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Macronutrients accumulation and growth of pineapple cultivars submitted to aluminum stress

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Key words:
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ABSTRACT
The objective was to determine the growth and accumulation of macronutrients of two pineapple cultivars submitted to different concentrations of aluminum (Al). For this, a study was conducted in plastic pots containing 4 L of nutrient solution, in a randomized block design, in a 2 x 5 factorial scheme, corresponding to two pineapple cultivars ('IAC Fantástico' and 'Vitória') and five Al concentrations (0, 21.6, 43.2, 64.8 and 86.4 mg of Al plant^{-1}), with four replicates. The following variables were evaluated: root length, dry matter of root, stem and leaf, stem diameter, number of leaves, chlorophyll content and accumulation of macronutrients at 60 days after treatment. The cv. 'Vitória' showed a linear decrease in chlorophyll content, root dry matter, root length and accumulation of N, P, K, Ca and Mg in most plant components promoted by the increase of Al concentration in the nutrient solution. The cv. 'IAC Fantástico' had lower total dry matter, stem dry matter, stem diameter and accumulation of N, Ca and Mg. However, the evaluated characteristics were not influenced by the increase of Al concentration, showing greater tolerance of this cultivar to Al in nutrient solution.

Palavras-chave:
Al
toxicidade
tolerância
solução nutritiva

Acúmulo de macronutrientes e crescimento de cultivares de abacaxizeiros submetidos ao estresse por alumínio

RESUMO
Objetivou-se, neste trabalho, determinar o crescimento e o acúmulo de macronutrientes de duas cultivares de abacaxi submetido a diferentes concentrações de alumínio; para isto foi realizado um estudo com vasos de plástico contendo quatro litros de solução nutritiva e utilizado o delineamento em blocos casualizados em esquema fatorial 2 x 5 sendo duas cultivares de abacaxi (IAC Fantástico e Vitória) cinco concentrações de alumínio (0, 21,6; 43,2; 64,8 e 86,4 mg de Al planta^{-1}) e quatro repetições. Foram avaliados o comprimento de raiz, a matéria seca da raiz, o talo e as folhas, diâmetro do talo, emissão de folhas, teor de clorofila e acúmulo de macronutrientes aos 60 dias após os tratamentos. A cv. Vitória apresentou decréscimo linear no teor de clorofila, matéria seca de raízes, comprimento da raiz e acúmulo de N, P, K, Ca e Mg, na maioria dos componentes da planta promovidos pelo aumento da concentração de Al na solução nutritiva. A cv. IAC Fantástico teve menor massa de matéria seca total, matéria seca do talo, diâmetro do talo e acúmulo de N, Ca e Mg; no entanto, as características avaliadas não foram influenciadas pelo aumento da concentração de Al indicando maior tolerância desta cultivar ao Al em solução nutritiva.

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Introduction

The pineapple crop represents an option of agricultural activity potentially profitable for semiarid regions like northern Minas Gerais, provided that the main local needs are met, such as low rainfall levels and seasonal rainfall distribution.

According to Guarçoni & Ventura (2011), the other aspect that plays an important role for the pineapple crop is the edaphic character, since the crop develops better in acidic soils, with pH range from 4.5 to 5.5. Normally, this range of acidic pH is correlated with high levels of aluminum (Al) that can be toxic to the main agricultural crops.

Al toxicity is characterized as one of the essential limiting factors of plant exploitation in weathered soils of tropical regions, especially for causing inhibition of the root system of plant species. Besides inhibiting the normal formation of roots, it interferes with enzymatic reactions and with the absorption, transport and use of nutrients by the plants (Tomás et al., 2006)

The technique of hydroponic cultivation provides advantages to the studies on the interaction of this element with plants, due to the easy access to the root system and to the possibility of monitoring and controlling pH, besides the concentrations of Al and of other ions that are relevant to the expression of reactions of sensitivity and tolerance (Rossiello & Jacob, 2006). In this context, this study aimed to evaluate the effect of different doses of Al on the growth and absorption of nutrients by the pineapple cultivars 'IAC Fantástico' and 'Vitória'.

