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Sources and doses of aluminum in experiments with rice in nutrient solution

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ABSTRACT: The aluminum source to produce toxicity in upland rice in nutrient solution experiments is not yet well established, although the aluminum potassium sulfate has been utilized source to produce aluminum toxicity. However, in recent studies have used aluminum chloride. The aim of this study was to evaluate the capacity of aluminum sources and doses to produce toxicity in upland rice plants grown in nutrient solution. The experiment was arranged in a block randomized design, in a 2 x 5 factorial scheme and four repetitions. The treatments were two aluminum sources (aluminum potassium sulfate - $\text{AlK}(\text{SO}_4)_3 \cdot 12\text{H}_2\text{O}$ and aluminum chloride - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) and five aluminum doses in nutrient solution (0, 370, 740, 1100 and 1480 $\mu\text{mol L}^{-1}$). The experiment was conducted in a greenhouse in Botucatu city, São Paulo state, Brazil, starting in April 2012, and was carried out for 56 days from transplanting of the seedlings. Using aluminum chloride, the rice plants show lower production of root and total dry weight, area and root volume, medium and thick root length, potassium and sulfur contents and accumulations. Using aluminum potassium sulfate, there are lower aluminum activity and availability, besides the formation of large amount of aluminum compounds non-toxic to the plants (aluminum sulfate) in the nutrient solution. The aluminum doses between 1100 to 1480 $\mu\text{mol L}^{-1}$, corresponding to aluminum activity of 336.8 to 429.0 $\mu\text{mol L}^{-1}$ of aluminum chloride as source, are more effective to produce aluminum toxicity in upland rice plants grown in nutrient solution.

Key words: *Oryza sativa*, ionic activity, Visual Minterq, root length

Fontes e doses de alumínio para utilização em experimentos com arroz em solução nutritiva

RESUMO: Não é ainda bem definida a fonte de alumínio para gerar toxidez em experimentos de solução nutritiva com plantas de arroz, embora o sulfato de alumínio e potássio tem sido utilizado como fonte para gerar toxidez por alumínio. Entretanto, em publicações mais recentes, tem-se utilizado o cloreto de alumínio. Assim, o objetivo do trabalho foi avaliar a capacidade de fontes e doses de alumínio em causar toxidez em plantas de arroz de terras altas cultivadas em solução nutritiva. O delineamento experimental foi o de blocos casualizados, disposto em esquema fatorial 2 x 5, com quatro repetições. Os tratamentos foram duas fontes de alumínio (sulfato de alumínio e de potássio - $\text{AlK}(\text{SO}_4)_3 \cdot 12\text{H}_2\text{O}$ e cloreto de alumínio - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) e cinco doses de alumínio em solução nutritiva (0, 370, 740, 1100 e 1480 $\mu\text{mol L}^{-1}$). O experimento foi conduzido em casa de vegetação, no município de Botucatu, São Paulo; foi iniciado em abril de 2012 e conduzido por 56 dias. Ao utilizar o cloreto de alumínio, as plantas de arroz apresentaram menor produção de massa seca total e de raiz; área e volume radicular; comprimento de raízes médias e grossas; teor e acúmulo de potássio e sulfato. Utilizando-se sulfato de alumínio e de potássio há menor atividade e disponibilidade de alumínio, além de formação de grande quantidade de compostos de alumínio não tóxico às plantas (sulfato de alumínio) na solução nutritiva. As doses de alumínio entre 1100 a 1480 $\mu\text{mol L}^{-1}$, correspondente à atividade de alumínio de 336,8 a 429,0 $\mu\text{mol L}^{-1}$, utilizando o cloreto de alumínio são mais eficazes em causar toxidez por alumínio em plantas de arroz de terras altas cultivadas em solução nutritiva.

Palavras-chave: *Oryza sativa*, atividade iônica, Visual Minterq, comprimento radicular



INTRODUCTION

Aluminum (Al) is toxic to the plant growth and is a problem in tropical regions (Kochian et al., 2004; Sun et al., 2010; Guo et al., 2012). Therefore, the baseline studies in order to evaluate Al toxicity in plants grown in nutrient solution are important to food security. Nutrient solution experiments have some benefits such as the possibility to be conducted in a shorter period of time and at a lower cost compared to field experiments, and it is possible to study individual factors and control the solution pH, which are essential characteristics to achieve the proposed objectives in experiments involving toxicity caused by Al.

