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Response of cowpea plants submitted to acid conditions: Aluminum and hydrogen stress

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ABSTRACT: Aluminum (Al) is an important abiotic stress factor constricting the production of cowpea (*Vigna unguiculata* (L.) Walp.) in acid soil conditions. This study investigated the effect of Al and pH levels on the photosynthesis, proline accumulation, and nutritional status of two cowpea cultivars. Two pot experiments under greenhouse conditions were performed concurrently. Each experiment was carried out using a completely randomized design, in a factorial scheme 2 × 3 +1, in which each cultivar was exposed to two levels of Al (0.0 and 1.0 mmol L^{-1}) and three levels of pH (3.3; 4.0, and 4.7), plus a control. There was an interaction effect of the factors Al and pH reducing significantly the internal CO_2 concentration (*Ci*) for BRS Caldeirão, and transpiration (E) and stomatal conductance (*gs*) for BRS Tracuateua, notably under growth conditions with Al (1 mmol L^{-1}) and pH value of 4.7. For BRS Caldeirão, proline content was significantly reduced by the isolated effect of the factors Al and pH on shoot tissue. For BRS Tracuateua, proline content was significant only in roots, indicating a metabolic response to water stress damage. Under growth conditions with Al and pH value of 3.3, there was an improvement in nutrient uptake and accumulation according to nutritional status of both cultivars. In summary, cowpea response pointed that physiological, biochemical, and nutritional aspects of both cultivars improved at the lowest pH of 3.3 under Al-stress, and also that cv. BRS Tracuteua was less tolerant than BRS Caldeirão.

Keywords: *Vigna unguiculata* (L.) Walp, abiotic stress, plant mineral nutrition, leaf gas Exchange, and proline.

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INTRODUCTION

Agricultural productivity is threatened by various stress factors which seriously impact global food security, and abiotic stress is one of the most important problems currently faced by agriculture (Godoy et al., 2021), causing losses of up to 70 % in major food crops (Mantri et al., 2012). Aluminum (Al) is one of the major abiotic stresses to plants (Kochian, 1995; Jaskowiak et al., 2019). It is associated with acid soil conditions, constituting the major factor limiting crop plant development worldwide (Horst et al., 2010). The health and nutrition of people are directly affected by the low quality of such cultivated plants (Rabel et al., 2018). Mineral acid soils result from parent material that is acidic or strongly leached from the soil profile (Samac and Tesfaye, 2003). It is reported that up to 50 % of the arable lands in the world are acidic (Szurman-Zubrzycka et al., 2021). About 70 % of the highly weathered soils in the tropics are acidic (Sanchez et al., 1982). Although several studies have been carried out to understand such impacts on food security, Al stress remains a hamper for increasing crop production in these regions.

In terms of crop food, cowpea (*Vigna unguiculata* (L.) Walp.) has great potential to contribute to global food security and nutritional security (Mekonnen et al., 2022). Cowpea is a grain legume with high-quality protein and high nutritional value (Akibode and Maredia, 2012) and one of the most important income sources for subsistence farming in the North and Northeast of Brazil (Honaiser et al., 2022). Despite its relevance to agriculture in the developing world and its resilience to stress, studies on cowpea are relatively scarce (Carvalho et al., 2017). Additionally, due to the wide range of genetic diversity and tolerance capacity to different abiotic stress factors among cowpea genotypes (Akinrinde et al., 2006; Soares et al., 2014), studies on cowpea resilience and tolerance have been highly recommended, including Al-stress response (Ologundudu et al., 2018). Despite that, there is still a lack of information about cowpea behavior submitted to Al and H stress conditions. Understanding these interactions is highly important in helping to improve crop growth and increase food production.

In most soils, a large part of Al occurs in the oxide and aluminosilicate forms, but these forms are not harmless to plants (Ma, 2005). However, under acid conditions ($pH<5.0$), Al is released from clay minerals in the form of $[Al(H_2O)_6]^{3+}$, commonly known as Al^{3+} (Sade et al., 2016). This mononuclear form (i.e., Al^{3+}) is considered to be the most toxic and strongly harmful to most plants, and several species are Al-sensitive (Delhaize and Ryan, 1995; Szurman-Zubrzycka et al., 2021), even at micromolar concentrations (Haridasan, 2006). Despite some research on Al phytotoxicity, the mechanisms involved in plant response still remain not fully understood (Liu et al., 2022). Some studies have emphasized that Al stress can affect the plants' cell metabolism and physiologic process, resulting in reduced growth and development. The negative effect of Al phytotoxicity on plant shoots is mainly due to injuries in the root systems (Ma, 2005). Aluminum accumulates, preferentially, in the root apex, causing inhibition of root growth and root elongation (Kochian et al., 2004). Thus, the root system becomes stunted and fragile, with low formation of root hairs and the apex becoming swollen and injured (Panda et al., 2009). As a result, these damages tend to increase the rigidity of the cell walls, which makes it difficult for water and nutrient uptake and transportation to shoot (Sade et al., 2016).

Moreover, Al stress affects the root system and photosynthetic behavior (Yang et al., 2015). Aluminum toxicity causes disturbance in the chloroplast structure and a decrease in photosynthetic performance due to the reduction of electron transport in photosystem II (PSII) (Panda et al., 2009). In Al-sensitive plants, Al stress reduces important physiological parameters, such as stomatal conductance and chlorophyll content, and therefore, the electron transport chain is damaged. This generally affects the photosynthetic apparatus and plant growth. In such plants under stress, the functioning and efficiency of the photosynthetic apparatus can be evaluated by using non-destructive techniques, such as chlorophyll *a* fluorescence and leaf gas exchange analysis (Strasser et al., 2010).

Some studies have also emphasized that Al-stress stimuli the production of reactive oxygen species (ROS), and that can cause lipid peroxidation and cell degradation (Sairam et al., 2005). In response, plant tissue can accumulate significant amounts of solutes (i.g., proline) that are non-toxic at high cellular concentrations (Hayat et al., 2012). The increase in proline biosynthesis and accumulation is a metabolic mechanism for protecting the integrity of enzymes and cell membranes (Jaleel et al., 2007). The amino acid proline acts as an excellent free radical scavenger (Delauney and Verma, 1993; Cruz et al., 2014), which may enhance metabolic functions under abiotic stress conditions, constituting an important indicator of plant stress. It has also been reported that proline content can indicate the tolerance behavior of plants so that stress-tolerant ones accumulate higher proline content (Yan et al., 2021). Addressing the abovementioned issue, the present study was performed to investigate the effect of aluminum and pH levels on photosynthesis, proline accumulation, and the nutritional status of two cowpea cultivars.

MATERIALS AND METHODS

Plant material and experimental design

Two pot experiments were carried out, concurrently, in a greenhouse located at the Faculty of Agricultural Sciences of the Federal University of Amazon (UFAM), Brazil (03° 06’ 01.94” S, 59° 58’ 34.59” W). For that, two cowpea cultivars with different growth habits and yield potentials, namely “BRS Caldeirão” and “BRS Tracuateua” were used. Both cultivars have been recommended for cultivation on upland and floodplain soils in the Amazon State by the Brazilian Agricultural Research Corporation - EMBRAPA. Each experiment consisted of only one cultivar (experiment 1 – BRS Caldeirão and experiment 2 – BRS Tracuateua) and was performed using a completely randomized design, in a factorial scheme with an additional treatment 2 × 3 + 1, composed of three replications and one plant per experimental unit, totaling 21 plots. Thus, each cultivar was exposed to two levels of Al (0.0 and 1.0 mmol L^{-1}) and three levels of pH (3.3, 4.0, and 4.7), plus a control. Based on that, the following treatment combinations were used: respectively (Al:pH); T1- (0:3.3); T2 - (0:4.0); T3 - (0:4.7); T4 - (1:3.3); T5 - (1:4.0); T6 - (1:4.7). Control treatment (C) – (0:5.8).

Environmental conditions

In this study, the plants were grown in a greenhouse under natural sunlight and environmental conditions with 612.3 µmol m^{-2} s^{-1} of photosynthetic photon flux density, photoperiod of 12:00 h/12:00 h (day/night), average temperature and relative humidity of around 35 °C and 70 %, respectively.

Substrate preparation, plant vessel and growth media

The plants were grown under sand culture technique using washed sand as substrate and fertilized with nutrient solution. The substrate was previously washed to remove any salts and algae. For that, the sand particles were washed several times with running water and then submerged in acid solution (0.5 mol L^{-1} HCl) for 24 h. Next, the substrate was again subjected to several washes with tap water and thereafter with deionized water until the leached presented a low electrical conductivity (EC) ($EC \leq 3$ µS cm^{-1}), according to procedures used by Silva (2020). Finally, these sand particles were air-dried and transferred into plastic vessels. It is pointed out that these pots were painted externally with aluminum metallic paint to avoid an increase in solution temperature due to sunlight incidence.

For this experiment, the hydroponic solution was formulated based on the complete solution proposed by Hoagland and Arnon (1950) (Table 1). The used growth solution was composed of the following sources: CH_4N_2O, $Ca(NO_3)_2.4H_2O$, K_2SO_4, KCl, KH_2PO_4,

$MgSO_4.7H_2O$, FeEDTA, $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, H_3BO_3, $CuSO_4.5H_2O$ and $H_2MoO_4.H_2O$. The Al treatment was prepared with $Al_2(SO_4)_3.6H_2O$. For solution preparation, deionized water was used. The supply of N was done in the forms of nitrate (80 %) and ammonium (20 %), while the pH of the solutions was adjusted according to each treatment, by adding a 0.5 mol L^{-1} NaOH or 0.5 mol L^{-1} HCl solution. A pH meter (Digimed, DM-23) was used to measure and set the pH values of the nutrient solutions.

Table 1. Chemical composition of nutrients in the solution proposed by Hoagland and Arnon (1950)

N-NO^{3-}	N-NH^{4+}	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn
mg L^{-1}												
196	14	31	234	160	48.6	70	0.5	0.02	5	0.5	0.01	0.05

Germination, plant growth, and harvesting

Cowpea seeds were germinated in a polypropylene tray using sand washed as substrate. At 15 days after sowing, healthy and uniform seedlings were selected and transplanted to pots filled with sand substrate. Over 30 days after transplanting, these seedlings were pre-grown in a greenhouse being irrigated with 1/10 Hoagland solution of pH 5.8 for 10 days, and thereafter with 1/4 solution using the same pH level for 20 days left, until acclimatized. Next, the plants were exposed to the treatments, i.e., Al and pH levels, for 27 days more, totalizing 57 days of cultivation. During the period of growth, they were irrigated up to 65 % of substrate saturation capacity in each pot. Moreover, weekly, the substrate was washed with deionized water and nutrient solution renewed to prevent substrate salinization.