Material and Methods

The experiment was carried out in a greenhouse at the State University of Montes Claros, Campus of Janaúba-MG, Brazil (15º 47’ 18” S; 43º 18’ 18” W; 510 m), in a randomized block design with four replicates in a 2 x 5 factorial scheme, corresponding to two pineapple cultivars (‘IAC Fantástico’ and ‘Vitória’) and five Al doses (0, 21.6, 43.2, 64.8 and 86.4 mg plant^{-1}). During the experiment, the greenhouse had relative air humidity of approximately 60% and temperature of 27 ± 2 ºC. The hydroponic cultivation was conducted in plastic pots with capacity for 4 L containing one plant per pot and the nutrient solution proposed by Hoagland & Arnon (1950). The pineapple seedlings were removed from the multiplication bed and subjected to initial characterization (Table 1).

Initially, the pineapple plants were acclimated in the nutrient solution for 30 days, period after which the treatments with the Al doses were added and the plants were cultivated for another 40 days for the beginning of the evaluations.

Table 1. Initial characterization of the pineapple cultivars through the determination of height, stem diameter, root length, plant weight and total number of leaves

Cultivar	Height	Stem diameter	Root length	Plant weight	Number of leaves
	(cm)			(g)	(unit)
‘IAC Fantástico’	41.80	2.66	12.61	186.71	12.00
‘Vitória’	40.55	3.46	12.59	243.75	13.00

During the acclimation, the pH of the nutrient solution of each experimental unit was daily regulated to values between 5.0 and 6.0; after the addition of the treatments, the pH of the solution was maintained between 4.0 and 4.5, for better simulation of the Al availability to plants, and was controlled using a portable digital pH meter during the permanence of the pineapple cultivar in the solution.

The nutrient solution and the treatments were renewed in an interval of 10 days and continuously aerated; after 40 days, the plants were collected, weighed and separated into roots, stem and leaves for the determination of fresh and dry matter.

The evaluated characteristics were: length of two roots marked with a sewing thread; plant fresh matter, on a precision scale; leaf length, with a graduated ruler; and total number of leaves, through direct count. In the last evaluation, the chlorophyll content was also analyzed, using a portable chlorophyll meter (SPAD-502), expressed in SPAD index in three measurements on the ‘D’ leaf.

The plant material was dried in a forced-air oven at 65 ºC for approximately 120 h until constant mass; then, the dry matter was determined using a digital electronic precision scale; after that, each sample was ground in a Wiley-type mill with a 1-mm-mesh sieve and subjected to nitric-perchloric digestion. The obtained extract was analyzed for the contents of total nitrogen through the Kjeldahl method; phosphorus (P), through colorimetry; calcium (Ca) and magnesium (Mg), through atomic absorption spectrophotometry, and potassium (K), through flame emission photometry.

The obtained data were subjected to analysis of variance (F test) and, when significant for Al doses, regression analysis was applied. The means of the cultivars were compared by F test at 0.05 probability level. The regression equations were fitted, selecting the best model to explain the phenomenon and using the statistical program SISVAR® (Ferreira, 2008).

Results and Discussion

The chlorophyll index (CI) was significantly influenced ($p \leq 0.05$) by the interaction between the factors cultivars and Al concentrations in the nutrient solution; thus, the CI in the leaves of the cv. ‘Vitória’ suffered linear reduction with the increase in Al concentrations, but the CI of 'IAC Fantástico' was not influenced by the presence of Al in the nutrient solution ($\bar{y}$ = 63.76), suggesting a lower susceptibility of 'IAC Fantástico' to Al toxicity (Figure 1). The reduction of CI in the leaves of the cv. ‘Vitória’ reflects the lower chlorophyll content. The toxicity caused by Al reduces the biosynthesis of chlorophyll in the leaves of the plants, leading to a lower content of chlorophyll and photosynthesis, but these phenomena depend on species, cultivar, time of exposure and Al concentration in the nutrient solution (Peixoto et al., 2007).

In the plants, there are various symptoms of injuries caused by Al interfering with the metabolism of N, which is an important element in the synthesis of amino acids and photosynthesizing pigments (Sphear & Souza, 2004). Al toxicity can cause generalized deficiency of nutrients that are essential to the plants and lead to disorders in the metabolism, evidenced in the reduction of the content of proteins and

