Osemwota et al. (2007) in a study on corn. The sulfate ions compete with the phosphate ones (H_2PO^-; HPO_4^{2-}) for uptake sites in the roots. Thus, the high sulfate (SO_4^{2-}) amounts added to the gypsum could interfere in P acquisition by the plant (Davidian & Kopriva, 2010). The B application (B2 treatment) under such condition has enabled the DM accumulation in very high acidity, with gypsum, to be equal to that recorded in very high acidity (Table 2). This result had indicated increased need of B. LeNoble et al. (1996) and Favaretto et al. (2007) had reported the beneficial effect of B in acidic soils; plants demand for B increases under stress conditions.

Overall, gramineous species, such as wheat, present lesser need for B than the leguminous ones; however, the gramineous are more susceptible to B excess (Broadley et al., 2012). Limited growth was more severe when the B excess, under different acidity and toxicity conditions was also higher in the acidity treatment (very high acidity with gypsum) (Table 2). Thus, the B excess was an additive to the stressful condition. Barbosa et al. (2013) found that besides growth, B toxicity in acidic soils may adversely affect the reproductive organ of wheat plants, thus delaying their development.

The high transpiration in plants grown in low acidity soil and the B effects (adequate, B2 and B3; toxicity, B5) on very high acidity, with gypsum, (Figure 1A) highlight that the results were strongly guided by DM variations (Figure 1C). Similarly, studies have found that transpiration is an important variable to determine the effects of B on plants (Ben-Gal & Shani, 2003; Mesquita et al., 2016).

On the other hand, water use efficiency presented more stable values (Figure 1B), since B application did not influence it. Therefore, the beneficial effect of B was not a mechanism related to water use efficiency, but only to the total amount of absorbed water. However, by considering the positive B effects observed in the treatment using more stressful soil conditions (very high acidity and gypsum), it would be very interesting to determine the water use efficiency under water deficit in future studies, since such condition affects B uptake (Broadley et al., 2012), besides being common in the field.

Boron application affected the root attributes: length, surface area and volume in the very high acidity, with gypsum, only. Both have shown to be beneficial and toxic (Figure 2). The increase root length was not significant; the acidity treatments only differed in B2 - the wheat plant roots in very high acidity, with gypsum, reached 14 m long, whereas, in low acidity, they only reached 10 m long.

Only the treatment in which B had a significant effect on the root growth in wheat plants has corroborated other studies (LeNoble et al., 1996; Favaretto et al., 2007). Boron may have favored root growth because it has increased the ascorbate and glutathione synthesis, which are antioxidants responsible for reducing the oxidative stress due to the high acidic condition and nutritional imbalance in the soil (Lubaszewski & Blevis,
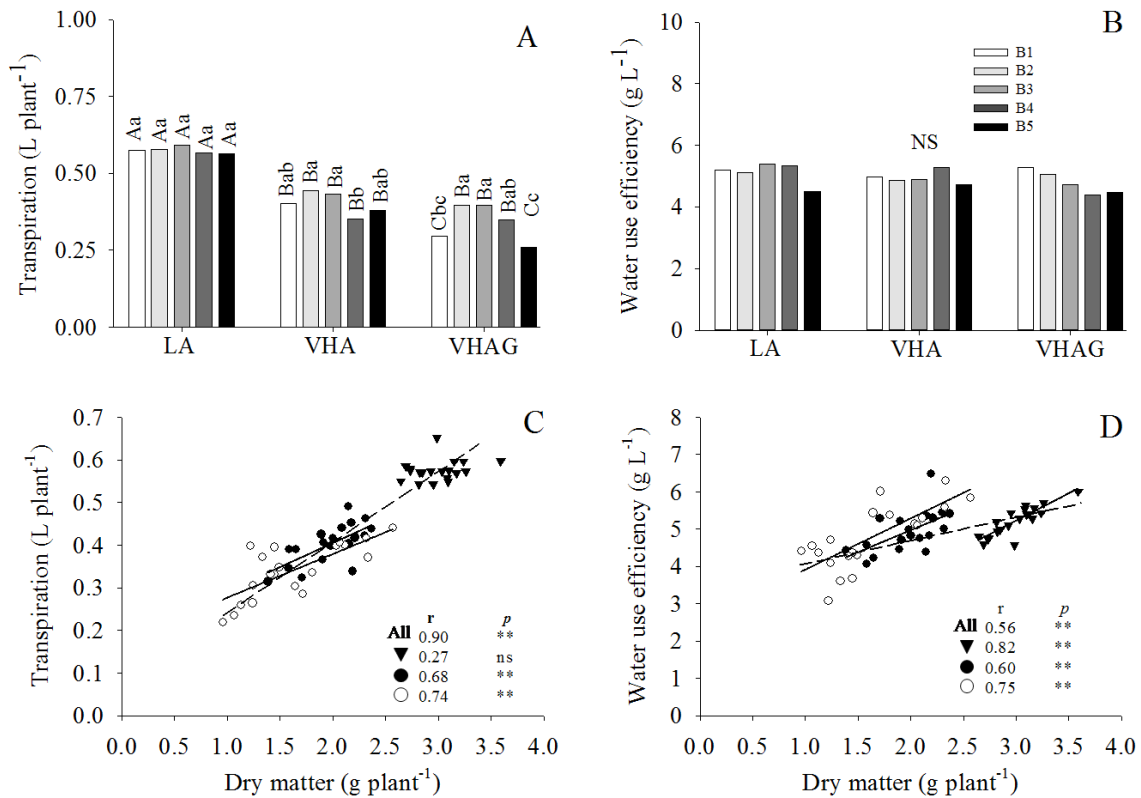
Figure 1. Transpiration (A) and water use efficiency (g dry matter per L of water) (B) of wheat due to soil acidity and boron application and the correlation between these variables (C; D) and the total dry matter. Different values for lowercase (B application) and uppercase letters (soil acidity) indicate significant difference through the Tukey test (p < 0.05). NS = not significant. LA - Low acidity (black triangle), VHA - very high acidity (black circle) and VHAG - very high acidity with gypsum (white circle).