Leaf gas exchange analysis

After 27 days of plant exposition to the treatments, the liquid photosynthesis rates (*A*), stomatal conductance to water vapor (*gs*), internal CO_2 concentration (*Ci*), and transpiration (*T*) were obtained between 8:00 and 12:00 h in the morning. These physiological parameters are usually measured in intact leaves (Krause and Weis, 1991). Thus, the data collected from the third leaf (from the apex of the plant) fully expanded the plants in each plot. A portable infrared gas analyzer (IRGA, LI-COR 6800) was used. The equipment was calibrated with settings to mean radiation (1500 µmol m^{-2} s^{-1}), ambient CO_2 concentration (400 µmol m^{-2} s^{-1}), temperature (27 °C) and relative humidity (50 %). From the data (*A* and *Ci*), the internal carboxylation efficiency (*ICE*) was accessed using the following formula: $EIC = A/Ci$.

Chlorophyll a fluorescence analysis

After 27 days of plant exposition to the treatments, the parameters of chlorophyll *a* fluorescence, ABS-based performance (PI_{abs}) and total performance index (PI_{total}) were obtained. Such parameters are usually measured in intact leaves (Krause and Weis, 1991). Thus, the data collected from the third leaf (from the apex of the plant) fully expanded the plants in each plot. A portable fluorometer (PEA, MK2–9600 Hansatech, Norfolk, UK) was used. Readings were performed in the leaves selected between 8:00 and 12:00 h in the morning, after a dark adaptation for 30 min by using strong light-induced O-J-I-P transients (JIP-test), according to the method proposed by Strasser et al. (2010). The maximum quantum efficiency of photosystem II (Fv/Fm ratio) was obtained from these data.

Proline content analysis

At 27 days after exposition to the treatments, all plants were harvested and separed into root and shoot. Each part was carefully washed using deionized water, placed in paper bags, and dried in a ventilated oven at 65 °C for 72 h. The plant materials (root and shoot) were analyzed for proline content, according to the method of Bates et al. (1973) with modifications proposed by Leite et al. (2000). Proline extraction was performed using 0.05 g of dry matter (DM), which was macered in a mortar and homogenized with 10 mL of 3 % (w/v) sufosalicylic acid. Next, the homogenate was centrifuged at 6000 rpm for 30 min. The supernatant was then collected and transferred to microcentrifuge tubes (15 mL), where 2 mL of ninhydrin acid solution were added. This mixture was heated at 100 °C for 1 h in a water bath. The reaction was then stopped by an ice bath, and the mixture was extracted with toluene. Proline quantification was estimated using a previously established standard curve with L-proline, and expressed in micromol of proline per gram of dry matter (μmol proline g^{-1} of DM).

Nutritional status

Plant materials (root and shoot) were ground, digested, and analyzed for N, K, Ca, Mg, P, S, Fe, Mn, Zn, B and Cu content. For nitrogen (N) determination, sulfuric digestion was performed and then analyzed by Kjeldahl method (Bremner, 1996), and the remaining nutrients with nitro-perchloric digestion. Potassium (K) was analyzed by flame photometry, and boron (B) by colorimetry at 540 nm wavelength, and calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) by atomic absorption spectrophotometry, and sulfur (S) by turbidimetry ($\lambda = 540$ nm), phosphorus (P) content by colorimetry at 725 nm wavelength, according to Malavolta et al. (1997).

Data analysis

The data obtained were submitted to tests of normality, according to Shapiro and Wilk (1965) ($p>0.05$), and then subjected to analysis of variance (ANOVA) by the F test, using a factorial scheme with an additional treatment. When significant ($p\leq0.05$), the factorial was unfolding and the means among treatments were compared by Tukey's test ($p\leq0.05$). Dunnet test was performed to compare each treatment with the control treatment at $p\leq0.05$.

RESULTS

Experiment 1. Photosynthetic characteristics (cv. BRS Caldeirão)

There was a significant interaction effect of the factors Al and pH on the internal CO_2 concentration (*Ci*) (Figure 1). It is noted that *Ci* value was higher in the absence of Al, particularly at pH values of 3.3 and 4.0 (Figure 1a). Under pH 4.7, growth solution, with and without Al, had no significant influence on this physiological parameter. The effect of the pH levels inside the Al concentrations showed that *Ci* was little dependent on Al presence, except in the solution without Al and pH value of 4.7, where this variable reduced significantly compared to that of pH 3.3 and 4.0 (Figure 1b).

The isolated effect of the factor pH influenced significantly the liquid photosynthesis rates (*A*) and internal carboxylation efficiency (*ICE*) (Figura 2). Plants exposed to pH of 4.7 presented a significant reduction in *A* and *EIC* indexes, notably compared to those treated with pH 3.3 and 4.0 (Figures 2a and 2b). Moreover, a significant reduction of around 18.8 % in *A* rates was observed for plants exposed to pH level of 4.7 as compared to pH 4.0. In turn, *EIC* parameter was reduced by about 20 % in relation to that found at pH level of 4.0.

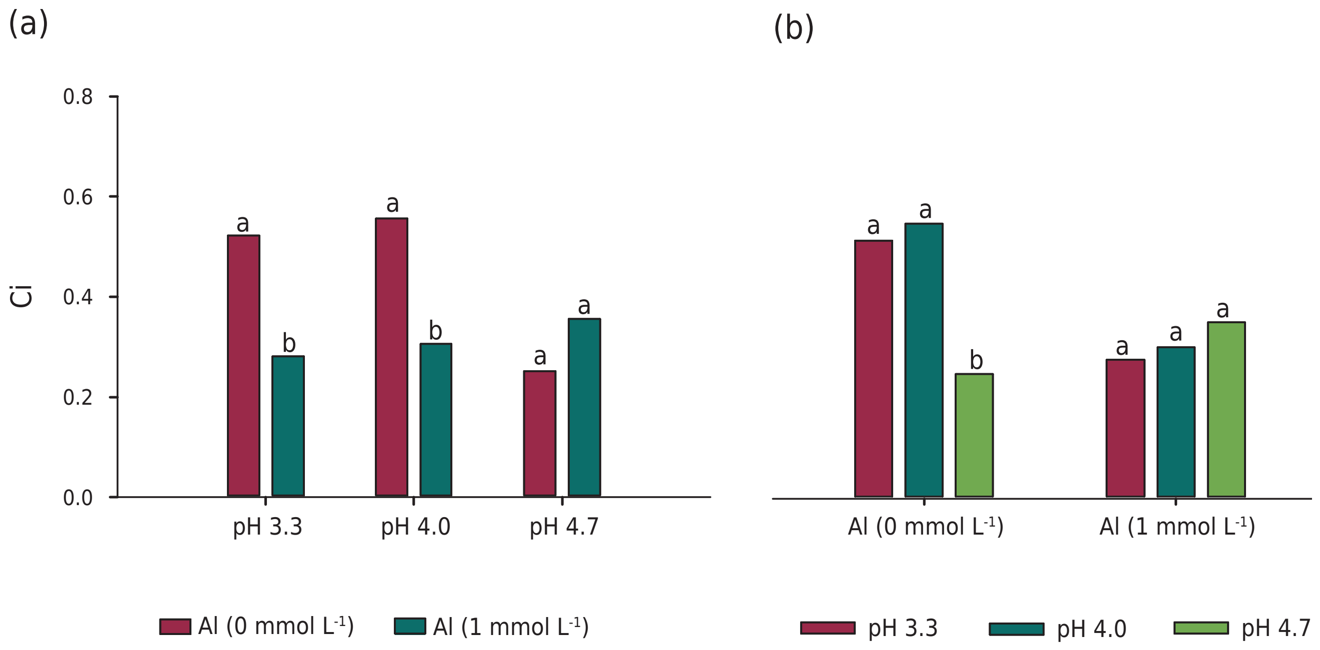

Figure 1. Effect of Al and pH levels on internal CO2 concentration (Ci) of cowpea cv. BRS Caldeirão, after 27 days of plant exposition to the treatments. Effect of Al concentrations inside the pH levels (a) and of pH inside the Al concentrations (b). Columns followed by the same letters are not significantly different ($p<0.05$), according to Tukey's Test.

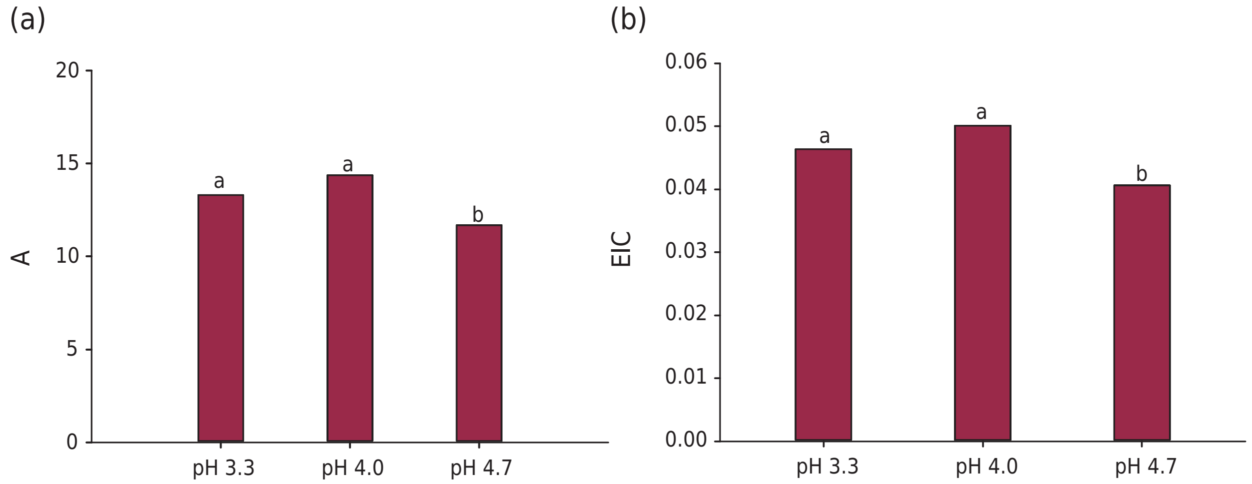

Figure 2. Isolated effect of pH levels on liquid photosynthesis rates (a) and internal carboxylation efficiency (ICE) (b) of cowpea cv. BRS Caldeirão, after 27 days of plant exposition to the treatments. According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

Regarding the mean contrast of the control treatment with other treatments, it was found that T5 (1;4.0) presented the lowest PI_{total} (Figure 3a), while other treatments did not differ significantly from the control. This reduction in photochemical performance for T5 (1;4.0) was nearly 32.16 %. On the other hand, with regard to *A* and EIC parameters, a lower performance for T3 (0;4.7) and T5 (1;4.0) compared to the control and other treatments was also detected. However, when compared with T4 (1;3.3), *A* index for T6 (1;4.7) was not statistically different. In comparison to the control, a reduction of about 25 and 17.3 % in EIC for T3 (0;4.7) and T6 (1;4.7) treatments were observed, respectively. While, in terms of *A* performance, it was about 25.33 % for T3 (0;4.7), 14.81 % for T4 (1;3.3), and 17.91 % for T6 (1;4.7).