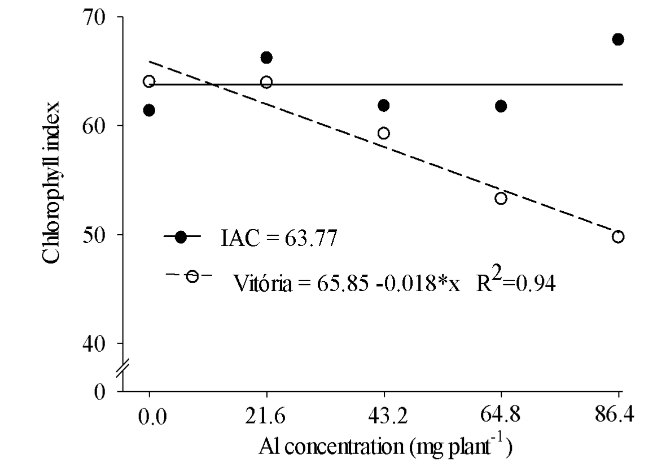


Figure 1. Chlorophyll index in the leaves of 'IAC Fantástico' and 'Vitória' pineapple cultivars after the addition of the aluminum (Al) concentrations in the nutrient solution

chlorosis in the leaves, besides other abnormalities (Miguel et al., 2010). Beckmann (1954) observed, for the first time in wheat and other cereals, symptoms of yellowing and reduction of plant growth, which was called "crestamento" (Al toxicity).

The inexistence of Al toxicity effects on the CI of leaves of 'IAC Fantástico' pineapple was possibly associated with the existence of more-efficient tolerance mechanisms, in comparison to the results obtained in the cultivar 'Vitória'. Mechanisms of tolerance to Al in different plant species are divided into two groups. The first one is related to mechanisms of exclusion, with exudation of organic ligands (mucilage, organic compounds of low molecular weight, etc.) by the roots, which are capable of complexing Al by the efflux of the Al accumulated in the roots and by the alteration in the pH of the rhizosphere (Langer et al., 2009). The second group of tolerance mechanisms is related to internal detoxification, by the fixation of Al in the cell wall, through the complexation in the symplast via organic ligands and by the accumulation of Al in the vacuole (Ryan et al., 2008).

The mean root dry matter significantly ($p \leq 0.05$) decreased with the increase in Al concentrations for the cv. 'Vitória'; however, for the cv. 'IAC Fantástico', there was no significant effect ($\bar{y}$ = 10.07), thus suggesting higher sensitivity of the 'Vitória' pineapple, and this effect may be a result of injuries on the apex of the root system, especially the radicles, which are generally the most affected part by the toxic effect of Al, resulting in the reduction of root dry matter (Figure 2A).

Similar effect was observed by Fung et al. (2008), who reported that the dose of 1 mmol of Al caused significant reductions in root dry matter of *Camellia sinensis* ("chá da Índia"). In spite of that, the lowest doses showed positive effect on the absorption of nutrients in this study.

When exposed to the cation, the roots suffer disintegration of tissues of the epidermis and of external portions of the cortex in the apices of the roots, and the cells wrinkle or, in more severe cases, collapse (Lin & Chen, 2013). There is also a reduction in the size of the root cap and derangement of the meristematic tissue, besides the formation of protoxylem and endoderm in regions close to the root apex with high contents of lignin (Miguel et al., 2010).

For root length, there was a significant ($p < 0.05$) and linear reduction with the increase in Al concentration in the nutrient solution for the cv. 'Vitória', while no significant ($p < 0.05$) response was observed in the cv. 'IAC Fantástico' (Figure 2B). These results can be explained by the fact that the plant, when subjected to any stress, uses most of its energy to the biosynthesis of secondary compounds and to triggering adaptive strategies that will provide it with tolerance or not (Taiz & Zeiger, 2009) to the presence of Al. In the cv. 'IAC Fantástico', it was possible to observe the accumulation of mucilage in the root growth zone, besides the increase in the pH of the nutrient solution, which was daily regulated to 4.5, which can be a result of exudation of organic ligands that complex the Al and reduce its toxicity in the plants.

In the presence of Al, plants secrete mucilage, which increases the pH in the root apex region and has high binding capacity, explained by the reactions of exchange with negative charges of the citrate and succinate, the main constituent of the mucilage (Taiz & Zeiger, 2009). Such material is mostly synthesized in the Golgi complex of the cells that are more external to the root cap. Among the exuded organic acids, citrate is the most effective, for being a tri-carboxylate anion that forms more-stable chelates with the trivalent Al. Besides citrate, other exudates, such as citric and malic acids, are capable of mitigating the effects caused by the toxicity (Hartwig et al., 2007).