However, it is not well established the best Al source and dose to formulate the nutrient solution, that can be used to perform experiments, such as evaluations of cultivars to Al tolerance, interactions between Al and macro and micronutrients, and many other kinds of evaluation. Thus, Furlani & Furlani (1988) indicate aluminum potassium sulfate ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) as the source to produce Al toxicity in rice, maize and sorghum plants experiments in nutrient solution. In this context, Guo et al. (2007), Lobão et al. (2007), Zhou et al. (2009, 2016), Ringo et al. (2010), Macedo et al. (2011), Okekeogbu et al. (2014), Desta et al. (2017) and Menezes et al. (2018) have used in their studies aluminum potassium sulfate as a source of Al toxicity to the plants.

On the other hand, studies dated before 1988 (Fageria & Zimmermann, 1979) and more recent publications have used aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) (Ma et al., 2002; Guimarães et al., 2006; Sun et al., 2010; Bitencourt, 2011; Garzon et al., 2011; Alvarez et al., 2012; Shen et al., 2014; Freitas et al., 2016, 2017a, b). Zonta (2003) used nutrient solution described by Furlani & Furlani (1988) in their study, but replacing the aluminum potassium sulfate by aluminum chloride. Therefore, there is no consensus about the source and dose of Al to use in nutrient solution experiments; consequently, it is necessary to define the most effective Al sources and doses to produce toxicity in upland rice plants. Thus, the aim of this study was to evaluate the capacity of Al sources and doses to produce toxicity in upland rice plants grown in nutrient solution.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Department of Soil and Environmental Resources of the Universidade Estadual Paulista in Botucatu, São Paulo state, Brazil (22° 51' 07" S; 48° 25' 57" W; altitude of 790 m). The experiment started on April 2012 and was carried out for 59 days, arranged in randomized complete block design, in a 2 x 5 factorial scheme with four repetitions. The treatments were two Al sources (aluminum potassium sulfate - $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and aluminum chloride - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) and five Al doses (0, 370, 740, 1100 and 1480 $\mu\text{mol L}^{-1}$). The upland rice cultivar Maravilha was used (Freitas et al., 2016).

The protocol of nutrient solution used in the experiment was described by Furlani & Furlani (1988) to study the Al tolerance of rice plants. The nutrient solution composition (mmol L^{-1}) used in both Al sources was: 1.42 Ca, 0.85 K, 0.33 Mg,

0.95 N- NO_3^- , 0.41 N- NH_4^+ , 0.01 P, 0.21 S, 0.21 Cl, 0.22 Fe, 0.009 Mn, 0.008 B, 0.00076 Zn and 0.00031 Cu. However, when the source of Al was aluminum chloride, the nutrient solution had the K concentration changed to 1.51 mmol L^{-1} , following Zonta (2003) recommendation. The original study indicated an Al concentration of 740 $\mu\text{mol L}^{-1}$ to be used in rice experiments.

The rice seeds were treated with carboxin + thiram (400 mL for 100 kg of seeds). In the sequence, the seeds were placed in a filter paper and the germination was conducted in a germination chamber at $\pm 25^\circ\text{C}$ and 8 h of light. After 72 h, initial radicle emergence was observed; then, the filter papers were placed vertically inside a pot filled with 1/5 diluted nutrient solution without Al. The pots were transferred to the countertops at the greenhouse with temperature between 22 to 27 $^\circ\text{C}$ with controlled humidity.

The filter papers were kept for seven days. The variables for seedling choice were related to the shape uniformity and size. The seedlings were transferred to the 4 L plastic pots filled of half strength nutrient solution. The plastic pot covers were made of Styrofoam plates. There were six holes distributed over the plates to fix one plant per hole (fixed by foam pieces), allowing roots to touch the nutrient solution. After seven days, the half strength nutrient solution was replaced by a full strength nutrient solution, in which the seedlings were grown for more seven days. Then, the nutrient solution was changed, Al treatments were added in the nutrient solution and seedlings were grown in this condition for 35 days, and were evaluated after this period.