1996; Ruiz et al., 2006). Nevertheless, root growth stimulations could be associated with the transportation of auxin by B (Bell, 1997). Thus, one of the reasons that could have favored B significant effect on the wheat plants grown (Table 2) under very high acidity, with gypsum, was that both the antioxidation and auxin systems could have been affected by the lack of Mg (Taiz et al., 2015).

The root CEC did not vary in response to B application to wheat plants, but values followed the order: low acidity > very high acidity with gypsum > very high acidity in the absence of micronutrients (Figure 2F).

The present results are linked to the effect of B and Al^{3+} on root CEC. Stass et al. (2007) reported that the root growth in bean plants under Al stress results from B and that it leads
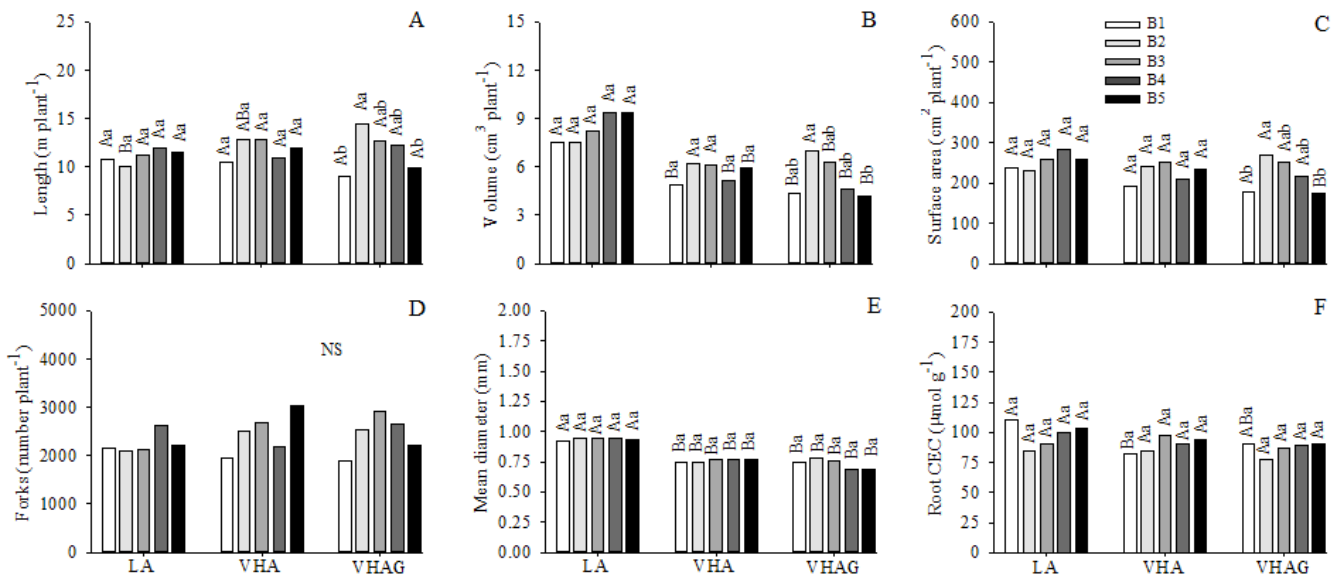

Figure 2. Length (A), volume (B), surface area (C), forks (D), mean diameter (E) and cation exchange capacity (CEC) (F) of wheat roots due to soil acidity and B application. Different values for lowercase (B application) and uppercase letters (soil acidity) indicate significant difference through the Tukey test (p < 0.05). NS = not significant. LA - Low acidity, VHA - very high acidity and VHAG - very high acidity with gypsum.

to 25 nmol carboxyl group reduction in the cell walls per root apex, thus reducing the CEC. Such negative charge reductions take place along with cell wall structural changes, keeping in mind that the formation of the rhamnogalacturonan-II dimer (RG-II) from a bridge among B and two rhamnogalacturonan-II monomers (RG-II) decreases the pore size of the cell wall (Horst et al., 2010). However, Al^{3+} binds strongly to the cation exchange sites on cell wall pectates, which also causes root CEC reduction. In other words, as the B and Al^{3+} binding property decreases in the root, the root CEC reaches the highest values (110 µmol g^{-1}) in the interaction between treatment B1 (zero B addition) and the soil Al^{3+} absence (low acidity treatment).

Overall, the results related to soil acidity treatments (Figure 2F) corroborate those by Ray & George (2011) in their study on wild grasses. They observed that the CEC increases in the roots as the soil pH also increases. Furthermore, Cezar et al. (2015) reported lower root CEC in black oat plants as the response to the application of other nutrients (Ca, Mg). However, the treatment with higher Ca availability (very high acidity, with gypsum) probably did not differ from the low acidity treatment (Figure 2F), because Ca competes with Al^{3+} for the cation exchange sites.

