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Selection of genotypes of *Jatropha curcas* L. for aluminium tolerance using the solution-paper method

Selección de genotipos Jatropha curcas *L. tolerantes al aluminio mediante el método papel-solución*

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ABSTRACT

The study objective was to determinate the concentration of aluminum that allow to distinguish genotypes of *Jatropha* for tolerance to this element, using the solution-paper method. For this study, seeds of four genotypes of *Jatropha*, were germinated at different Al concentrations (0, 15, 40, 80 and 120 mg L^{-1}). After 14 days, the root length was measured for all treatments. It was observed that there are different tolerance levels for the genotypes. The root length decreased exponentially with the increase of the Al concentration. The solution-paper method allows the selection of genotypes of *Jatropha* in relation to aluminum tolerance and the concentration of 40 mg L^{-1} of Al is recommended to discriminate the tolerance of genotypes to this element.

Key words: *Jatropha curcas*, mineral nutrition, toxicity, genetic variability.

RESUMEN

El objetivo de la investigación fue evaluar el efecto de la concentración del aluminio en solución que mejor diferencia la tolerancia al aluminio en genotipos de Jatropha*, utilizando el método papel-solución. Semillas de cuatro genotipos diferentes de* Jatropha *(CNPAE-248, CNPAE-301,CNPAE-308 y CNPAE-144) fueron germinadas en diferentes concentraciones de Al (0, 15, 40, 80 y 120 mg L^{-1}) usando la técnica de papel-solución. Después de 14 días se midió el tamaño de la raíz primaria en todos los tratamientos. Los resultados aquí presentados muestran que hay un comportamiento diferente de los genotipos para la tolerancia donde el tamaño de la raíz primaria de estos disminuye exponencialmente en relación con el aumento de los niveles de Al. El método de papel-solución permite la selección de los genotipos de* Jatropha *en relación con la tolerancia al aluminio; recomienda utilizar la concentración de 40 mg L^{-1} de Al para discriminar diferencias en la tolerancia a este elemento.*

Palabras clave: Jatropha curcas*, nutrición mineral, toxicidad, variabilidad genética.*

Introduction

The cultivation of *Jatropha* has high potential for oil production due to the high tolerance in adverse conditions of climate and soil, making this a good option for cultivation in savannah/cerrado conditions and in semi-arid regions (Saturnino *et al.*, 2005; Reichel *et al.*, 2013).

Despite being considered a rustic plant, *Jatropha* requires some environmental conditions for its cultivation (Amaral *et al.*, 2013). This creates the need for studies that can provide information about the crop development in areas with adverse soil characteristics, such as soils with high acidity and high content of aluminum (Silva *et al.*, 2007; Martins *et al.*, 2012).

Being abundant in tropical soils, the aluminum (Al) is one of the major obstacles to the cultivation on these regions, especially in soils with high clay content (Martins *et al.*, 1999). The absorption and accumulation of Al affect cells and organelles in physiological, morphologic and cytogenetic level, reducing nutrient absorption, growth and development of roots and shoots, significantly impairing the establishment of the crop and its yield (Braccini *et al.* 2000). This condition can be overcome with

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the correction of the soil, together with the use of species or cultivars that are tolerant to Al.

Numerous studies have reported genotypes of the same species exhibiting differential behavior in soils with high Al content, which is important for breeding programs, in the selection of tolerant genotypes (Martins *et al.*, 1999; Braccini *et al.*, 2000; Custodio *et al.*, 2002; Crestani *et al.*, 2009; Macedo & Lopes, 2008; Rosado *et al.*, 2012). The importance of selecting genotypes tolerant to Al is even greater when you want to cultivate on soils of savanna/cerrado, where the costs for the neutralization of Al toxicity can be high and is not always efficient to make a correction in the subsurface soil layers.

Studies for the selection of plants tolerant to Al are complex due to the need for strict pH control to maintain a constant concentration of Al during the test. This created the need for indirect techniques of detection that allow measuring different characters of the seedlings in the laboratory, distinguishing phenotypically which genotypes are sensitive and which are tolerant to Al (Braccini *et al.*, 2000).

A widely cited technique in the literature for detection of genotypes tolerant to Al is the method of solution- paper, based on measurements of root length (Konzak *et al.*, 1976; Braccini *et al.*, 2000; Macedo & Lopes, 2008; Rosado *et al.*, 2012). Initially Konzak *et al.* (1976) successfully used this method in plants of wheat, barley, rice, sorghum, corn and soybeans. They described its advantages as the easy pH control, simplicity, speed and cost.