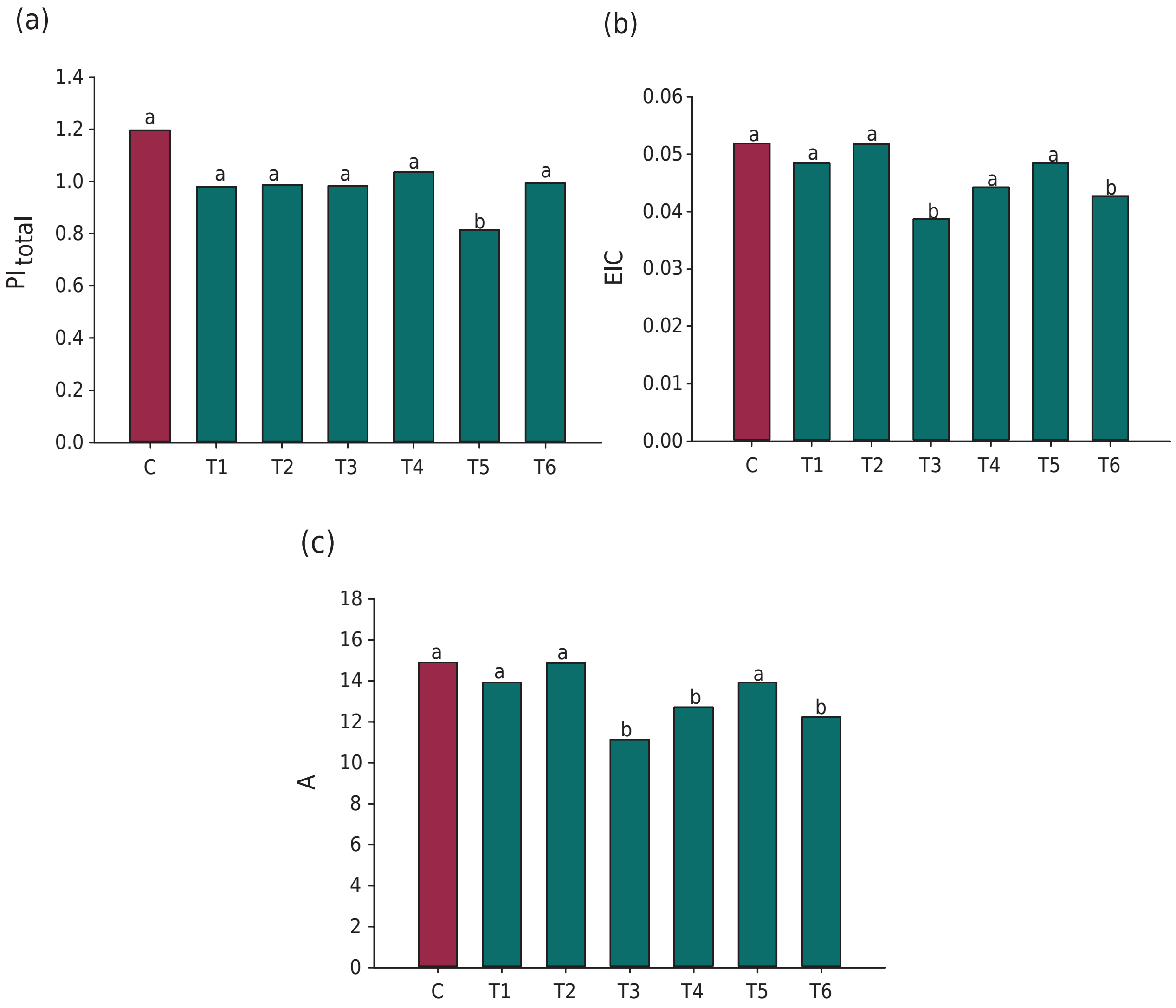

Figure 3. Effect of Al and pH levels on PItotal index (a), internal carboxylation efficiency (b) and liquid photosynthesis rates (ICE) (c) of cowpea cv. BRS Caldeirão, after 27 days of plant exposition to the treatments. Control treatment (C) and treatments (Al:pH), respectively; T1 = (0:3.3); T2 = (0:4.0); T3 = (0:4.7); T4 = (1:3.3); T5 = (1:4.0); T6 = (1:4.7). Columns followed by the same letters are not significantly different (p<0.05), according to Dunnett's Test.

Experiment 2. Photosynthetic characteristics (cv. BRS Tracuateua)

There was a significant interaction effect of the factors Al and pH on transpiration (*E*) and stomatal conductance to water vapor (*gs*) (Figure 4). This interaction showed that *E* rate of cowpea grown at pH level of 4.7 and treated with Al (1 mmol L^{-1}) was statistically different and lower than other treatments studied (Figure 4a). It also revealed that *E* rate was not affected significantly, as compared to different pH levels tested, regardless of the Al concentration (Figure 4b).

Regarding *gs* performance, plants treated with Al solution of pH 4.7 were affected with a significant reduction of this parameter (Figure 4c). On the other hand, *gs* of those plants exposed to pH levels of 3.3 and 4.0 were not statistically different when treated with and without Al. Besides that, *gs* of plants grown without Al were not significantly different among pH levels evaluated (Figure 4d). Nevertheless, when this cultivar was grown in the presence of Al, there was a significant reduction in *gs*, specially at pH value of 4.7 compared to that of those under pH condition of 3.3. Moreover, this physiological aspect was not affected significantly when the plants were subjected to the solution containing Al and a level of pH 4.0.

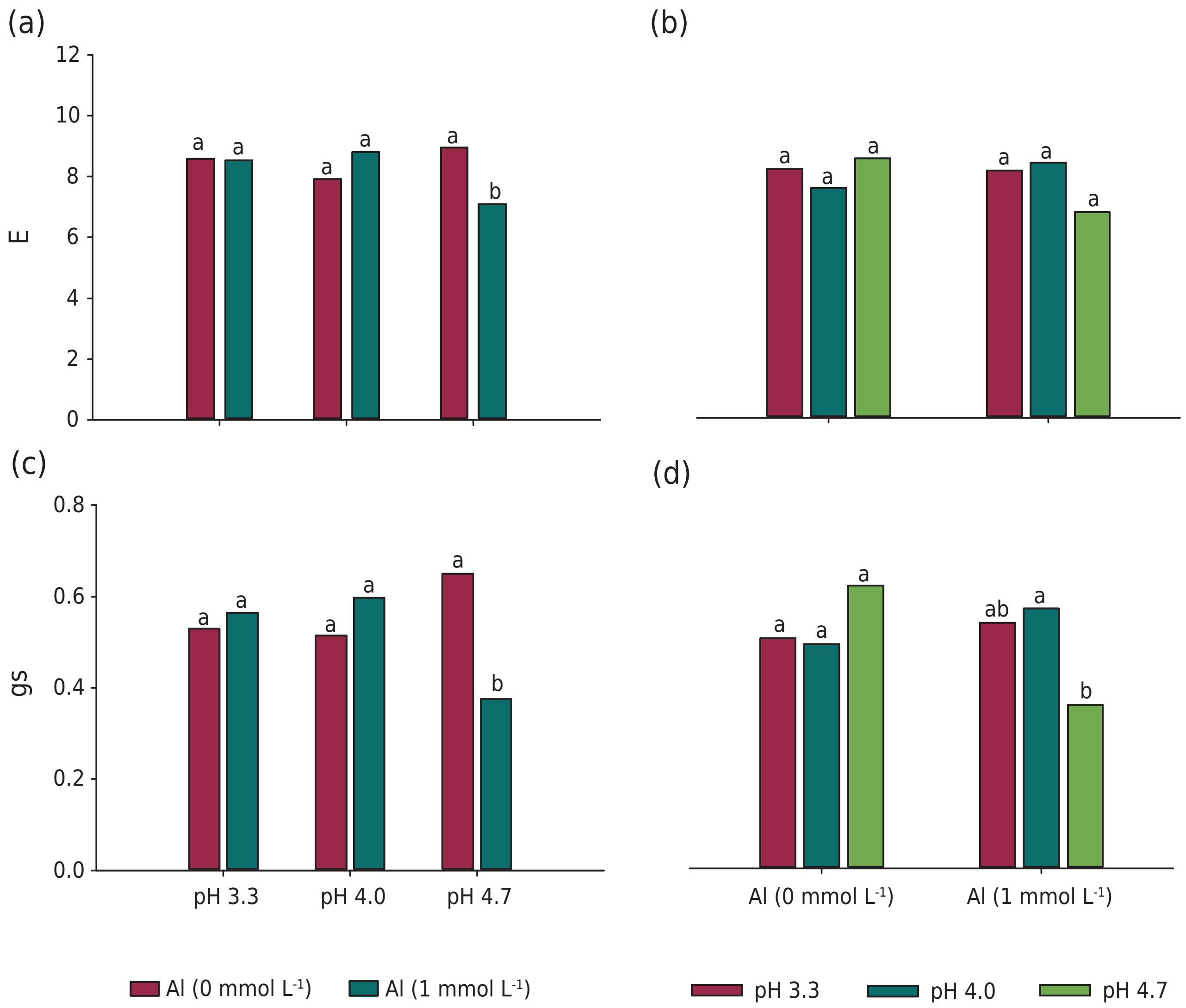

Figure 4. Effect of Al and pH levels on internal CO^2 concentration (Ci) of cowpea cv. BRS Tracuateua, measured after 27 days of plant exposition to the treatments evaluated. Effect of Al concentrations inside the pH levels (a and c) and of pH inside the Al concentrations (b and d). According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

Experiment 1. Proline content (cv. BRS Caldeirão)

In this cultivar, there were isolated effects of the factors Al and pH on proline content in the shoot (Figure 5). It was noted that factor pH had a significant effect ($p<0.05$) on the biosynthesis and accumulation of this amino acid. At pH 4.0, the lowest content was found, but it did not differ statistically from plants subjected to pH conditions of 4.7 (Figure 5a). At pH levels of 3.3 and 4.7, proline content was 40.05 and 38.98 μmol g^{-1} of DM, respectively. Notably, when the plants were cultivated at a pH level of 4.0, the lower content represented a reduction of nearly 15.48 %, compared to the higher accumulation observed under a pH of 3.3.

In addition, proline content was also strongly affected under conditions with Al (1 mmol L^{-1}), in comparison to those that were treated without Al (Figure 5b). The proline biosynthesis and accumulation were significantly lower in the shoot of plants exposed to Al stress. In these plants, the content was lower at about 13.67 %.