The borated fertilization was not essential to the alleviation of the stress condition caused by plant cultivation in acid soil (Figure 3A-C) since alkalinization did not dependent on it.
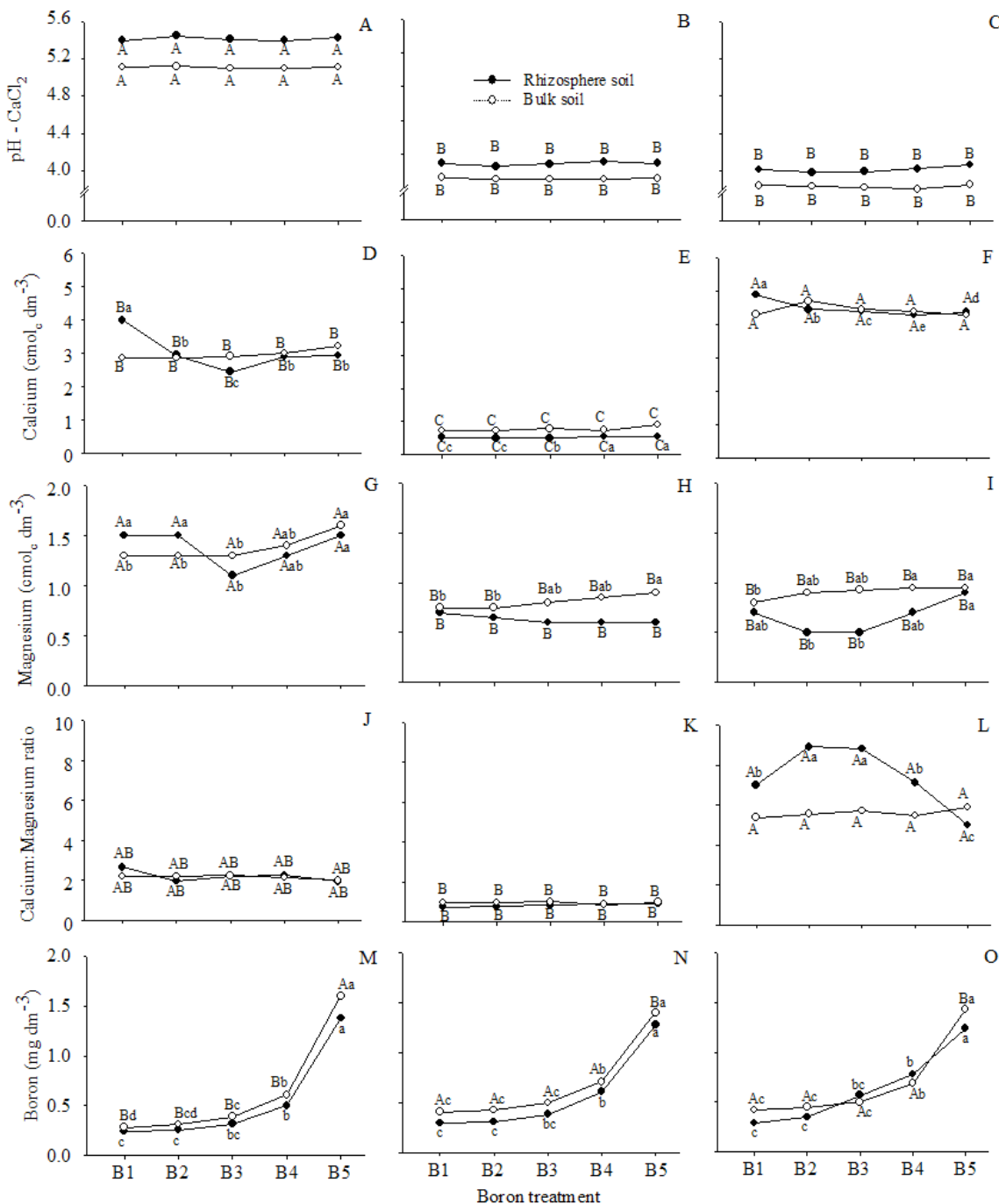

Figure 3. pH-$CaCl_2$ (A, B, C), calcium (D, E, F), magnesium (G, H, I), calcium: magnesium ratio (J, K, L) and boron (M, N, O) of wheat plants sown in bulk and rhizosphere soils. LA - Low acidity = A, D, G, J, M. VHA - Very high acidity = B, E, H, K, N. VHAG - Very high acidity with gypsum = C, F, I, L, O. Different values for lowercase (B application) and uppercase letters (soil acidity) indicate significant difference through the Tukey test (p < 0.05).

Also, a difference between the pH-CaCl$_2$ in the rhizosphere and bulk soils occurred in all soil acidity treatments, but with greater intensity in the low acidity soils.

The higher soil pH could promote nitrifying bacteria activity in the low acidity treatment. Such activity keeps more N in the nitrate (NO$_3^-$) form (Higgins et al., 2013). Thus, the more nitrate is absorbed to balance the electrochemical cell potential, the greater the hydroxyl extrusion (OH$^-$) in the rhizosphere, the fact that leads to alkalinization (Neumann & Römheld, 2012). Furthermore, plants grown in soil without exchangeable acidity are supposed to accumulate more dry matter (Table 2), and, possibly, uptake more N. However, the nitrification process also generates acid, so the magnitude of the alkalizing effect may be lower. On the other hand, it is likely that nitrification in treatments using higher soil acidity (very high acidity and very high acidity with gypsum) is low; whereas the NH$_4^+$ uptake is higher. The opposite effect is seen for NO$_3^-$ uptake, i.e., more rhizosphere acidification often takes place (Neumann & Römheld, 2012). Therefore, the results may vary due to a whole range of factors, regardless of the prevailing alkalinization in the rhizosphere found in the current study.

The Ca concentration reduction in rhizospheric soils added with B for the treatments in very high acidity and very high acidity, with gypsum (Figure 3J-L), shows that the lack of B (B1) in a soil with high Ca availability may lead to lower Ca uptake. According to Oliveira et al. (2010), Ca, which gets in contact with the root system - preferably through mass flow -, may accumulate in the rhizosphere, and its amount gets larger than that absorbed by the plant (Neumann & Römheld, 2012).