Throughout the whole period of the experiment the nutrient solution was aerated and the pH monitored daily, in order to keep its value equal to 4.0 (± 0.1) and NaOH (0.1 mol L^{-1}) and HCl (0.1 mol L^{-1}) solutions were used to correct the pH. The nutrient solution was replaced weekly by adding the treatments, and the evapotranspiration loss from the plants was replaced daily using demineralized water.

The following variables were evaluated: activity, availability and forms of Al estimated by the software Visual Minteq 3.0 (Gustafsson, 2012); total root length, root diameter and root length in each diameter classification (fine - diameter < 0.5 mm; medium - diameter between 0.5 to 1.00 mm; thick - diameter > 1.00 mm) (WinRhizo); plant height and number of tillers per plant; root, shoot and total plant dry weight production; root and shoot potassium (K) and sulfur (S) concentration and accumulation.

The data were subjected to analyses of variance (ANOVA) using F test. The Al source averages were compared by the t test (LSD) at $p \leq 0.05$, while the Al doses effects were evaluated by the regression models chosen based on the regression coefficient significance, using the t test at $p < 0.05$ and determination coefficient.

RESULTS AND DISCUSSION

According to Visual Minteq 3.0 results, the Al concentrations applied, 0, 370, 740, 1110 and 1480 $\mu\text{mol L}^{-1}$, correspond to the Al activity in the solution of 0, 85.1, 144.1, 191.2, 229.7 $\mu\text{mol L}^{-1}$ (aluminum potassium sulfate), and 0, 117.6, 233.0, 336.8,

429.0 $\mu\text{mol L}^{-1}$ (aluminum chloride), respectively (Table 1). About the Al availability regarding the Al concentration, it was equal to 0, 50.7, 45.5, 42.3, 39.8% (aluminum potassium sulfate); 0, 72.4, 78.4, 81.9, 84.2% (aluminum chloride), respectively (Table 1). Thus, analyzing all Al doses and sources studied (Tables 1 and 2), the aluminum chloride showed higher Al activity and availability (toxicity form to the plants) compared to the aluminum potassium sulfate; therefore, it is able to produce more Al toxicity to the plants.

The Al form may change in the solution and turn into complex compound with other ions (non-toxic to the plants), that depends on several factors, such as nutrient solution composition and pH (Sposito, 1996). However, it is important to highlight that only the Al^{3+} form is toxic to plant growth (Sposito, 1996). According to estimations generated by the software Visual Minteq 3.0 applying aluminum potassium sulfate in the solution, the aluminum can be in these forms:

Table 1. Aluminum concentration, activity and availability in nutrient solution using aluminum potassium sulfate ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) and aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), estimated by software Visual Minteq 3.0

Al concentration ($\mu\text{mol L}^{-1}$)	Al activity in solution	Al availability in solution (%)
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ (%)		
0	0	0
370	85.1	50.7
740	144.1	45.5
1110	191.2	42.3
1480	229.7	39.8
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$		
0	0	0
370	117.6	72.4
740	233.0	78.4
1110	336.8	81.9
1480	429.0	84.2

pH of nutrient solution 4.0

Table 2. Aluminum forms in the nutrient solution at different concentrations using aluminum potassium sulfate ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) and aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), estimated by software Visual Minteq 3.0

Forms (Al)	370	740	1110	1480
	($\mu\text{mol L}^{-1}$)			
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ (%)				
Al^{+3}	50.7	45.5	42.3	39.8
AlOH^{+2}	3.2	2.8	2.5	2.3
$\text{Al}(\text{OH})_2^{+}$	0.1	0.1	0.09	0.08
$\text{Al}_2(\text{OH})_2^{+4}$	0.03	0.05	0.06	0.07
AlSO_4^{+}	42.4	48.8	52.4	55.1
$\text{Al}(\text{SO}_4)_2^{-}$	0.5	0.8	1.1	1.4
AlHPO_4^{+}	2.1	1.1	0.7	0.5
$\text{Al}_2\text{PO}_4^{+3}$	0.6	0.6	0.5	0.5
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$				
Al^{+3}	72.4	78.4	81.9	84.2
AlOH^{+2}	4.6	4.7	4.7	4.6
$\text{Al}(\text{OH})_2^{+}$	0.1	0.1	0.1	0.1
$\text{Al}_2(\text{OH})_2^{+4}$	0.06	0.1	0.2	0.3
AlCl^{+2}	0.02	0.04	0.06	0.07
AlSO_4^{+}	19.3	14.3	11.2	9.2
$\text{Al}(\text{SO}_4)_2^{-}$	0.08	0.04	0.03	0.02
AlHPO_4^{+}	2.2	1.0	0.6	0.4
$\text{Al}_2\text{PO}_4^{+3}$	1.0	1.0	0.9	0.8
$\text{Al}_3(\text{OH})_4^{+5}$	-*	-*	0.02	0.04