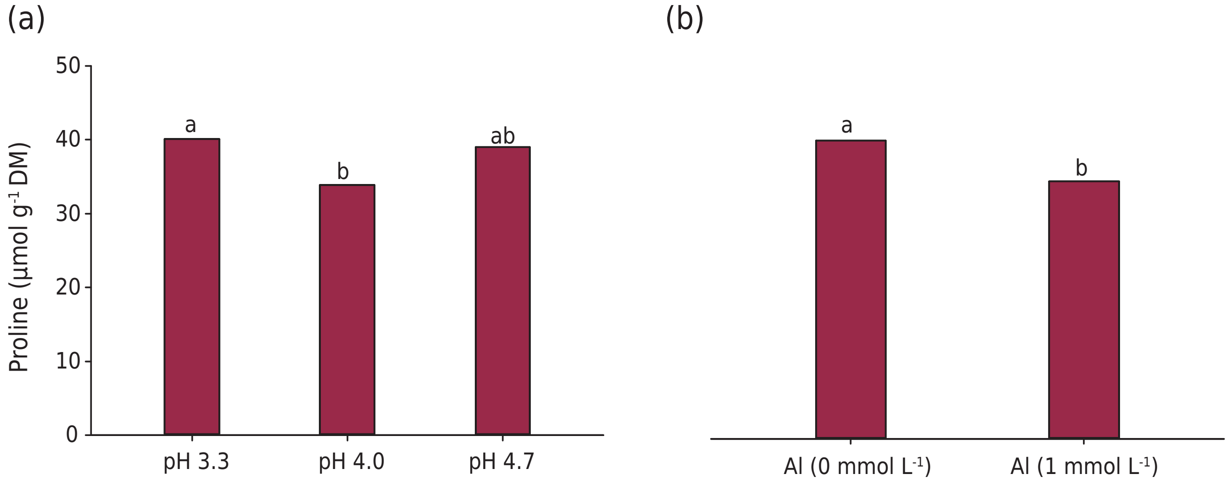

Figure 5. Isolated effects of pH (a) and Al (b) on proline content in the shoot of cowpea cv. BRS Caldeirão, after 27 days of plant exposition to the treatments. According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

Experiment 2. Proline content (cv. BRS Tracuateua)

In shoot and root tissues, there was no interaction effect of the factors Al and pH. However, in roots, a significant difference ($p\leq0.05$) between the means of the factorial and control was observed. The comparison of all six treatments with the control showed that proline accumulation was statistically higher for the control, with the exception of T4 (1;3.3) and T6 (1;4.7) treatments (data not shown).

Experiment 1. Nutritional status (cv. BRS Caldeirão)

There was a significant interaction effect of the factors Al and pH on amounts of P, S, and Fe in the shoot tissue of this cultivar (Figure 6). The results showed that the contents of P and Fe were greater in plants grown without Al, compared to those with Al 1 mmol L^{-1}, whatever pH level (Figures 6a and 6e). On the other hand, the levels of Al did not significantly affect S content of these plants, except as exposed to pH level of 4.7, in which the content of this nutrient was greater without Al (Figure 6c). Additionally, the pH levels had no significant influence on the contents of P and S, especially when cultivated without Al; however, with Al in solution, there was a reduction of the amounts with increasing pH levels (Figures 6b and 6d). Under growth conditions without Al and pH value of 4.0, increased Fe uptake and accumulation in the shoot significantly, while pH 3.3 and 4.7 had no significant impact on Fe accumulation of this tissue (Figure 6f).

In the shoot, the isolated effect of the factor Al had a significant impact on Ca, Mg, and Mn accumulation, while the isolated effect of the factor pH had a significant influence on Mn content (Figure 7). It was noted that uptake and accumulation of these nutrients were higher and significant in the absence of Al (Figures 7a, 7b, and 7c). In plants subjected to Al stress, a significant reduction of 32.9, 21.2, and 12.9 % was observed, respectively for Ca, Mg, and Mn content. Regarding the effect of the pH levels on Mn accumulation, it a reduction was noted with an increase in pH values (Figure 7d), occurring a greater Mn nutrition at pH level of 3.3 compared to 4.7.

In roots, the Al levels had an isolated effect on P, K, Ca, and Mg contents (Figures 7e, 7f, 7g and 7h). In general, a significant increase in accumulation of these nutrients was observed when cultivated without Al. In the presence of Al (1 mmol L^{-1}), a reduction of around 26.62, 12.06, 33.07, and 27.69 %, respectively, for P, K, Ca and Mg, as compared to that of without Al.

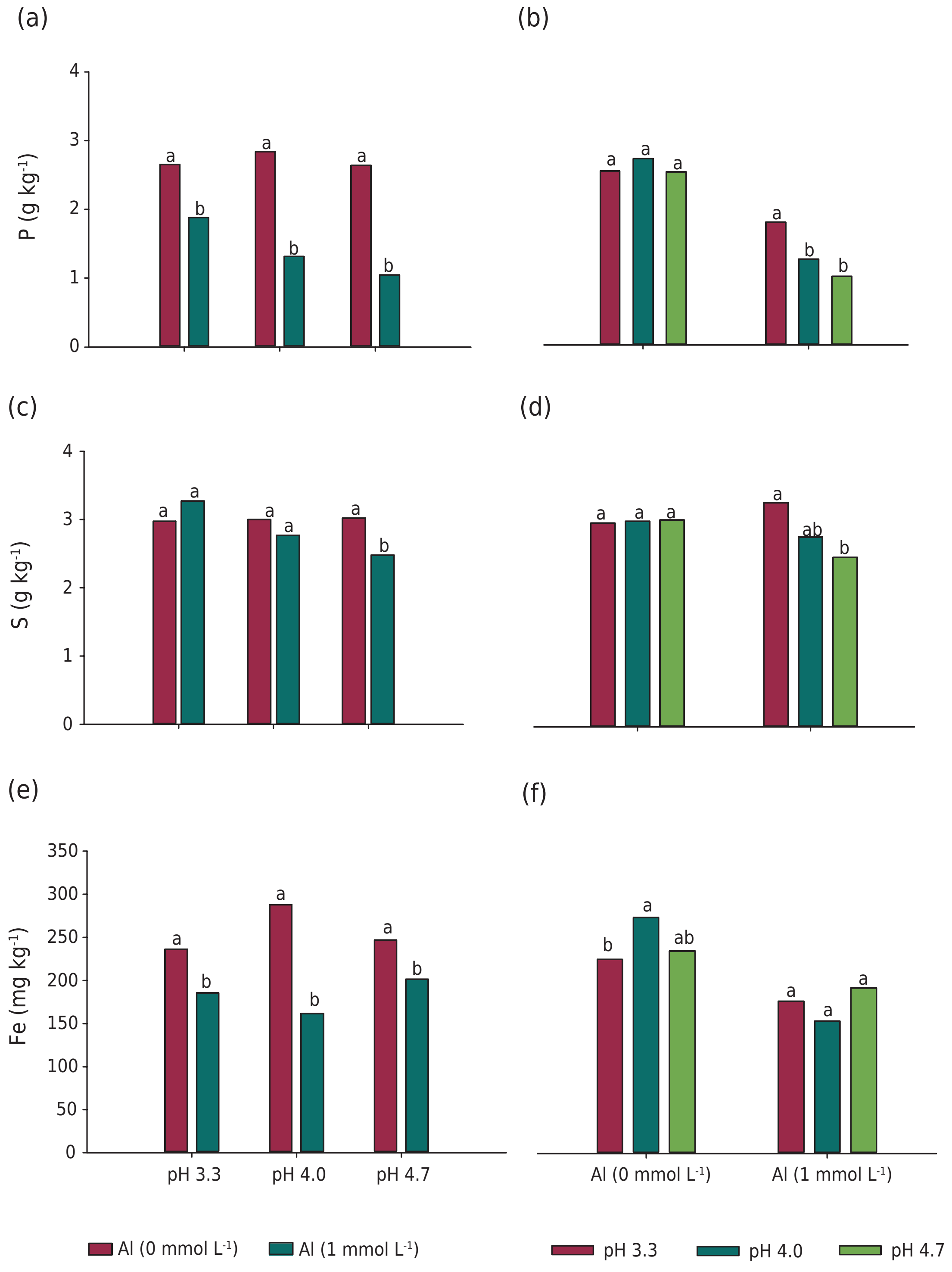

Figure 6. Contents of P, S, and Fe in shoot of cowpea cv. BRS Caldeirão, after 27 days of plant exposition to the treatments. Effect of Al concentrations inside the pH levels (a, c, and e) and of pH levels inside the Al concentrations (b, d, and f). According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

For this cultivar, there was also a significant interaction between control and other treatments concerning the contents of P, Ca, Mg, S, and Mn, while in roots, this interaction had a significant impact on amounts of N, Ca, and B (data not shown). It was found that P and Ca amounts were lower in the shoot when exposed to Al, compared to the control and treatments without Al. A similar behavior was observed for Mg and Mn status, indicating that these nutrients were significantly lower in plants subjected to Al-stress and with higher pH levels, i.e., T5 (1;4.0) and T6 (1;4.7), compared with control and other treatments. For S content, only T6 (1;4.7) presented a significant difference, displaying