The B2 and B3 treatments in the rhizosphere soil have shown the lowest Mg concentration in the very high acidity, with gypsum, whereas the low acidity was real in B3, only (Figure 3G-I). The Mg comes into contact with the roots mainly via mass flow just as the Ca (Oliveira et al., 2010). Therefore, treatments using lower Mg concentration indicate high uptake of such element by the plants. Such results are consistent with the DM increase caused by B (B2 and B3) found in the very high acidity with gypsum treatment; thus, the Mg extraction by the wheat plants was higher.

In parallel, the higher Ca/Mg ratio in the plant rhizospheres of the B2 and B3 treatments, under the most extreme soil acidity (very high acidity with gypsum), was consistent with the Mg depletion from the rhizosphere soil (Figure 3J-L). The relationship among these cations was a rhizospheric soil attribute that has clearly indicated the positive effect of B on the most stressful condition (very high acidity and gypsum) due to the Mg variation in the rhizospheric soil.

Boron concentrations in the rhizosphere and bulk soils change according to the amounts of B applied as boric acid. However, B differs among the acidity treatments in the bulk soils, its levels were slightly lower in the low acidity condition than in the other treatments, except for B5 (Figure 3M-O). Such outcome evidences that the soil acidity effect on B availability was more important in the first four treatments (B1, B2, B3 and B4), since the B availability was lower when the pH was higher, fact that is related to increased B adsorption of the soil (Broadley et al., 2012).

About the B5 treatment, wherein the B concentration in the soil significantly increased due to the application of B, it is possible that the soil acidity effect did not remain due to an expressive B absorption in the treatments using very high acidity and very high acidity, with gypsum (Figure 3M-O). These results were favored by the higher B availability B in the soil with more acidic pH (Broadley et al., 2012).

The wheat plants subjected to treatments with high soil acidity presented B concentration (Figure 4A-C) consistent with the B variations recorded for the soil (Figure 3M-O). Plants had greater uptake in treatment B5 due to the higher B availability in the soil with lower pH. Consequently, there was lower B concentration in the soil after plant cultivation under treatments with very high acidity and very high acidity with gypsum. Accordingly, it is possible assuming that there are more chances of having a high B dose causing wheat toxicity under acid soil condition, as it is verified in the wheat flower development level (Barbosa et al., 2013).

The Al concentrations decreased in the stem+leaves fraction added with B in the treatments using very acidic soil, mainly in the treatment with very high acidity (Figure 4E), thus corroborating Jiang et al. (2009). However, the reduction in Al concentration was not significantly related to the greater plant growth in the present study.

On the other hand, the B5 treatment has favored the Al increase in the inflorescence of plants cultivated in very acidic soils (very high acidity and very high acidity with gypsum) (Figure 4F). At first glance, such result seems paradoxical to the effect of Al decrease on the stem+leaves fraction in response to B addition to the soil. However, these fractions represent organs with different functions, which are expected to have different compositions. Nevertheless, it is possible to find an Al accumulation effect due to the low DM in the inflorescences (Table 2) and to the relatively low Al transportation in susceptible species (Kochian et al., 2015).

Over time, the low acidity treatment has presented the lowest K levels in roots and stem+leaf (Figure 4H-I), probably due to greater Ca and Mg uptake. The increase in one of these nutrients often reduces the absorption of other nutrients (Neumann & Römheld, 2012) due to antagonistic relations among these elements' cationic forms in the soil solution (Ca^{2+}, Mg^{2+}, and K$^+$).

On the other hand, the lower Mg concentration in very high acidity, with gypsum (Figure 4M-O), may be related to the higher Ca/Mg ratio in the soil (Table 1), the fact that corroborates Osemwota et al. (2007), in their study on corn plants. However, no B effect on Mg concentration in plants was observed, despite the higher Mg extraction in the rhizospheric soil (Figure 3G-I). The dilution effect, due to higher plant growth, may have contributed to such result.