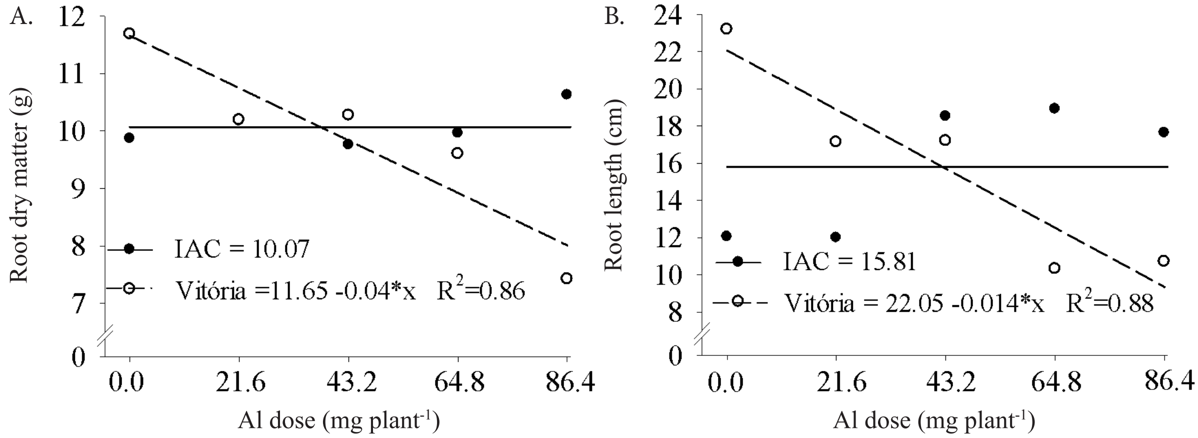


Figure 2. Dry matter (A) and root length (B) in 'IAC Fantástico' and 'Vitória' pineapple after the addition of the aluminum (Al) concentration in the nutrient solution

The first important publications related to this mechanism were presented in studies on wheat from the beginning of the 1990s. Rincón & Gonzalez (1992) observed that the level of sensitivity to Al was correlated with its concentration in the meristems, suggesting that the metabolic exclusion in the meristems was an important mechanism of tolerance. Delhaize et al. (1993) investigated the function of the organic acids and observed that the presence of Al in the nutrient solution stimulated the secretion of malic acid. These authors also found that the final 3 to 5 mm of the roots formed a primary source of the secreted organic acid; thus, there was a correlation between tolerance and high secretion of root exudates.

There was no effect of Al concentrations on the dry matter accumulation of leaves, stem, roots and total, root/shoot dry matter ratio, number of leaves and stem diameter for both pineapple cultivars; however, there was significant difference between the cultivars for the variables stem dry matter, total dry matter, root/shoot dry matter ratio and stem diameter. The 'Vitória' pineapple showed higher total dry matter due to the greater accumulation of stem dry matter, in comparison to 'IAC Fantástico'; however, the cv. 'IAC Fantástico' showed higher root/shoot dry matter ratio, which indicates greater allocation of photoassimilates in the roots (Table 2). These results were attributed to the genetic characteristics of both studied cultivars.

The cv. 'Vitória' showed greater accumulation of macronutrients in the stem, Mg in the leaves, N and Ca in the roots, N, Ca and Mg in the entire plant, besides greater ratio of N accumulation between roots and shoots. However, 'IAC Fantástico' allocated higher K content in the roots and had higher ratio of the accumulation of P, K and Mg between roots and shoots (Table 3), indicating greater proportion of P, K and Mg allocated in the roots, in comparison to the cv. 'Vitória'. In studies evaluating Al doses in *Camellia sinensis,* Fung et al. (2008) also reported increase of P, K and Mg in the roots of plants cultivated at doses of up to 0.5 mM. The greater proportion of these elements in the roots, observed in the cv. 'IAC Fantástico', is possibly related to the mechanisms of defense to Al toxicity, since elements like P can help in the formation of insoluble compounds, such as $Al_4(PO_4)_3$, retarding the entry of Al in the apoplast (Costa et al., 2014).