pH of nutrient solution 4.0; * At the Al concentrations of 370 and 740 $\mu\text{mol L}^{-1}$ there is no presence of $\text{Al}_3(\text{OH})_4^{+5}$

Al^{+3} , AlOH^{+2} , $\text{Al}(\text{OH})_2^{+}$, $\text{Al}_2(\text{OH})_2^{+4}$, AlSO_4^{+} , $\text{Al}(\text{SO}_4)_2^{-}$, AlHPO_4^{+} , $\text{Al}_2\text{PO}_4^{+3}$. Regarding AlSO_4^{+} (non-toxic forms to plants), this Al form represents 42.4% of Al available in solution (dose 370 $\mu\text{mol L}^{-1}$ of Al) and 55% (1480 $\mu\text{mol L}^{-1}$ of Al). On the other hand, when using aluminum chloride, the Al appears in these forms: Al^{+3} , AlOH^{+2} , $\text{Al}(\text{OH})_2^{+}$, $\text{Al}_2(\text{OH})_2^{+4}$, AlCl^{+2} , AlSO_4^{+} , $\text{Al}(\text{SO}_4)_2^{-}$, AlHPO_4^{+} , $\text{Al}_2\text{PO}_4^{+3}$, $\text{Al}_3(\text{OH})_4^{+5}$ (Table 2).

The complexed compounds formed with Al in the solution when aluminum potassium sulfate is used (Table 2), especially AlSO_4^{+} , may contribute to explaining why Al availability (%) is lower at the higher Al doses (Table 1). This occurs because higher Al doses of aluminum potassium sulfate also supply S to the solution; thus, more sulfate bonds with Al will occur, which decreases Al availability and Al^{+3} form.

The ionic activity obtained in the Visual Minteq evidence the higher ability of the aluminum chloride to produce Al toxicity to the plants, especially due to the fact that it leads to the highest Al activity and availability in the solution (Table 1), besides the higher amount of Al^{+3} (Al toxic form) (Table 2). The use of aluminum potassium sulfate in nutrient solution experiment may not be interesting due to the low Al activity and availability (toxicity form) and formation of a higher amount of non-toxic compounds to the plants (AlSO_4^{+}).

Using aluminum chloride caused lower root weight at the Al dose of 370 $\mu\text{mol L}^{-1}$ (Table 3) and root length (Table 4) at the Al dose of 1100 $\mu\text{mol L}^{-1}$, shoot and total plant dry weights at the Al dose of 1480 $\mu\text{mol L}^{-1}$ (Table 3), compared to the use of aluminum potassium sulfate. Although there is no statistical difference between the Al sources at all doses, except shoot and root dry weight and total plant dry weight, the absolute value of aluminum chloride is higher at all Al doses than that of aluminum potassium sulfate.

Lower root length was expected using the aluminum chloride source, due to the lower root dry weight caused by this source, especially at the Al dose of 370 $\mu\text{mol L}^{-1}$ (Table 3), but it was not observed. There was difference between Al sources when measuring root diameter at Al dose of 1480 $\mu\text{mol L}^{-1}$ (Table 4).

There was no difference between Al sources for plant height and number of tillers (Table 3). However, root variables are more important to define the intensity level of Al toxicity, because the primary target of the toxicity is the root (Kochian et al., 2004).