The phosphorus concentration did not change among the studied factors related to root and stem+leaf; however, the lowest values in very high acidity, with gypsum, were detected in the inflorescence (Figure 5A-C). These results are congruent because the sulfate ions compete with the phosphate ones (H$_2$PO$^-$; HPO$_4^{2-}$) for uptake sites in the roots. Accordingly, the high amounts of sulfate (SO$_4^{2-}$), added with the gypsum, could interfere with the P acquisition by the plant (Davidian & Kopriva, 2010).
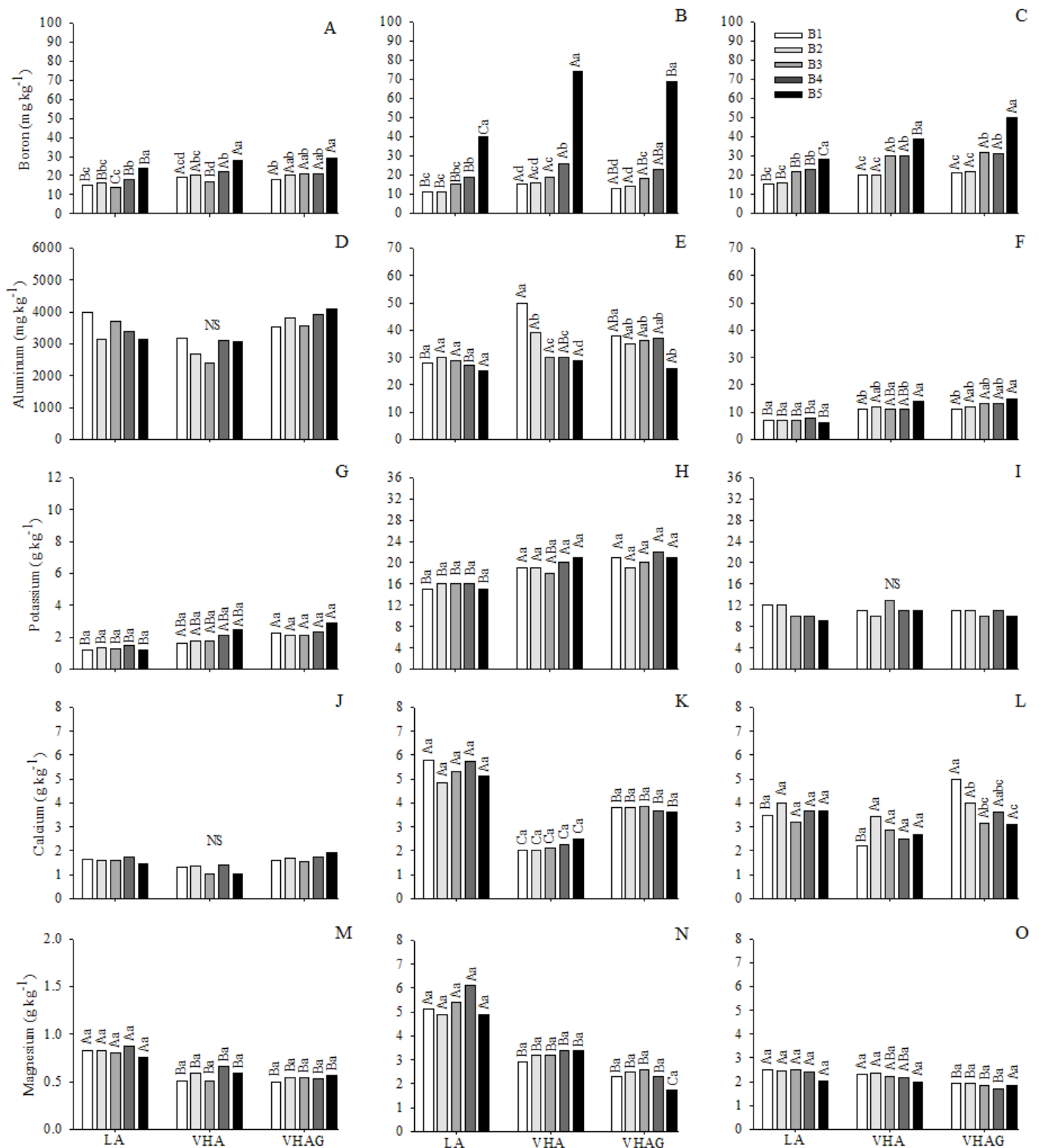
Figure 4. Boron (A, B, C), aluminum (D, E, F), potassium (G, H, I), calcium (J, K, L) and magnesium (M, N, O) concentration in wheat plant roots, and in stem+leaf and inflorescence fractions, according to soil acidity and B application. Different values for lowercase (B application) and uppercase letters (soil acidity) indicate significant difference through the Tukey test (p < 0.05). NS = not significant. LA - Low acidity, VHA - very high acidity and VHAG - very high acidity with gypsum.

The Mn concentration showed clear difference among the soil acidity treatments, the highest levels were found in the very high acidity and very high acidity with gypsum treatments (Figure 5G-I). Similarly, Zn, in stem+leaf (Figure 5K); and Cu, in root and inflorescence (Figure 5M-O) were more concentrated in the high soil acidity treatments. Thus, in

addition to the Al toxicity, the high Mg concentrations and, to a lesser extent, Cu and Zn, may have favored the oxidative stress in wheat plants (Millaleo et al., 2010; Broadley et al., 2012).