The increasing concentrations of Al in the nutrient solution caused reduction in the accumulation of macronutrients in most of the components (leaves, stem and roots) of 'Vitória' pineapple and did not influence the accumulation of nutrients in the tissues of 'IAC Fantástico' pineapple (Figure 3). In the cv. 'Vitória', there was linear decrease in the accumulation of K and Mg in the roots, K in the stem and N, P, and K in the plant (sum of the components) with the increase in Al concentrations,

Table 3. Accumulation of macronutrients (mg plant^{-1}) in the dry matter of leaves, stem, roots and plant, and ratio between the accumulation of nutrients in the roots and shoots of 'IAC Fantástico' and 'Vitória' pineapple cultivars, cultivated in nutrient solution

Cultivar	Leaves	Stem	Roots	Plant	Roots/ Shoots
			Nitrogen		
'IAC'	343.85 a	89.35 b	13.16 b	446.37 b	0.03 b
'Vitória'	387.06 a	132.17 a	22.55 a	541.78 a	0.04 a
CV (%)	29.79	20.55	49.67	23.07	37.23
			Phosphorus		
'IAC'	77.65 a	42.85 b	17.48 a	137.97 a	0.16 a
'Vitória'	90.21 a	53.13 a	14.44 a	157.78 a	0.11b
CV (%)	38.74	29.12	49.23	23.04	57.98
			Potassium		
'IAC'	744.41 a	122.26 b	25.51 a	892.18 a	0.03 a
'Vitória'	728.06 a	177.05 a	21.46 b	926.56 a	0.02 b
CV (%)	20.11	33.62	26.15	37.38	20.11
			Calcium		
'IAC'	221.48 a	155.47 b	21.99 b	398.94 b	0.06 a
'Vitória'	224.11 a	247.22 a	26.48 a	497.80 a	0.06 a
CV (%)	31.20	20.82	20.65	18.42	32.10
			Magnesium		
'IAC'	76.25 b	29.02 b	15.75 a	121.03 b	0.17 a
'Vitória'	107.00 a	47.08 a	15.66 a	169.74 a	0.11 b
CV (%)	44.28	19.43	24.65	27.79	49.12

Means followed by the same letter in the column do not differ by F test at 0.05 probability level

which indicates the suppressive effect of Al on the absorption of the macronutrients.

The decrease in the absorption of macronutrients by the cv. 'Vitória' can be associated with the increase of injuries caused by Al in the root system. In addition, N metabolism is highly dependent on the produced energy and plants, when subjected to stress, paralyze the photosynthetic processes, which leads to energy deficit; hence, the plant probably paralyzed N absorption due to the lack of energy.