To obtain better understanding of the effect of Al in the roots, root length was evaluated in three different root diameter classes (Table 4). The root lengths of fine at 1100 $\mu\text{mol L}^{-1}$, medium at 740 $\mu\text{mol L}^{-1}$ and thick at 370 $\mu\text{mol L}^{-1}$, were lower when aluminum chloride was used compared to aluminum potassium sulfate. This demonstrated that the aluminum chloride effect was more severe.

Increasing Al doses caused a reduction in root and total plant dry weights, plant height and number of tillers per plant at both Al sources used (Table 3). About shoot dry weight production, there was an increase up to the Al dose of 460 $\mu\text{mol L}^{-1}$; however, from that point there was a decrease, with more toxicity at Al dose of 1480 $\mu\text{mol L}^{-1}$ (Table 3). This effect occurs only for the aluminum chloride source.

There was decrease of total, fine, medium and thick root length when the Al doses were raised in both Al sources (Table 4). This occurs due to the root growth inhibition caused by Al

Table 3. Plant height, number of tillers per plant, dry weight production of shoot, root and total plant of upland rice as affected by aluminum doses and sources (aluminum potassium sulfate - $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and aluminum chloride - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$)

Source	Doses ($\mu\text{mol L}^{-1}$)					Regression model of Al doses	CV (%)
	0	370	740	1100	1480		
Plant height (cm)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	55.0 a	54.5 a	51.6 a	52.3 a	48.8 a	$\hat{y} = 55.419 - 0.0039^{***}x$ ($R^2 = 0.86$)	3.6
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	55.0 a	52.6 a	52.9 a	50.1 a	50.5 a	$\hat{y} = 54.581 - 0.0031^{***}x$ ($R^2 = 0.83$)	
Number of tillers per plant							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	3.0 a	2.9 a	2.9 a	2.8 a	2.7 a	$\hat{y} = 3.066 - 0.00019^{***}x$ ($R^2 = 0.93$)	3.6
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	3.0 a	3.0 a	2.9 a	2.9 a	2.7 a	$\hat{y} = 3.085 - 0.00020^{***}x$ ($R^2 = 0.85$)	
Shoot dry weight (g pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	7.6 a	7.6 a	7.8 a	7.5 a	7.5 a	Ns	4.5
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	7.6 a	7.9 a	7.6 a	7.6 a	6.8 b	$\hat{y} = 7.96 + 0.00092x - 0.000001^{***}x^2$ ($R^2 = 0.92$)	
Root dry weight (g pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	4.0 a	3.7 a	3.2 a	2.7 a	2.2 a	$\hat{y} = 4.146 - 0.0012^{***}x$ ($R^2 = 0.99$)	12.8
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	4.0 a	2.9 b	3.0 a	2.4 a	1.7 a	$\hat{y} = 3.845 - 0.0013^{***}x$ ($R^2 = 0.90$)	
Total plant dry weight (g pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	11.7 a	11.3 a	11.1 a	10.2 a	9.8 a	$\hat{y} = 11.854 - 0.0013^{***}x$ ($R^2 = 0.96$)	4.0
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	11.7 a	10.9 a	10.7 a	10.0 a	8.6 b	$\hat{y} = 11.811 - 0.0019^{***}x$ ($R^2 = 0.92$)	

Values followed by the same letters in the columns are not significantly different at $p \leq 0.05$ according to the LSD test. ** Significant at $p \leq 0.01$, by F test

Table 4. Total root length, root length in the each diameter classification and root diameter of upland rice plants as affected by aluminum doses and sources (aluminum potassium sulfate - $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and aluminum chloride - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$)