Furthermore, different from what was observed for Al (Figure 4D-F), Mn presented high concentration in stem+leaves. However, Fe, Zn, and Cu were more similar to
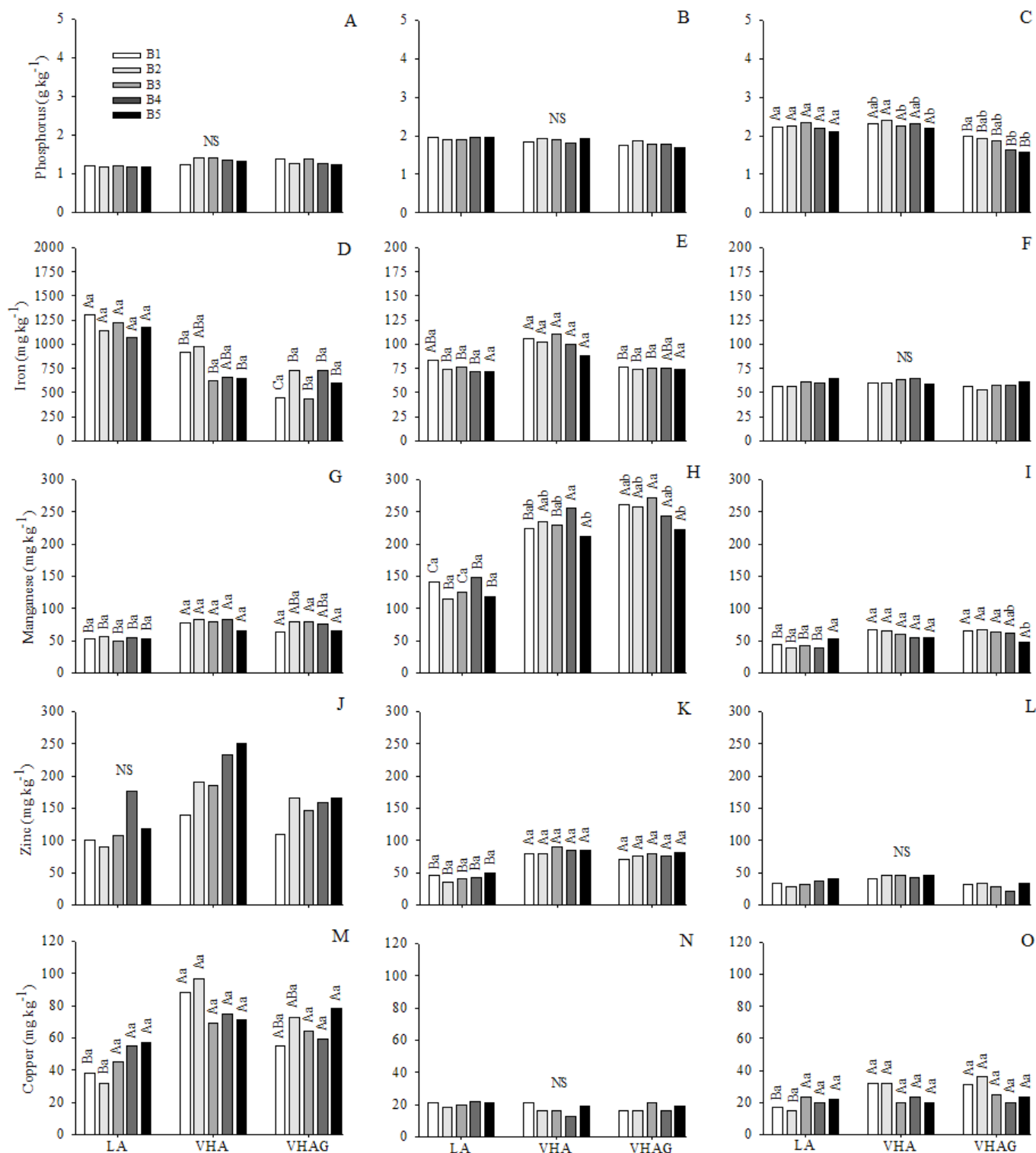
Figure 5. Phosphorus (A, B, C), iron (D, E, F), manganese (G, H, I), zinc (J, K, L) and copper (M, N, O) concentration in wheat root, stem+leaf and inflorescence fractions, according to soil acidity and B application. Different values for lowercase (B application) and uppercase letters (soil acidity) indicate significant difference through the Tukey test (p < 0.05). NS = not significant. LA - Low acidity, VHA - very high acidity and VHAG - very high acidity with gypsum.

Al, since they showed higher levels in the roots (Figure 5). Therefore, there was a clear difference in these metals between the root and stem+leaves. Such difference is associated with the strength of the bond between the metal and the root tissues, as well as with the low Al transportation to the shoot via xylem (Wu & Hendershot, 2009; Millaleo et al., 2010; Kochian et al., 2015).

However, the metal concentrations in the wheat inflorescences (Figure 4, Figure 5) indicate a limitation to Al and Mn transportation. By considering the potential of these two metals to cause toxicity to most species cultivated in acid soils, it is likely that the limitation in the transportation may be regarded as a mechanism of the species to avoid transferring Al and Mn to the reproductive organs.
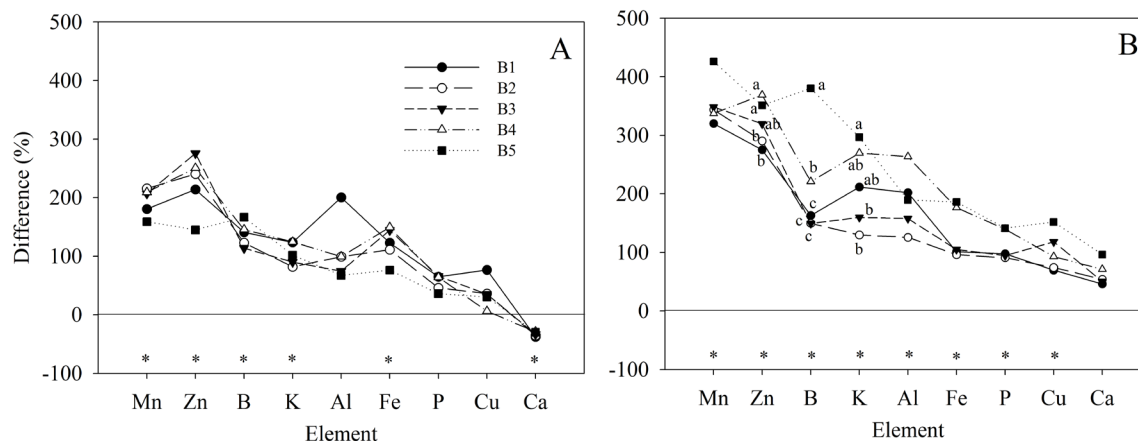
Figure 6. Difference (%) in element: Mg ratio in stem+leaves in the very high acidity (A) and very high acidity, with gypsum (B) treatment in comparison to the low acidity treatment. Different values for lowercase letters (B application) and asterisk (soil acidity) indicate significant difference through the Tukey test (p < 0.05).

Higher cellular oxidation levels, with plant physiological function losses associated with high metal concentration, have been reported (Broadley et al., 2012; Kochian et al., 2015). However, Ruiz et al. (2006) indicated that B reduces the oxidative stress induced by Al. Thus, although B did not affect the metal levels in the present study, it may have affected the wheat plant antioxidant system, the fact that has affected the results, mainly in the treatment using very high acidity with gypsum.