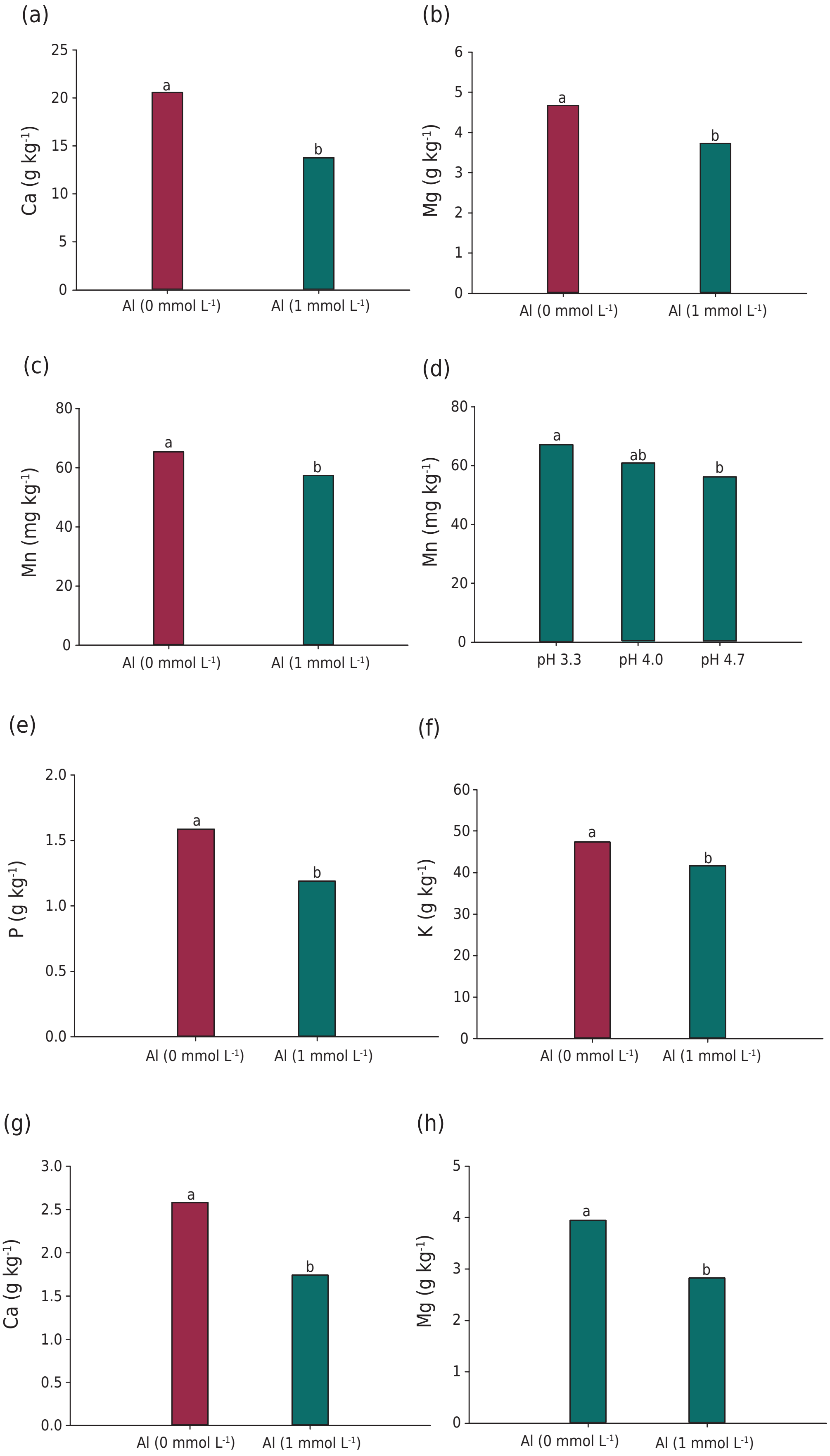

Figure 7. Contents of Ca, Mg, and Mn in shoot (A, B, C and D) and of P, K, Ca, and Mg (E, F, G and H) in roots of cowpea cv. BRS Caldeirão, after 27 days of plant exposition to the treatments. Isolated effect of Al (A, B, C, E, F, G and H), and isolated effect of pH (D). Columns followed by the same letters are not significantly different (p<0.05), according to Tukey´s Test.

a lower content in relation to the control and other treatments tested. In roots, it is noted that N accumulation was significantly reduced only for T1 (0;3.3), T3 (0;4.7), and T6 (1;4.7), as compared to the control, while other treatments did not differ. For Ca, only T5 (1;4.0) was significantly reduced. Concerning the control, B accumulation was significantly lower for T3 (0;3.3), T5 (1;4.0), and T6 (1;4.7) treatments.

Experiment 2. Nutritional status (cv. BRS Tracuateua)

In the shoot of this cultivar, there was a significant interaction effect of the factors Al and pH on K content (Figure 8). Also, the isolated effect of the factor Al on P, Ca, Mg, Fe, and Zn, as well as of the factor pH on Mg and Zn contents, were observed (Figure 9). Potassium accumulation was significantly greater at pH 3.3 without Al, compared to those plants grown with Al (Figure 8a). When plants were cultivated with Al, pH levels had no significant impact on K content; however, without Al at pH level of 3.3, the greatest content was found, while at pH 4.0 and 4.5 were lower and with similar nutritional behavior (Figure 8b).

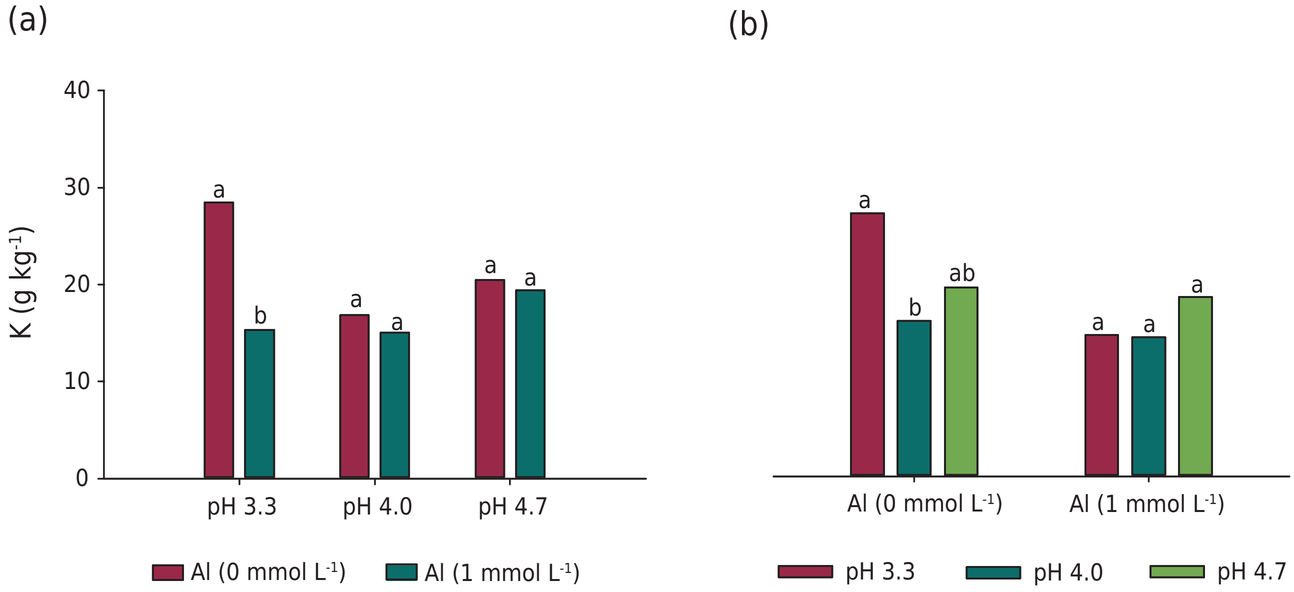

Figure 8. Content of K in shoot of cowpea cv. BRS Tracuateua, after 27 days of plant exposition to the treatments. Effect of Al concentrations inside the pH levels (a) and of pH levels inside the Al concentrations (b). According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

Nutrients influenced significantly by the isolated effect of Al were reduced by 42.73, 22.88, 14.15, 23.45, and 17.18 %, respectively, for P, Ca, Mg, Fe, and Zn (Figures 9a, 9b, 9c, and 9d). The reduction was generally higher for P, followed by Fe, Ca, Zn, and Mg. Concerning the isolated effect of pH levels, it was noted that Mg decreased at pH levels of 3.3 and 4.0. An opposite behavior was found for Mn, indicating a trend of absorption increase with increased pH levels (Figures 9e and 9f).

In roots, the factor Al had an isolated effect on P, Ca, and Fe contents (Figures 10a, 10b, and 10c). The contents of these nutrients were reduced when cultivated with Al by 34.37, 19.55, and 23.31 % for P, Ca, and Fe, respectively. Thus, there was a higher reduction in P accumulation, followed by Ca and Fe. The isolated effect of the factor pH significantly impacted Ca content, indicating that Ca uptake and accumulation improved with increasing pH levels of the solution (Figure 10d). At pH 4.7, the improvement in Ca status was 24.4 % compared to those at a pH level of 3.3.

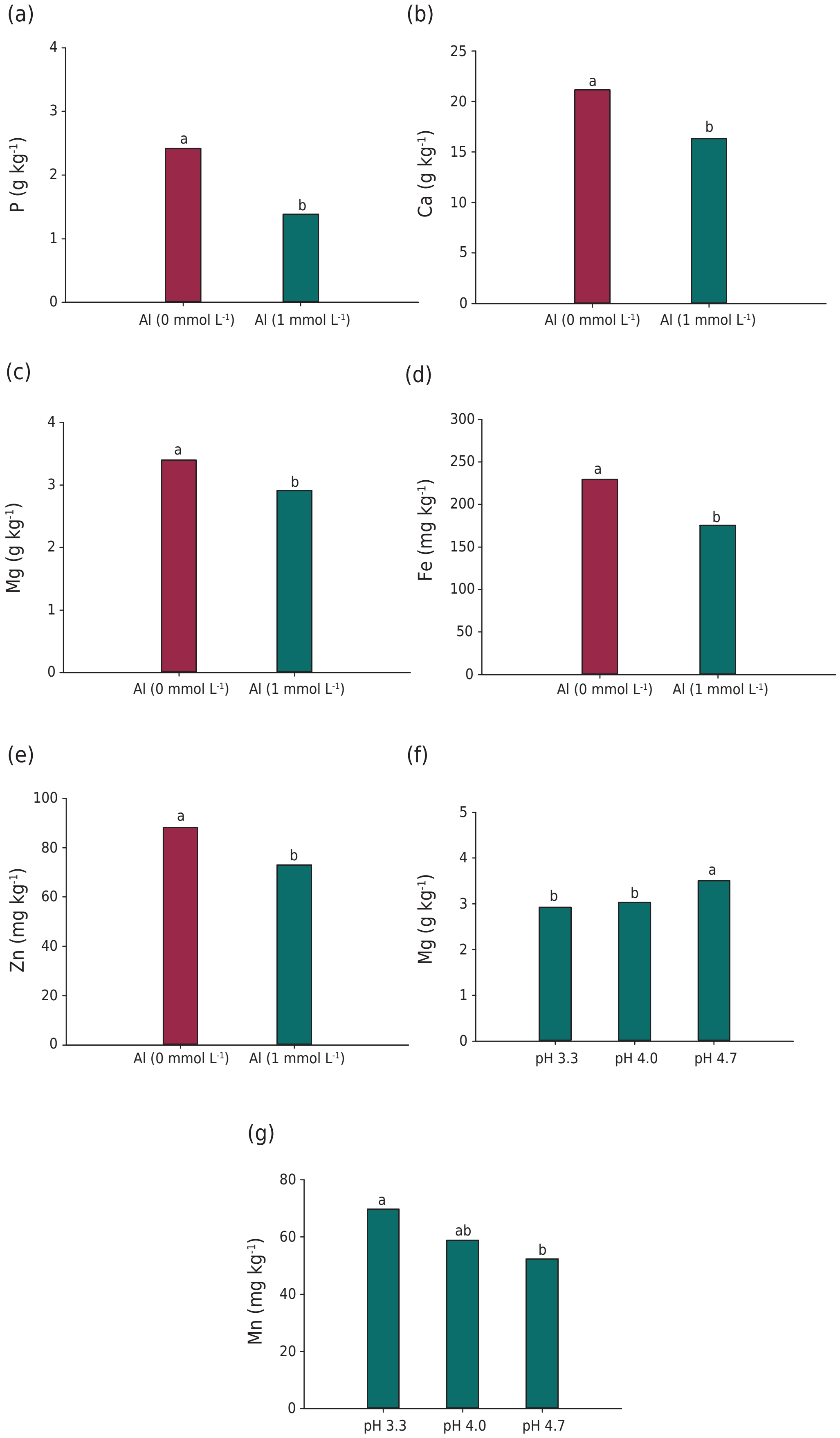

Figure 9. Contents of P, Ca, Mg, Fe, Zn, and Mn in the shoot of cowpea cv. BRS Tracuateua, after 27 days of plant exposition to the treatments. Isolated effect of Al (a, b, c, and e), and isolated effect of pH (f and g). According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