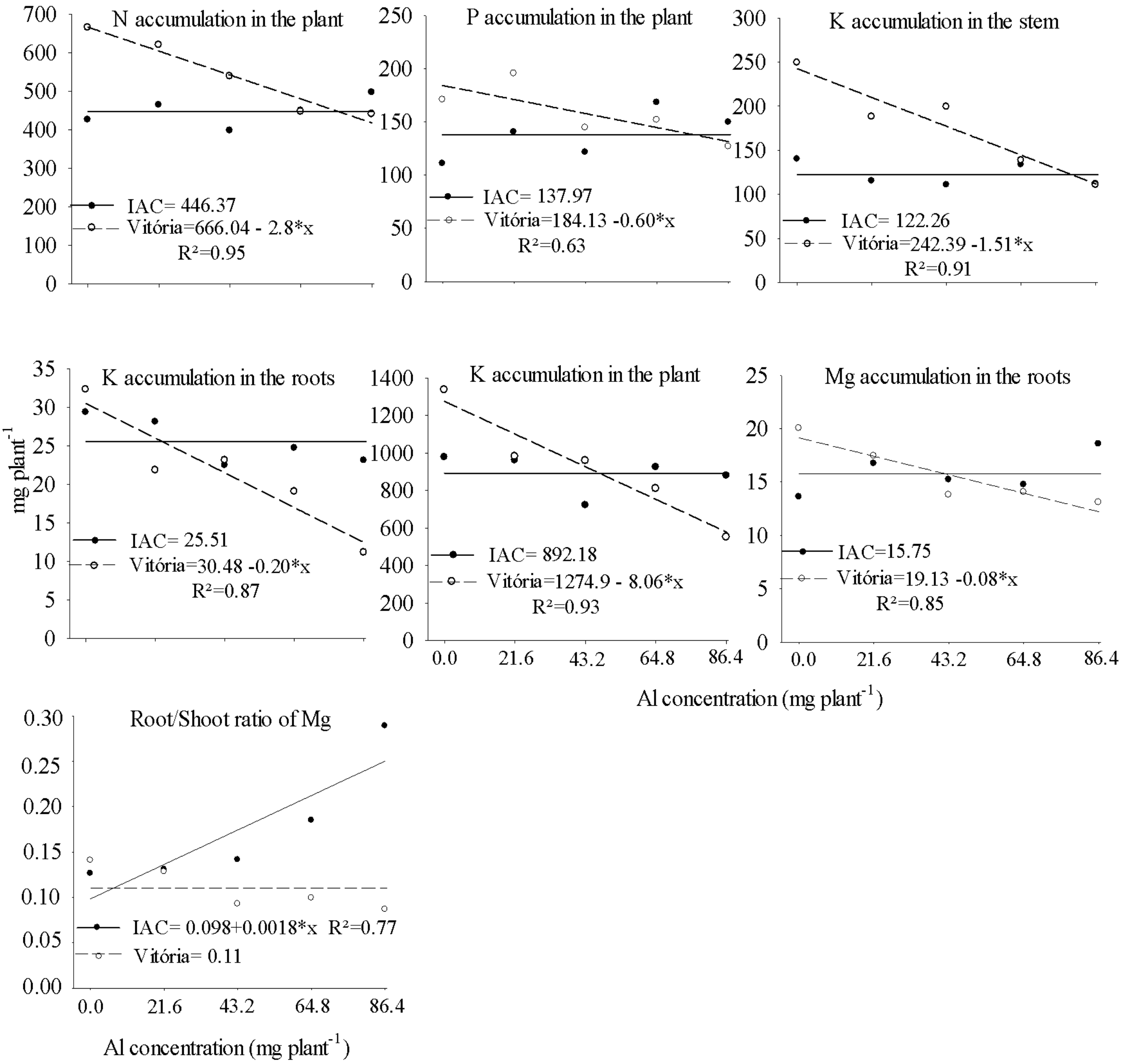
According to Basso et al. (2007), the excess of Al, besides inhibiting the normal root growth, blocks mechanisms of acquisition and transport of nutrients by the plants, in particular, P, an element of extreme importance for plants, found in the DNA and RNA. In the energetic metabolism, in the ATP molecule, P may become less soluble to the plants for binding to Al, forming aluminophosphate. The authors also reported reduction in the absorption and accumulation of P, Ca and Mg in plants sensitive to Al, Freitas et al. (2006) in rice genotypes, Peixoto et al. (2007) in sorghum and Nolla et al. (2007) in the soybean crop.

The inexistence of toxic effect of Al on the cv. 'IAC Fantástico' suggests that it has physiological mechanisms of tolerance, such as: exclusion mechanisms, in which Al is prevented from reaching its toxicity sites in the plant by the formation of organo-mineral complexes or chelates with

Table 2. Leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), total dry matter (TDM), root/shoot dry matter ratio (R/S), number of leaves (NL) and stem diameter (SD)

Cultivar	LDM	SDM	RDM	TDM	R/S	NL	SD
	g plant^{-1}					unit	cm
'IAC Fantástico'	32.34 a	17.06 b	10.08 a	59.48 b	0.21 a	5.90 a	2.71 b
'Vitória'	34.74 a	25.08 a	9.83 a	69.65 a	0.17 b	6.50 a	3.47 a
CV (%)	23.34	16.94	16.81	14.96	16.81	29.27	14.53

Means followed by the same letter in the column do not differ by F test at 0.05 probability level



*0.05 probability level by F test

Figure 3. Accumulation of macronutrients in the tissues (leaves, stems, roots and the entire plant) of 'IAC Fantástico' and 'Vitória' pineapple cultivars after the addition of the aluminum (Al) concentrations in the nutrient solution

organic compounds of low molecular weight, exuded in the region of the rhizosphere or apoplast by the plants; internal or repair mechanism, which allows the penetration of Al in the cell, but its phytotoxic action is neutralized by enzymes or even isolated inside the vacuole, where the complexation of the cations occurs (Van & Masuda, 2004).

Conclusions

1. The cv. 'IAC Fantástico' was not influenced by the increasing concentrations of Al in the nutrient solution.

2. The cv. 'Vitória' showed reduction in the chlorophyll index, root dry matter, root length and accumulation of N, P, K, Ca and Mg in the plant components (leaves, stems and roots) with the increase of Al concentrations in the nutrient solution.

3. The cv. 'Vitória' showed higher total dry matter, stem dry matter, stem diameter and accumulation of N, Ca and Mg in the plant.

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