The ratio among the five elements (Mn, Zn, B, K, Fe) and Mg increased (275% to 6%) in the very high acidity condition, whereas the Ca: Mg presented -32% reduction in comparison to the treatment using low acidity (Figure 6A). On the other hand, the ratio of the eight elements (Mn, Zn, B, K, Al, Fe, P, Cu) and Mg increased (426% to 60%), and the application of B mainly affected the K: Mg, Zn: Mg and B: Mg ratio in the very high acidity with gypsum treatment (Figure 6B). The difference concerning the number of the element: Mg ratios were already expected if one considers the low Mg values and low Ca: Mg ratio in the soil presenting very high acidity with gypsum (Figure 4N).

Nutritional imbalances in the Mn: Mg, Zn: Mg, Al: Mg and Cu: Mg ratios were higher since these relationships were more critical for wheat in very high acidity with gypsum than in the very high acidity condition. The excessive concentration of these metals improves the lack of Mg in plants because they compete for uptake sites on cell membranes - a complex formation of ATP and enzyme activation (Broadley et al., 2012).

On the other hand, the imbalance in the K: Mg ratio was also decisive for wheat growth, given the antagonism between the two nutrients (Jezek et al., 2015). The very high acidity with gypsum was the treatment presenting the best response to B application (Table 2, Figure 2 and 3). Accordingly, treatments B2 and B3 (both with higher growth than the others) may have favored the wheat plants by reducing K: Mg. This nutritional factor certainly adds to others, as highlighted in the text, and, to some extent, to wheat plants grown in the treatment using very high acidity with gypsum in treatment B2.

Conclusion

The high addition of gypsum in the acid soil caused a nutritional imbalance (mainly Mg) in the wheat plants; thus, it led to lower dry matter accumulation values.

The high acidity with gypsum was the only soil condition presenting stress alleviation by B. However, the soil presenting very high acidity and gypsum were also more favorable to the occurrence of B toxicity.

The wheat plants cultivation under the most stressful conditions showed a better response to B.

Acknowledgements

Literature Cited

Baquy, M. A.; Li, J. Y.; Xu, C. Y.; Mehmood, K.; Xu, R. K. Determination of critical pH and Al concentration of acidic Ultisols for wheat and canola crops. Solid Earth, v. 8, n. 1, p. 149-159, 2017. http://dx.doi.org/10.5194/se-8-149-2017.

Barbosa, J. Z.; Consalter, R.; Pauletti, V.; Motta, A. C. V. A magnitude da toxidez de B é dependente da condição de acidez do solo. Cultivando o Saber, v.6, n.3, p.113-121, 2013. http://www.fag.edu.br/upload/revista/cultivando_o_saber/52fbd00153a92.pdf. 09. Apr. 2016.

Bell, R. W. Diagnosis and prediction of boron deficiency for plant production. Plant and Soil, v.193, n.1-2, p.149-168, 1997. http://dx.doi.org/10.1023/a:1004268110139.

Ben-Gal, A.; Shani, U. Water use and yield of tomatoes under limited water and excess boron. Plant and Soil, v.256, n. 1, p.179-186, 2003. http://dx.doi.org/10.1023/a:1026229612263.

Broadley, M.; Brown, P.; Cakmak, I.; Rengel, Z.; Zhao, F. Functional of nutrients: micronutrients. In: Marschner, P. (Ed.). Marschner's mineral nutrition of higher plants. London: Elsevier, 2012. p. 191-248.

Cezar, R. M.; Barbosa, J. Z.; Pauletti, V. Expressão da capacidade de troca de cátions radicular e nutrição de aveia-preta (*Avena strigosa* Schreb.) sob deficiência de nutrientes. Científica, v.44, n.1, p.83-90, 2015. http://dx.doi.org/10.15361/1984-5529.2016v44n1p83-90.

Crooke, W. M. The measurement of the cation-exchange capacity of plants roots. Plant and Soil, v.21, n.1, p.43-49, 1964. http://dx.doi.org/10.1007/bf01373871.

Davidian, J.C.; Kopriva, S. Regulation of sulfate uptake and assimilation - the same or not the same? Molecular Plant, v.3, n.2, p.314-325, 2010. http://dx.doi.org/10.1093/mp/ssq001.

Favaretto, N.; Motta, A. C. V.; Barcik, C.; Lustosa, S. B. C.; Comin, J. J. Shoot and root responses of *Trifolium vesiculosum* to boron fertilization in an acidic Brazilian soil. Brazilian Archives of Biology and Technology. v.50, n.4, p.597-604, 2007. http://dx.doi.org/10.1590/s1516-89132007000400005.

Guigues, S.; Bravin, M. N.; Garnier, C.; Masion, A.; Doelsch, E. Isolated cell walls exhibit cation binding properties distinct from those of plant roots. Plant and soil, v. 381, n. 1-2, 367-379, 2014. http://dx.doi.org/10.1007/s11104-014-2138-1.

Heidarabadi, M. D.; Ghanati, F.; Fujiwara, T. Interaction between boron and aluminum and their effects on phenolic metabolism *Linum usitatissimum* L. roots. Plant Physiology and Biochemistry, v.49, n.12, p.1377-1383, 2011. http://dx.doi.org/10.1016/j.plaphy.2011.09.008.

Higgins, S.; Laughlin, R. J.; Watson, C. J. Antecedent effect of lime on nitrous oxide and dinitrogen emissions from grassland soils. Nutrient Cycling in Agroecosystems, v.95, n.2, p.219-229, 2013. http://dx.doi.org/10.1007/s10705-013-9559-8.

Horst, W. J.; Wang, Y.; Eticha, D. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Annals of Botany, v.106, n.1, p.185-197, 2010. http://dx.doi.org/10.1093/aob/mcq053.