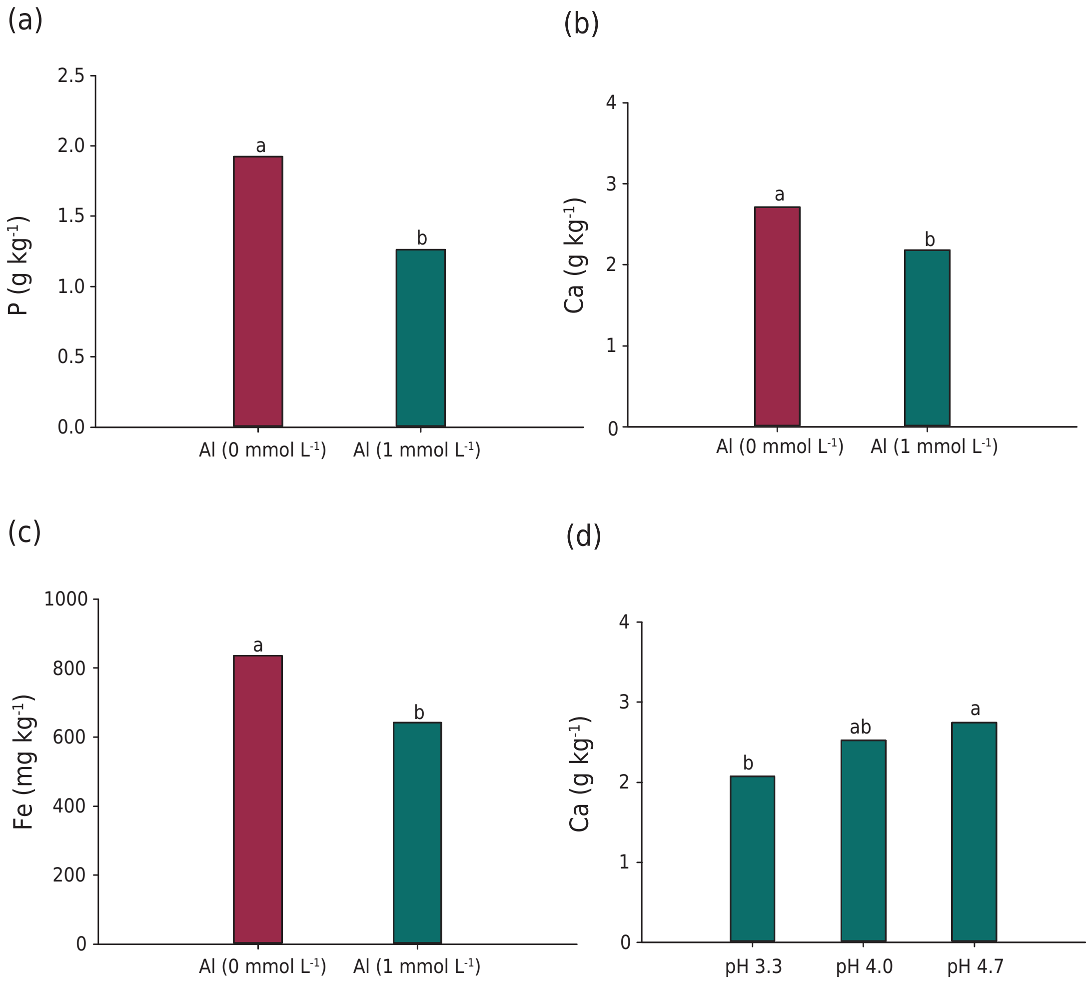

Figure 10. Contents of P, Ca, and Fe in roots of cowpea cv. BRS Tracuateua, after 27 days of plant exposition to the treatments. Isolated effect of Al (a, b and c), and isolated effect of pH (D). According to Tukey's Test, columns followed by the same letters are not significantly different ($p<0.05$).

Additionally, it was also found a significant difference between control and other treatments in N, Ca, and B content in roots (data not shown). In regard to the control, N accumulation of the treatments T1 (0;3.3), T3 (0;4.7), and T6 (1;4.7) was significantly lower, while Ca accumulation was lower only for T4 (1;3.3). For B content, treatments T3 (0;4.7), T5 (1;4.0), and T6 (1;4.7) were also significantly lower compared to the control.

DISCUSSION

Experiment 1. Photosynthetic characteristics (cv. BRS Caldeirão)

Photosynthesis behavior can be understood as a physiological and biochemical process, which is highly important in promoting plant growth, particularly when *A* rates present high values. In this cultivar, a greater physiological performance regarding *A* and *EIC* parameters was observed, notably under growth conditions with the lowest pH level of 3.3, which is associated to high H^+ ions concentration on cell surface roots. According to Kinraide and Parker (1987), high concentrations of hydrogen could reduce the phytotoxicity of other cations like Al^{3+}, due to the amelioration by H^+ ions and a higher effectiveness for high-affinity binding sites; thus the binding reduces negative surface charges of the cell surface. The improvement in liquid photosynthesis rates under an acidic solution could also be related to the increase in H^+ extrusion by proton pump, which is considered largely essential for growth and cell extension (Yan et al., 1992).

On the other hand, the lower *A* rate at the level of pH 4.7 has been explicated by a significant reduction in *Ci* concentration when these plants were cultivated at this acid pH condition. This strong reduction was probably promoted by the lower influx and CO_2 input in the sub-stomatal chamber, which can also be related to a decreasing regulation capacity of the stomatal opening in the leaves. In addition, this consequently points to a low amount of substrate available in the sub-stomatal chamber, decreasing the activity of enzymes associated with the carbon metabolism, i.e., ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity, as well as biomass production. According to Krause and Weis (1991), plants submitted to stress conditions normally tend to close the stomata as a defense mechanism to prevent water loss, which also can result in reduced stomatal conductance, and that could improve plant resistance.

It has been well documented that Chl *a* fluorescence can be a useful tool for obtaining information on the structure and functioning of the photosynthetic apparatus (Krause and Weis, 1991; Strasser et al., 2004). Regarding the photochemical performance of the factorial plants, it seems that greater PI_{total} of the control treatment can indicate a greater efficiency of electron transport flux through PSII. On the other hand, a reduction in photochemical performance for T5 (1;4.0) treatment results from photodamages to the photosynthetic apparatus of the thylakoid structure. Such a lower photochemical performance can indicate photodamages to the photosynthetic apparatus (Yusuf et al., 2010). Furthermore, a study carried out by Oliveira et al. (2018) on cowpea also observed decreasing in this index, especially when plants were exposed to higher levels of salt stress. Moreover, exposition of plants to stress situation by toxic ions like Al at acidic pH, have a negative effect on the photosynthesis process and electron transport rate through PSII (Zheng, 2010), generating reactive oxygen species (ROS), which in turn, can cause photochemical damages to PSII reaction centers (Guidi et al., 2019).

The maximum potential quantum efficiency of PSII, accessed by Fv/Fm ratio, presented values of nearly 0.8, which means optimal values for most plant species, according to Krause et al. (2001). Lower values than 0.83 indicate stress and/or photoinhibition (Björkman and Demmig, 1987). Although T5 (1;4.0) treatment revealed a low photochemical performance pointed by PI_{total} index, when looking at Fv/Fm ratio, it was therefore observed values in the optimal interval. This parameter did not detect seriously photodamages to the photosynthetic apparatus, which also indicated tolerance and adaptation mechanisms under acid stress situations. Other studies on cowpea (Souza et al., 2011; Andrade et al., 2019) also did not detect a significant variation related to Fv/Fm ratio, pointing to stress tolerance. It is pointed out that results obtained from the Chl *a* fluorescence parameters have been used to select tolerant plants (Azevedo Neto et al., 2011), such as tolerance to Al-stress, as reported by (Moustakas et al., 1995).

Experiment 2. Photosynthetic characteristics (cv. BRS Tracuateua)

The effect of Al and pH levels on transpiration (*E*) and stomatal conductance to water vapor (*gs*) significantly reduced the photosynthesis performance of this cultivar, probably due to the closeting of the stomatal in the leaves. Usually, this behavior indicates a response mechanism to a stress situation. It can be used as a defense mechanism by plants to avoid water loss (Szabados and Savouré, 2010). Therefore, the reduction in *E* and *gs* parameters also results in a low photoassimilate production, consequently decreasing growth and plant yield. This fully agrees with the negative impacts observed on this cultivar's growth and development, especially as compared to BRS Caldeirão cultivar, indicating higher sensitivity under acid stress conditions. However, when such plants were exposed to the lowest pH levels, the negative impact on *E* and *gs* performance was attenued, promoted by a higher H ion concentration, which also contributed to amelioration in mineral nutrition and physiological process of the plants.

Experiment 1. Proline content (cv. BRS Caldeirão)

Under abiotic stress conditions, plants can produce and accumulate high amounts of amino acids such as proline (Szabados and Savouré, 2010), which is a biochemical mechanism to protecting the integrity of enzymes and cell membranes (Jaleel et al., 2007). When submitted to growth conditions with Al (1 mmol L^{-1}), the lower proline accumulation can result from Al toxicity, which is in accordance with results observed by other studies (Cruz et al., 2014). The authors have attributed such metabolic behavior to lower efficiency in ammonium assimilation (NH^{4+}) by plants affected with Al-stress, which resulted in NH^{4+} accumulation in leaves. This lower metabolic efficiency has been attributed the low activity of enzymes that catalyze the reduction of nitrate N to organic compounds within the plant, such as nitrate reductase (Justino et al., 2006; Cruz et al., 2014).

In this cultivar, a greater accumulation of proline when cultivated at pH level of 3.3 was obtained, indicating an improvement of amino acid biosynthesis under such conditions. At this acid pH condition, plants also presented a higher efficiency of the carboxylation reactions, in accordance with physiological behavior. Thus, as discussed previously, this physiological and biochemical performance is attributed to a lower stress damage on the root system, probably due to H amelioration.