The breeding programs of *Jatropha* in Brazil are at the beginning, and accord to Laviola *et al.* (2010), there is high genetic variability among the genotypes, thus becoming highly relevant to standardize a method that permits the selection of a large number of genotypes in a short period, saving resources.

The objectives of this study were: 1) To evaluate the tolerance of genotypes of *Jatropha* to Al. 2) Evaluate a fast procedure to distinguish genotypes of *Jatropha* for tolerance to Al. 3) Determine the concentration of this element in the solution that allows better discrimination of the tolerance between genotypes.

Methodology

The experiment was conducted at the Laboratory of Seed Analysis of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), located in Alegre municipality, Espírito Santo State, Brazil.

The experiment was designed in completely randomized design, with four replications of 25 seeds each. The treatments were arranged in a factorial scheme 4 x 5, composed of four genotypes of *Jatropha* (CNPAE-248, CNPAE-301, CNPAE-308 and CNPAE-144) and five concentrations of aluminum in solution (0, 15, 40, 80 and 120 mg L^{-1} of Al as $Al_2(SO_4)_3.16H_2O$).

The chosen genotypes (CNPAE-248, CNPAE-301, CNPAE-308 e CNPAE-144) are from different regions of the country and were supplied by the Brazilian research institute "Embrapa Agroenergia". The seeds were characterized by germination, moisture content, weight of 1000 seeds, width and length (Table 1) according to the methodology proposed by Brazil (2009).

The Al concentrations (0, 15, 40, 80 and 120 mg L^{-1}) were chosen based on preliminary tests and results reported on the literature (Rosado *et al.*, 2012). In addition to the various Al concentrations, the nutrient solution was composed by 0.1 mmol of $MgSO_4$ L^{-1}, 0.1 mmol KNO_3 L^{-1}, 0.15 mmol NH_4NO_3 L^{-1} and 8.0 mmol $KHC_8H_4O_8$ L^{-1}

Table 1. Characterization of seeds of *Jatropha* genotypes

Genotype	Origin	Yield[1] (g plant^{-1})	GER[2]	MOI[3] (%)	W1000[4] (g)	WID[5]	L1[6] (mm)	L2[7] (mm)
CNPAE-248	Minas Gerais	885	96.0	5.89	639.78	17.07	10.48	8.36
CNPAE-301	Rio G. do Sul	1200	96.0	5.92	628.62	16.84	10.33	8.25
CNPAE-308	Paraíba	400	96.0	5.97	620.41	16.95	10.40	8.28
CNPAE-144	Maranhão	550	92.0	6.05	656.26	17.20	10.89	8.23

[1] Grain production in the 3th year (g plant^{-1}), data supplied by Embrapa Agroenergia for the year 2012 in Planaltina, Brasília, Brazil; [2]Germination; [3]Moisture; [4]Weight of 1000 seeds; [5]Width; [6]Greater length; [7]Shorter length.

(potassium biphtalate to maintain the pH around 4.0). The nutrient solution was replaced weekly. Due to the short time of the essay and considering the existence of reserves in seeds, phosphorus was omitted from the solution to avoid precipitation of Al.

The osmotic potential of the nutrient solution was kept constant and equal to the treatment with 120 mg L^{-1} of Al, using PEG 6000. The concentrations of PEG were determined by the equation: Ψos - (1,18 x 10^{-2}).C - (1,18 x 10^{-4}).C^2 + (2,67 x 10^{-4}).CT + (8,39 x 10^{-7}).C^2T (Michel & Kaufmann, 1973). Resulting in: 33.5 g L^{-1}, 15.8 g L^{-1}, 11.2 g L^{-1}, 5.8 g L^{-1} and 0.0 g L^{-1} of PEG 6000, respectively, for the treatments of 0, 15, 40, 80 and 120 mg L^{-1} of Al.

For the entire study, the method of paper rolls for germination was used (Konzak *et al.*, 1976), arranging the rolls vertically within plastic containers, filled with 250 mL of solutions prepared with different concentrations of aluminum, maintained in growth chamber with alternating temperature of 20-30 ºC.

After seven days, five seedlings were carefully selected, having rootlets length of 1 centimeter long, those seedlings were replaced in the respective solutions. Seven days after the standardization selection, the root length was measured in all treatments using a digital caliper. The root length is the character that has greater efficiency for differentiating genetic constitutions for sensitivity and tolerance to aluminum (Konzak *et al.*, 1976; Crestani *et al.*, 2009).