Source	Doses ($\mu\text{mol L}^{-1}$)					Regression model of Al doses	CV (%)
	0	370	740	1100	1480		
Total root length (m pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	837.3 a	571.0 a	495.0 a	315.2 a	155.5 a	$\hat{y} = 798.699 - 0.438^{***}x$ ($R^2 = 0.97$)	16.2
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	821.6 a	608.8 a	408.9 a	181.0 b	163.1 a	$\hat{y} = 785.213 - 0.472^{***}x$ ($R^2 = 0.95$)	
Fine root length (m pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	731.6 a	481.2 a	421.1 a	265.2 a	118.3 a	$\hat{y} = 692.042 - 0.390^{***}x$ ($R^2 = 0.97$)	18.2
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	721.8 a	539.1 a	353.0 a	142.2 b	135.9 a	$\hat{y} = 691.768 - 0.424^{***}x$ ($R^2 = 0.94$)	
Medium root length (m pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	82.4 a	73.1 a	63.9 a	39.7 a	31.4 a	$\hat{y} = 85.242 - 0.036^{***}x$ ($R^2 = 0.96$)	20.5
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	80.8 a	62.5 a	45.3 b	30.0 a	22.5 a	$\hat{y} = 78.086 - 0.040^{***}x$ ($R^2 = 0.98$)	
Thick root length (m pot ⁻¹)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	23.1 a	16.6 a	9.9 a	10.2 a	8.2 a	$\hat{y} = 20.914 - 0.0098^{***}x$ ($R^2 = 0.85$)	32.1
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	18.9 a	7.0 b	10.4 a	8.7 a	4.6 a	$\hat{y} = 15.348 - 0.0072^{***}x$ ($R^2 = 0.61$)	
Root diameter (mm)							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	0.30 a	0.32 a	0.31 a	0.34 a	0.42 a	$\hat{y} = 0.285 + 0.00007^{***}x$ ($R^2 = 0.75$)	12.0
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	0.29 a	0.28 a	0.30 a	0.40 a	0.34 b	$\hat{y} = 0.282 + 0.00005^{***}x$ ($R^2 = 0.47$)	

Values followed by the same letters in the columns are not significantly different at $p \leq 0.05$ according to the LSD test. ** Significant at $p \leq 0.01$, by F test

(Kochian et al., 2004). It is important to highlight that aluminum chloride source had a bigger effect on the total and fine root length at the Al dose of 1100 $\mu\text{mol L}^{-1}$, compared to aluminum potassium sulfate (significant difference between Al sources).

Despite the decrease of fine, medium and thick root lengths, the length of fine roots showed greater trend towards reduction. This fact occurs because the difference between the Al doses of 0 and 1480 $\mu\text{mol L}^{-1}$ (Al sources average) was 600 m in fine root, while in medium roots the decrease was 54.6 m and in thick roots 14.6 m, representing reductions of 82.5, 67 and 69.5%, respectively. This indicated that the Al may interfere in a more intense way with the growth of fine roots, which are the most responsible for water and nutrient uptake (Wang et al., 2006; Zonta et al., 2006). Lower root length may have led to a lower root dry weight production (Table 3).

There was increase in root diameter with the increase of Al doses, for both Al sources (Table 4). This occurred because the roots that were under Al toxicity became stunted due to the damage caused to the root meristem and the increase of the cell wall rigidity and thickness (Vitarello et al., 2005).

Potassium and sulfur concentration and accumulation in the shoot and root (Tables 5 and 6) were higher when

the aluminum potassium sulfate was used compared to aluminum chloride. The better plant nutrition with K and S at the increasing doses of aluminum potassium sulfate may explain it, due to the formulation containing these nutrients ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$).

The higher concentration of K and S in plants grown under toxicity caused by aluminum potassium sulfate may explain the higher shoot, root and total plant dry weights and total root length obtained by this treatment in comparison to the aluminum chloride (Table 3). If the nutrient solution is not balanced, these characteristics become undesirable for an Al toxicity experiment, since it will supply plants with nutrients (K and S) instead of damaging them (Al toxicity). Therefore, aluminum chloride is more effective to produce Al toxicity in upland rice plants, because it does not increase K and S concentrations in the plant, as occurs with aluminum potassium sulfate (Tables 5 and 6); higher Al activity and availability (Tables 1 and 2); and lower values of the growth variables (Tables 3 and 4).