Experiment 2. Proline content (cv. BRS Tracuateua)

In plants, most of its physiological and biochemical processes can be affected by water restriction (Taiz and Zeiger, 2006). Notably, this cultivar presented a significant effect of proline content in roots, which agrees with results found, showing that acid stress condition caused damage principally to this tissue. It can also be reinforced, specially, by the observed reduction in physiological parameters (*E* and *gs*). The high levels of osmolitytes (i.g., proline), have been associated with an osmotic adjustment to maintain cell turgor under osmotic stress (Delauney and Verma, 1993). In addition, the positive effect of proline accumulation under stress has been well documented (Szabados and Savouré, 2010).

Therefore, accumulation of high levels of proline is often associated with metabolic response as an adaptation strategy to promote water influx in the roots under water stress conditions. On the other hand, as mentioned previously, lower proline content is associated to reduction in activity of enzymes in nitrogen metabolic pathway, explaining a lower accumulation in control plants compared to other treatments, except for T5 (1:4.0) treatment grown under acid conditions with Al 1 mmol L^{-1} and pH value of 4.0.

Experiment 1. Nutritional status (cv. BRS Caldeirão)

According to Kochian et al. (2004), Al in acid solution can cause negative effects on root growth, reducing the uptake of macro- and micronutrients by plants. In this study, acid stress conditions had a significant impact on plant mineral nutrition, reducing the uptake of P and S, notably when they were grown at pH level of 4.7, which can be related to a higher aluminum ion activity at this acid condition. In contrast, the lower toxicity effect observed at pH value of 3.3, likely is due to a higher H concentration in the solution, which reduced Al toxicity on root cells, improving nutrient uptake and transportation to shoot.

For tropical acid soils, it has been reported that the inhibitory effect of Al on P uptake and mineral nutrition has implications on plant cultivation, resulting in reduced crop yield (Fageria and Carvalho, 1982). It is important to point out that the P element is involved in the maintenance of the stability of cell membranes and also in the synthesis of DNA and RNA, in addition to being an essential nutrient for respiration and photosynthesis processes (Taiz and Zeiger, 2006).

Regarding the accumulation of S, a reduction of this nutrient in the shoot can lead to nutritional deficiency, and as a consequence, it decreases protein synthesis and plant growth (Vitti et al., 2006). The plant growth under acid stress showed an interaction effect of the Al and pH levels on Fe uptake, reducing its content, mainly in the presence of Al. Also, at acid pH conditions (Foy and Fleming, 1982) reported that Al leads to iron deficiency and inhibits the absorption and/or use of Fe by plants.

The observed low amounts of Ca, Mg, and Mn in shoot tissue, pointed to a harmful effect of Al on growth and mineral nutrition of cowpea. Ryan and Kochian (1993) have reported that Al ions normally compete with Ca and Mg by the absorption sites on transporter proteins, inhibiting nutrient influx in the cell and transportation to shoot. In addition, according to Marschner (1995), Ca has an important role in stabilizing the cell walls of plants, while Mg is an essential component of chlorophyll molecules. In plants, the appropriate amount of Ca ranges from 4 to 40 g kg^{-1} (Fernandes, 2006), depending on specie, tissue, and vegetal organ (Marschner, 2012).

However, in this study, Ca content was below the minimum amount, attributed to exposition to acid stress conditions. In plants, low absorption and transportation of nutrients when exposed to toxic ions like Al can induce mineral Ca deficiency in the shoot (Foy et al., 1978). Under such conditions, Ca status also negatively affects root development, ion uptake, and permeability of cell membranes (Rengel and Zhang, 2003).

Concerning micronutrients in the shoot, a higher Mn accumulation, as cultivated at pH level of 3.3, indicated that H ions are involved in the improvement of the physiological behavior of the plants, as pointed out by the liquid photosynthesis of this cultivar. Additionally, according to (Foy 1973), at acid conditions, the availability of Mn increases, enhancing absorption and accumulation. This nutrient is highly important as a component of the structure of photosynthetic proteins and enzymes (Millaleo et al., 2010), which can explain the improvement observed.

In roots, reductions in P, K, Ca, and Mg content have been attributed to the toxic effect of Al ions. The lower absorption of these nutrients negatively impacted the plants' metabolism and growth. It was also noted that the amounts were lower in root than shoot, indicating a higher nutrient accumulation, mainly P and Ca. Such nutritional behavior can be associated with tolerance under acid stress conditions.

Experiment 2. Nutritional status (cv. BRS Tracuateua)

The levels of Al and pH had effect on K status of this cultivar, indicating that under acid situation with absence of Al, there was a higher accumulation of K in shoot. The result indicated that K accumulation improved when exposed to the lowest pH level of 3.3. In contrast, a low amount of K under pH conditions of 4.7 and Al (1 mmol L^{-1}) contributed to the reduction in *E* and *gs* parameters, which also pointed to water stress damage. The K^+ ions are essential in reducing water loss and the transpiration process of plants under water stress (Shimazaki et al., 2007). Moreover, this nutrient acts as an osmoregulator within the cell, being involved in regulating the opening and closeting of stomata, and also plays an important role in cell turgor, carbohydrate transport, and respiration process of the plants (Epstein and Bloom, 2006).

The isolated effect of the factor Al promoted a reduction in the contents of some elements in the shoot, such as P, Ca, Mg, Fe, Zn, and Mn, showing that the presence of this metal resulted in a lower nutritional quality due to Al toxicity at the highest pH level. While, the isolated effect of the factor pH on Mg and Mn status reduced the availability of Mg under acid stress conditions, while promoting a higher Mn uptake.

Concerning contents of nutrients in roots, the decreasing of P, Ca, and Fe caused by the isolated effect of Al, and also pH effect inhibiting Ca influx, can result from competition by absorption sites on roots. While, the higher Ca accumulation probably is due to a lower inhibition effect and higher availability with increasing pH level. Therefore, as found for BRS Caldeirao, this cultivar also presented tolerance under acid stress conditions, however, it was lower, possibly due to higher nutrient precipitation in root tissue, which decreased transportation and accumulation in the shoot, mainly Fe.

CONCLUSIONS

The approach used to investigate cowpea response under Al^{3+} and H^+ stress conditions revealed that the physiological, biochemical, and nutritional aspects of both genotypes presented a greater performance with Al (1 mmol L^{-1}) when exposed to pH level of 3.3. In contrast, with Al and pH value of 4.7, the internal CO_2 concentration for cv. BRS Caldeirão was significantly reduced, as well as *E* and *gs* parameters for cv. BRS Tracuateua. The isolated effect of pH decreased *A* and *EIC* parameters for BRS Caldeirão.

Under growth conditions studied, cv. BRS Tracuateua was less tolerant than BRS Caldeirão, as indicated mainly by the lower photosynthetic and biochemical efficiency, as well as a lower crop nutrient accumulation. The BRS Tracuateua cultivar showed significantly lower K accumulation in the shoot when exposed to Al-stress and a pH value of 4.7 than plants cultivated without Al^{3+}, which reflected in lower photosynthetic performance.

The isolated effect of Al^{3+} and pH on shoot promoted a significant accumulation of proline for cv. BRS Caldeirão, which is an indicative of higher tolerance under abiotic stress conditions, especially compared to cv. BRS Tracuateua. While for cv. BRS Tracuateua, proline content was significantly affected in roots, indicating a metabolic response to water stress damage.

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REFERENCES

Akibode CS, Maredia MK. Global and regional trends in production, trade and consumption of food legume crops. Michigan: Michigan State University; 2012.

Akinrinde EA, Iroh L, Obigbesan GO, Hilger T, Neumann G, Romheld V. Differential expression of aluminium tolerance mechanisms in cowpea genotypes under phosphorus limitation. J Appl Sci. 2006;6:854-9. https://doi.org/10.3923/jas.2006.854.859

Andrade JR, Júnior SOM, Barbosa JWS, Alencar AEV, Jovino RS, Nascimento R. Chlorophyll fluorescence as a tool to select salinity-tolerant cowpea genotypes. Comun Sci. 2019;10:319-24. https://doi.org/10.14295/cs.v10i2.3012

Azevedo Neto AD, Pereira PPA, Costa DP, Santos ACC. Fluorescência da clorofila como uma ferramenta possível para seleção de tolerância à salinidade em girassol. Rev Cienc Agron. 2011;42:893-7. https://doi.org/10.1590/S1806-66902011000400010

Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973;39:205-7. https://doi.org/10.1007/BF00018060

Björkman O, Demmig B. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta. 1987;170:489-504. https://doi.org/10.1007/BF00402983

Bremner JM. Nitrogen-total. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME, editors. Methods of soil analysis: Part 3 Chemical methods. Madison: Soil Science Society of America; 1996. p. 1085-121. https://doi.org/10.2136/sssabookser5.3.c37

Carvalho M, Lino-Neto T, Rosa E, Carnide V. Cowpea: A legume crop for a challenging environment. J Sci Food Agr. 2017;97:4273-84. https://doi.org/10.1002/jsfa.8250

Cruz FJR, Almeida HJ, Santos DMM. Growth, nutritional status and nitrogen metabolism in '*Vigna unguiculata*' (L.) Walp is affected by aluminum. Aust J Crop Sci. 2014;8:1132-9.

Delauney AJ, Verma DPS. Proline biosynthesis and osmoregulation in plants. Plant J. 1993; 4:215-23.

Delhaize E, Ryan PR. Aluminum toxicity and tolerance in plants. Plant Physiol. 1995;107:315-21. https://doi.org/10.1104/pp.107.2.315

Epstein E, Bloom AP. Nutrição mineral de plantas: Princípios e perspectivas. Londrina: Editora Planta; 2006.

Fageria NK, Carvalho JRP. Influence of aluminum in nutrient solutions on chemical composition in upland rice cultivars. Plant Soil. 1982;69:31-44. https://doi.org/10.1007/BF02185701

Fernandes MS. Nutrição mineral de plantas. Viçosa, MG: Sociedade Brasileira de Ciências do Solo; 2006.

Foy CD. Manganese and plants. Manganese. In: NRC Committee on Biological Effects of Atmospheric Pollutants. Washington, DC: Manganese, National Academy of Sciences; 1973. p. 51-76.

Foy CD, Chaney RL, White MC. The physiology of metal toxicity in plants. Ann Rev Plant Physiol. 1978;29:511-66.

Foy CD, Fleming AL. Aluminum tolerances of two wheat genotypes related to nitrate reductase activities. J Plant Nutr. 1982;5:1313-33. https://doi.org/10.1080/01904168209363064

Godoy F, Olivos-Hernández K, Stange C, Handford M. Abiotic stress in crop species: Improving tolerance by applying plant metabolites. Plants. 2021;10:186. https://doi.org/10.3390/plants10020186

Guidi L, Lo Piccolo E, Landi M. Chlorophyll fluorescence, photoinhibition and abiotic stress: Does it make any difference the fact to be a C3 or C4 species? Front Plant Sci. 2019;10:174. https://doi.org/10.3389/fpls.2019.00174

Haridasan M. Alumínio é um elemento tóxico para as plantas nativas do cerrado. In: Prado CHBA, Casali CA, editors. Fisiologia vegetal: Práticas em relações hídricas, fotossíntese e nutrição mineral. Barueri, SP: Editora Manole; 2006. p. 1-10.