Hossain, A. K. M. Z.; Asgar, A.; Hossain, M. A.; Tosaki, T.; Koyama, H.; Hara, T. Boron-calcium synergically alleviates aluminum toxicity in wheat plants (*Triticum aestivum* L.). Soil Science and Plant Nutrition, v.51, n.1, p.43-49, 2005. http://dx.doi.org/10.1111/j.1747-0765.2005.tb00005.x.

Jezek, M.; Geilfus, C. M.; Bayer, A.; Mühling, K. H. Photosynthetic capacity, nutrient status, and growth of maize (Zea mays. L.) upon $MgSO_4$ leaf-application. Frontiers in Plant Science, v. 5, article 781, 2015. http://dx.doi.org/10.3389/fpls.2014.00781.

Jiang, H. X.; Tang, N.; Zheng, J. G.; Chen, L. S. Antagonistic actions of boron against inhibitory effects of aluminum toxicity on growth, CO_2 assimilation, ribulo-1,5-biphosphate carboxylase/oxygenase, and photosynthetic electron transport probed by the JIP-test, of *Citrus grandis* seedlings. BioMed Central Plant Biology, v.9, p.102, 2009. http://dx.doi.org/10.1186/1471-2229-9-102.

Kochian, L. V.; Piñeros, M. A.; Liu, J.; Magalhães, J. V. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. Annual Review of Plant Biology, v.66, n.1, p.571-598, 2015. http://dx.doi.org/10.1146/annurev-arplant-043014-114822.

LeNoble, M. E.; Blevins, D. G.; Miles, R. J. Prevention of aluminum toxicity with supplemental boron. II. Stimulation of root growth in acidic, high aluminum subsoil. Plant, Cell and Environment, v.19, n.10, p.1143-1148, 1996. http://dx.doi.org/10.1111/j.1365-3040.1996.tb00429.x.

Mesquita, G. L.; Zambrosi, F. C.; Tanaka, F. A.; Boaretto, R. M.; Quaggio, J. A.; Ribeiro, R. V.; Mattos, D. Anatomical and physiological responses of citrus trees to varying boron availability are dependent on rootstock. Frontiers in Plant Science, v. 7, article 224, 2016. http://dx.doi.org/10.3389/fpls.2016.00224.

Millaleo, R.; Reyes-Díaz, M.; Ivanov, A.G.; Mora, M.L.; Alberdi, M. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. Journal of Soil Science and Plant Nutrition, v.10, n.4, p.476-494, 2010. http://dx.doi.org/10.4067/s0718-95162010000200008.

Neumann, G.; Römheld, V. Rhizosphere chemistry in relation to plant nutrition. In: Marschner, P. (Ed.). Marschner's mineral nutrition of higher plants. London: Elsevier, 2012, p. 347-368.

Oliveira, E. M. M.; Ruiz, H. A.; Alvarez, V. H.; Ferreira, P. A.; Costa, F. O.; Almeida, I. C. C. Nutrient supply by mass flow and diffusion to maize plants in response to soil aggregate size and water potential. Revista Brasileira de Ciência de Solo, v.34, n.2, p.317-327, 2010. http://dx.doi.org/10.1590/s0100-06832010000200005.

Osemwota, I. O.; Omueti, J. A. I.; Ogboghodo, A. I. Effect of calcium/magnesium ration in soil on magnesium availability, yield, and yield components. Communications in Soil Science and Plant Analysis, v. 38, n.19-20, p.2849-2860, 2007. http://dx.doi.org/10.1080/00103620701663081.

Ray, J. G.; George, K. J. Cation exchange capacity of roots of wild grasses and the ecological implications. Journal of Ecology and Noosphereology, v.22, n.3-4, p.58-72, 2011. http://uenj.cv.ua/Ecology_Noo_2011_22_3-4/22_tom-3_4.html. 09. Apr. 2016.

Ruiz, J. M.; Rivero, R. M.; Romero, L. Boron increases synthesis of glutathione in sunflower plants subjected to aluminum stress. Plant and Soil, v.279, n.1-2, p.25-30, 2006. http://dx.doi.org/10.1007/s11104-005-7931-4.

Stass, A.; Kotur, Z.; Horst, W. J. Effect of boron on the expression of aluminium toxicity in *Phaseolus vulgaris*. Physiologia Plantarum, v.131, n.1-2, p.283-290, 2007. http://dx.doi.org/10.1111/j.1399-3054.2007.00957.x.

Taiz, L.; Zeiger, E.; Møller, I. M.; Murphy, A. Plant physiology and development. Sunderland, Massachusetts: Sinauer Associates, 2015. 761p.

Tang, C.; Conyers, M. K.; Nuruzzaman, M.; Poile, G.J.; Liu, D.L. Biological amelioration of subsoil acidity trough managing nitrate uptake by wheat crops. Plant and Soil, v.338, n.1, p.383-397, 2011. http://dx.doi.org/10.1007/s11104-010-0552-6.

Wu, Y.; Hendershot, W. H. Cation exchange capacity and proton binding properties of pea (Pisum sativum L.) roots. Water, Air, and Soil Pollution, v. 200, n. 1-4, 353-369, 2009. http://dx.doi.org/10.1007/s11270-008-9918-2.

Zhou, X. X.; Yang, L. T.; Qi, Y. P.; Guo, P.; Chen, L. S. Mechanisms on Boron-Induced Alleviation of Aluminum-Toxicity in Citrus grandis Seedlings at a Transcriptional Level Revealed by cDNA-AFLP Analysis. PloS One, v.10, n.3, e0115485, 2015. http://dx.doi.org/10.1371/journal.pone.0115485.