Therefore, the suggestion is to use the nutrient solution described by Furlani & Furlani (1988), adapted by Zonta (2003), with aluminum chloride as the Al source in order

Table 5. Potassium concentration and accumulation in the shoot and root of upland rice plants as affected by aluminum doses and sources (aluminum potassium sulfate - $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and aluminum chloride - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$)

Source	Doses ($\mu\text{mol L}^{-1}$)					Regression model of Al doses	CV (%)
	0	370	740	1100	1480		
Shoot concentration (g kg^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	19.3 a	22.4 a	21.0 a	21.3 a	20.2 a	$\hat{y} = 19.76 + 0.0053x - 0.00000082x^2$ ($R^2 = 0.58$)	4.2
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	18.8 a	19.3 b	20.4 a	19.1 b	19.2 a	ns	
Shoot accumulation (mg pot^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	148.1 a	170.8 a	165.0 a	160.1 a	153.0 a	$\hat{y} = 151.25 + 0.044x - 0.000030x^2$ ($R^2 = 0.74$)	4.5
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	143.8 a	154.1 b	156.3 a	145.1 b	131.4 b	$\hat{y} = 144.25 + 0.037x - 0.000032x^2$ ($R^2 = 0.98$)	
Root concentration (g kg^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	19.2 a	22.7 a	21.5 a	20.5 a	20.1 a	$\hat{y} = 19.85 + 0.0056x - 0.00000x^2$ ($R^2 = 0.58$)	10.8
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	20.8 a	19.4 b	15.1 b	14.8 b	14.9 b	$\hat{y} = 20.346 - 0.0044x$ ($R^2 = 0.81$)	
Root accumulation (mg pot^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	78.4 a	84.8 a	70.7 a	56.6 a	44.9 a	$\hat{y} = 86.153 - 0.0257x$ ($R^2 = 0.85$)	14.5
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	84.0 a	55.3 b	46.4 b	35.9 b	26.6 b	$\hat{y} = 76.509 - 0.0363x$ ($R^2 = 0.92$)	

Values followed by the same letters in the columns are not significantly different at $p \leq 0.05$ according to the LSD test. ** and * Significant at $p \leq 0.01$ and $p \leq 0.05$, by F test, respectively

Table 6. Sulfur concentration and accumulation in the shoot and root of upland rice plants as affected by aluminum doses and sources (aluminum potassium sulfate - $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ and aluminum chloride - $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$)

Source	Doses ($\mu\text{mol L}^{-1}$)					Regression model of Al doses	CV (%)
	0	370	740	1100	1480		
Shoot concentration (g kg^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	3.0 b	3.5 a	3.7 a	4.0 a	3.7 a	$\hat{y} = 3.06 + 0.0015x - 0.000001x^2$ ($R^2 = 0.94$)	3.3
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	3.3 a	2.6 b	2.5 b	2.5 b	2.7 b	$\hat{y} = 3.26 - 0.0016x + 0.000001x^2$ ($R^2 = 0.92$)	
Shoot accumulation (mg pot^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	23.5 a	27.2 a	29.0 a	30.2 a	28.6 a	$\hat{y} = 23.50 + 0.012x - 0.000006x^2$ ($R^2 = 0.99$)	5.7
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	25.4 a	21.0 b	19.8 b	19.2 b	18.6 b	$\hat{y} = 25.07 - 0.010x + 0.000004x^2$ ($R^2 = 0.96$)	
Root concentration (g kg^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	1.8 b	2.4 a	2.9 a	2.8 a	3.2 a	$\hat{y} = 2.065 + 0.00083x$ ($R^2 = 0.86$)	9.1
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	2.2 a	2.0 b	2.0 b	2.2 b	2.0 b	ns	
Root accumulation (mg pot^{-1})							
$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	7.6 a	9.2 a	9.7 a	7.9 a	7.1 a	$\hat{y} = 7.805 + 0.0047x - 0.000004x^2$ ($R^2 = 0.81$)	17.9
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	9.2 a	5.8 b	6.3 b	5.5 b	3.6 b	$\hat{y} = 8.442 - 0.0031x$ ($R^2 = 0.81$)	

Values followed by the same letters in the columns are not significantly different at $p \leq 0.05$ according to the LSD test. ** Significant at $p \leq 0.01$, by F test

to produce toxicity in the rice plants. The suggestion is not only for rice experiments, but also for maize and sorghum Al experiments, as described by Furlani & Furlani (1988).

There was an initial increase of K concentration and accumulation in the shoot with afterwards decrease of K concentration when the Al doses increased using aluminum potassium sulfate as the Al source (Table 5). However, the decrease may not be considered detrimental, because the K concentration and accumulation at the Al dose of $1480 \mu\text{mol L}^{-1}$ was practically the same as the one obtained at the Al dose 0.