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: A review. Plant Signal Behav. 2012;7:1456-66. https://doi.org/10.4161/psb.21949

Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. California: California Agricultural Experiment Station; 1950. (Circular 347).

Honaiser TC, Rossi GB, Rocha MM, Arisi ACM. Comparison of grain protein profiles of Brazilian cowpea (*Vigna unguiculata*) cultivars based on principal component analysis. Food Prod Process Nutr. 2022;4:16. https://doi.org/10.1186/s43014-022-00095-z

Horst WJ, 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. Ann Bot. 2010;106:185-97. https://doi.org/10.1093/aob/mcq053

Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, Gopi R, Somasundaram R, Panneerselvam R. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus* roseus exposed to water deficit. Colloid Surfaces B: Biointerfaces. 2007;59:150-7. https://doi.org/10.1016/j.colsurfb.2007.05.001

Jaskowiak J, Kwasniewska J, Milewska-Hendel A, Kurczynska EU, Szurman-Zubrzycka M, Szarejko I. Aluminum alters the histology and pectin cell wall composition of barley roots. Int J Mol Sci. 2019;20:3039. https://doi.org/10.3390/ijms20123039

Justino GC, Cambraia J, Oliva MA, Oliveira JA. Absorção e redução de nitrato em duas cultivares de arroz na presença de alumínio. Pesq Agropec Bras. 2006;41:1285-90. https://doi.org/10.1590/S0100-204X2006000800011

Kinraide TB, Parker DR. Cation amelioration of aluminum toxicity in wheat. Plant Physiol. 1987;83:546-51. https://doi.org/10.1104/pp.83.3.546

Kochian LV. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol. 1995;46:237-60.

Kochian LV, Hoekenga OA, Pineros MA. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol. 2004;55:459-93. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Krause GH, Weis E. Chlorophyll fluorescence and photosynthesis: The basics. Annu Rev Plant Physiol Plant Mol Biol. 1991;42:313-49.

Krause SA, Loupart M-L, Vass S, Schoenfelder S, Harrison S, Heck MM. Loss of cell cycle checkpoint control in *Drosophila Rfc4* mutants. Mol Cell Biol. 2001;21:5156-68. https://doi.org/10.1128/MCB.21.15.5156-5168.2001

Leite ML, Virgens Filho JS, Rodrigues JD. Variação dos níveis de prolina em folhas de caupi (*Vigna unguiculata* (L.) Walp) submetido a diferentes déficits hídricos. Biotemas. 2000;13:21-33.

Liu H, Zhu R, Shu K, Lv W, Wang S, Wang C. Aluminum stress signaling, response, and adaptive mechanisms in plants. Plant Signal Behav. 2022;17:2057060. https://doi.org/10.1080/15592324.2022.2057060

Ma JF. Plant root responses to three abundant soil minerals: Silicon, aluminum and iron. Crit Rev Plant Sci. 2005;24:267-81. https://doi.org/10.1080/07352680500196017

Malavolta E, Vitti GC, Oliveira SA. Avaliação do estado nutricional das plantas: Princípios e aplicações. Piracicaba: Potafos; 1997.

Mantri N, Patade V, Penna S, Ford R, Pang E. In: Ahmad P, Prasad M, editors. Abiotic Stress Responses in Plants. New York: Springer; 2012. p. 1-19. https://doi.org/10.1007/978-1-4614-0634-1_1

Marschner H. Mineral nutrition of higher plants. 3rd ed. London: Academic Press; 2012.

Marschner H. Mineral nutrition of higher plants. 2nd ed. Germany: Institute of Plant Nutrition University of Hohenheim; 1995.

Mekonnen TW, Gerrano AS, Mbuma NW, Labuschagne MT. Breeding of vegetable cowpea for nutrition and climate resilience in sub-saharan Africa: Progress, opportunities, and challenges. Plants. 2022;11:1583. https://doi.org/10.3390/plants11121583

Millaleo R, Reyes-Diaz M, Ivanov AG, Mora ML, Alberdi M. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J Soil Sci Plant Nutr. 2010;10:470-81. https://doi.org/10.4067/S0718-95162010000200008

Moustakas M, Ouzounidou G, Lannoye R. Aluminum effects on photosynthesis and elemental uptake in an aluminum-tolerant and non-tolerant wheat cultivar. J Plant Nutr. 1995;18:669-83. https://doi.org/10.1080/01904169509364930

Oliveira WJ, Souza ERD, Santos HRP, Silva EFFE, Duarte HHF, Melo DVM. Fluorescência da clorofila como indicador de estresse salino em feijão caupi. Rev Bras Agric Irrigada. 2018;12:2592-603. https://doi.org/10.7127/rbai.v12n300700

Ologundudu F, Ajayi Oluwatoyin, Ajayi Oluwaseun, Ajani I, Oladipupo S. Aluminium tolerance: a determinant factor to cowpea *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae) productivity. Braz J Biol Sci. 2018;5:105-13. https://doi.org/10.21472/bjbs.050911

Panda SK, Baluška F, Matsumoto H. Aluminum stress signaling in plants. Plant Signal Behav. 2009;4:592-7. https://doi.org/10.4161/psb.4.7.8903

Rabel DO, Motta ACV, Barbosa JZ, Melo VF, Prior SA. Depth distribution of exchangeable aluminum in acid soils: A study from subtropical Brazil. Acta Sci Agron. 2018;40:e39320. https://doi.org/10.4025/actasciagron.v40i1.39320

Rengel Z, Zhang W-H. Role of dynamics of intracellular calcium in aluminium-toxicity syndrome. New Phytol. 2003;159:295-314. https://doi.org/10.1046/j.1469-8137.2003.00821.x

Ryan PR, Kochian LV. Interaction between aluminum toxicity and calcium uptake at the root apex in near-isogenic lines of wheat (*Triticum aestivum* L.) differing in aluminum tolerance. Plant Physiol. 1993;102:975-82. https://doi.org/10.1104/pp.102.3.975

Sade H, Meriga B, Surapu V, Gadi J, Sunita MSL, Suravajhala P, Kavi Kishor PB. Toxicity and tolerance of aluminum in plants: tailoring plants to suit to acid soils. Biometals. 2016;29:187-210. https://doi.org/10.1007/s10534-016-9910-z

Sairam RK, Srivastava GC, Agarwal S, Meena RC. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biol Plant. 2005;49:85-91. https://doi.org/10.1007/s10535-005-5091-2

Samac DA, Tesfaye M. Plant improvement for tolerance to aluminum in acid soils–a review. Plant Cell, Tissue Organ Culture. 2003;75:189-207. https://doi.org/10.1023/A:1025843829545

Sanchez PA, Bandy DE, Villachica JH, Nicholaides JJ. Amazon Basin soils: Management for continuous crop production. Science. 1982;216:821-7. https://doi.org/10.1126/science.216.4548.821

Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965;52:591-611. https://doi.org/10.2307/2333709

Shimazaki K, Doi M, Assmann SM, Kinoshita T. Light regulation of stomatal movement. Annu Rev Plant Biol. 2007;58:219-47. https://doi.org/10.1146/annurev.arplant.57.032905.105434

Silva FWR. Resposta do feijão caupi a condições ácidas: interações alumínio x hidrogênio e manganês x hidrogênio [thesis]. Manaus: Universidade Federal do Amazonas; 2020.

Soares BL, Ferreira PAA, Oliveira-Longatti SM, Marra LM, Rufini M, Andrade MJB, Moreira FMS. Cowpea symbiotic efficiency, pH and aluminum tolerance in nitrogen-fixing bacteria. Sci Agric. 2014;71:171-80. https://doi.org/10.1590/S0103-90162014000300001

Souza RP, Machado EC, Silveira JAG, Ribeiro RV. Fotossíntese e acúmulo de solutos em feijoeiro caupi submetido à salinidade. Pesq Agropec Bras. 2011;46:587-92. https://doi.org/10.1590/S0100-204X2011000600003

Strasser RJ, Tsimilli-Michael M, Qiang S, Goltsev V. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant Haberlea rhodopensis. Biochim Biophys Acta. 2010;1797:1313-26. https://doi.org/10.1016/j.bbabio.2010.03.008

Strasser RJ, Tsimilli-Michael M, Srivastava A. Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou GC, Govindjee, editors. Chlorophyll a fluorescence: A signature of photosynthesis. Dordrecht: Springer; 2004. p. 321-62.

Szabados L, Savouré A. Proline: A multifunctional amino acid. Trends Plant Sci. 2010;15:89-97. https://doi.org/10.1016/j.tplants.2009.11.009

Szurman-Zubrzycka M, Chwiałkowska K, Niemira M, Kwaśniewski M, Nawrot M, Gajecka M, Larsen PB, Szarejko I. Aluminum or low pH–Which is the bigger enemy of barley? Transcriptome analysis of barley root meristem under Al and low pH stress. Front Genet. 2021;12:712. https://doi.org/10.3389/fgene.2021.675260

Taiz L, Zeiger E. Fisiologia vegetal. 3rd ed. Porto Alegre: Artmed; 2006.

Vitti GC, Lima E, Cicarone F. Cálcio, magnésio e enxofre. In: Fernandes MS, editor. Nutrição mineral de plantas. Viçosa, MG: Sociedade Brasileira de Ciências do Solo, 2006. p. 299-325.

Yan F, Schubert S, Mengel K. Effect of low root medium pH on net proton release, root respiration, and root growth of corn (*Zea mays* L.) and broad bean (*Vicia faba* L.). Plant Physiol. 1992;99:415-21. https://doi.org/10.1104/pp.99.2.415

Yan L, Li S, Riaz M, Jiang C. Proline metabolism and biosynthesis behave differently in response to boron-deficiency and toxicity in Brassica napus. Plant Physiol Biochem. 2021;167:529-40. https://doi.org/10.1016/j.plaphy.2021.08.029

Yang M, Tan L, Xu Y, Zhao Y, Cheng F, Ye S, Jiang W. Effect of low pH and aluminum toxicity on the photosynthetic characteristics of different fast-growing *Eucalyptus* vegetatively propagated clones. PLoS One. 2015;10:e0130963. https://doi.org/10.1371/journal.pone.0130963

Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Sarin NB. Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll a fluorescence measurements. Biochim Biophys Acta. 2010;1797:1428-38. https://doi.org/10.1016/j.bbabio.2010.02.002

Zheng SJ. Crop production on acidic soils: overcoming aluminium toxicity and phosphorus deficiency. Ann Bot. 2010;106:183-4. https://doi.org/10.1093/aob/mcq134