The K concentration in the shoot was not affected when the aluminum chloride was used (Table 5). On the other hand, there was a slight increase in the K accumulation in the shoot when the aluminum chloride was used up to the Al dose of $578 \mu\text{mol L}^{-1}$. However, when using higher doses, there was decrease in the K accumulation (Table 5).

Despite the initial increase of K accumulation in the shoot and then its decrease, in the root the K concentration and accumulation were reduced with the increase in Al doses (Table 5). Therefore, it may occur due to a possibility of the rice plants being more effective to transport K to the plant shoots up to an Al concentration in the solution (both Al sources), but when the Al doses were raised this behavior was not observed, probably due to the toxicity effects of Al at higher doses.

In the root it was evident the detrimental effect of aluminum chloride when the dose of Al was increased (Table 5). When this Al source was used there was a decrease of K concentration

and accumulation. The K concentration and accumulation decrease was more evident with aluminum chloride, while the roots were benefiting from aluminum potassium sulfate, due to the K concentration used in the formulation of this reagent. It may confirm one more time the more detrimental effect of aluminum chloride compared to aluminum potassium sulfate.

When the Al doses were increased using aluminum potassium sulfate there was an increase of S concentration and accumulation in the shoot and root up to $1100 \mu\text{mol L}^{-1}$ (Table 6). As occurs for K, there is S in the aluminum potassium sulfate formulation, so when the doses were increased, more S availability was expected in the system and higher concentration and accumulation in the plant. In addition, there is a strong tendency of Al to establish bonds with S (Cameron et al., 1986), more precisely the sulfate, so besides providing S to the plant, there is the possibility of formation of AlSO_4^+ (non-toxic to the plant), especially at the higher Al doses of aluminum potassium sulfate (Table 2).

Using the aluminum chloride, it is possible to see that there was a decrease of S concentration and accumulation in the shoot and of S accumulation in the root (Table 6), demonstrating one more favorable feature in the use of aluminum chloride, as there is no extra S supply.

It is evident for K and S concentration and accumulation results that, if nutrient solution balancing is not done properly, the aluminum potassium sulfate may supply extra K and S to the plants. This may negatively interfere in experiments with

Al doses, since with the increase in Al dose, the concentrations of K and S in the nutrient solution had changed, so it may have an influence on the results. However, it is worth mentioning that it would not be interesting to perform the balancing of the nutrient solution using aluminum potassium sulfate, because this procedure is not convenient and may cause changes in the ionic speciation of the nutrient solution, due to the supply of K and S from different sources of the original solution. Moreover, the solution composition proposed by Furlani & Furlani (1988), was developed specifically to study the Al tolerance of rice plants.

Given the results, especially of root growth (Table 4), it was found that Al doses between 1100 to 1480 $\mu\text{mol L}^{-1}$ of aluminum chloride were able to produce a substantial toxicity in the Al-susceptible upland rice cultivar Maravilha. These Al doses in the source selected (aluminum chloride) are equivalent to the Al activities of 336.8 and 429.0 $\mu\text{mol L}^{-1}$, respectively (Table 1).

It should be emphasized that the Al dose and source recommended by Furlani & Furlani (1988) is 740 $\mu\text{mol L}^{-1}$ and aluminum potassium sulfate, respectively. This dose and this source match the Al activity of 144.1 $\mu\text{mol L}^{-1}$. However, for possible upland rice cultivars tolerant to Al this dose and activity of this Al source may not be detrimental to plant growth, whereas the Al dose of 740 $\mu\text{mol L}^{-1}$ was used in this study the roots of cultivar Maravilha were able to obtain a reasonable growth.

CONCLUSIONS

1. The aluminum doses between 1100 and 1480 $\mu\text{mol L}^{-1}$ (Al activity of 336.8 to 429.0 $\mu\text{mol L}^{-1}$) of the aluminum chloride are more effective to produce aluminum toxicity in the upland rice plants grown in nutrient solution.

2. Using aluminum potassium sulfate leads to lower activity and availability of aluminum, also a large amount of non-toxic compounds to the plant in the nutrient solution.

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