The data were subjected to analysis of variance ($p \leq 0.05$), using the software SISVAR (Ferreira, 2008) and, when the variation source shown statistical significance, the Tukey test ($p \leq 0.05$) were used to study the qualitative factors and regression analysis to quantitative factors. Regression models were chosen based on the significance of the coefficients, using the Student t test ($p \leq 0.05$), and also considering the coefficient of determination (R^2).

Results and Discussion

Significant interaction was found ($p \leq 0.05$) among the genotypes of *Jatropha* and aluminum concentrations, therefore, the study of the factors within the levels of each other was performed.

The study of the influence of each concentration of Al over the root growth is presented on Table 2. In lower concentrations of aluminum (0 and 15 mg L^{-1}), the genotype CNPAE-308 had better root development, followed by CNPAE-301 and CNPAE-144. The genotype CNPAE-248 showed small root development when compared to the other genotypes.

At concentrations up to 15 mg L^{-1}, the Al caused reduction in the root growth, but this effect was similar in all genotypes. Therefore the discrimination between genotypes occurred due to the genetic difference between their growth rate, not being efficient to identify possible levels of tolerance to Al.

At the concentration of 40 mg L^{-1} of Al, the genotype CNPAE-301 averaged higher root length and in opposite CNPAE-144 showed reduced root system development. The genotypes CNPAE-308 and CNPAE-248 had intermediate means of root length. At the concentrations over 80 mg L^{-1}, the Al greatly limited the development of all genotypes, not allowing the seedlings to express differences in the root growth.

Overall, the root system of *Jatropha* showed different tolerance levels among the genotypes, indicating the existence of genetic control for Al tolerance. This fact can be explained by the high genetic variability existing between genotypes of *Jatropha*. The genetic characteristics that govern the Al tolerance are not yet fixed in the germplasm bank, due to the early stadium that the breeding program for this culture is actually in Brazil. This

Table 2. Means of root length (cm) of seedlings of *Jatropha* in each concentration of Al (0, 15, 40, 80 and 120 mg L^{-1}), using the solution-paper method

Genotypes	Al concentration (mg L^{-1})					Regression	R^2
	0	15	40	80	120		
CNPAE-248	15.10 d	10.07 d	4.70 b	2.57 a	0.51 a	CR = $130.7e^{-0.01x}$	0.952
CNPAE-301	17.16 b	12.50 b	6.27 a	2.83 a	0.57 a	CR = $162.5e^{-0.02x}$	0.988
CNPAE-308	24.93 a	16.57 a	4.77 b	2.40 a	0.50 a	CR = $236.7e^{-0.03x}$	0.955
CNPAE-144	16.17 c	10.93 c	3.97 c	2.54 a	0.48 a	CR = $161.1e^{-0.03x}$	0.997

Means followed by the same letter in the column don't differ significantly by Tukey test at 5% of probability.

high phenotypic variability was observed and described by Laviola *et al.* (2010), studying genetic parameters of 110 genotypes of *Jatropha*.

Differential tolerance levels for stress with Al has also been shown in other species, such as oats (Silva *et al.*, 2007; Crestani *et al.*, 2009), coffee (Macedo *et al.*, 2011) and maize (Martins *et al.*, 1999). According to Martins *et al.* (1999), it is possible that the susceptibility is the dominant feature (additive and polygenic) over the Al tolerance, which would make it more difficult to keep the phenotypic stability of tolerant genotypes.

It was possible to discriminate the genotypes of *Jatropha* for the tolerance to Al in the concentration of 40 mg L^{-1}, and concentrations above this level resulted in no significant difference in root length of genotypes. It is noteworthy that there were differences in the initial levels studied, however, the statistic conformation for the concentration of 15 mg L^{-1} was the same than the observed in the control (0 mg L^{-1}). Therefore, the influence of aluminum wasn't limiting to the point of showing a discriminatory level for tolerance to this element. Low concentrations of Al may not influence and may even stimulate root elongation, without causing toxic effects over the initial development of the plant (Yamamoto *et al.*, 2002; Rosado *et al.*, 2012).

The found value of 40 mg L^{-1} differs from the results of Rosado *et al.* (2012), these authors; studying similar methodology, found that the concentration of 85 mg L^{-1} of Al would be suitable to discriminate tolerance to Al in *Jatropha*. However, their work didn't study aluminum levels between the control and the concentration of 85 mg L^{-1}, which led to an overestimation of the concentration. This adjustment of the level can cause reduction in root growth of susceptible genotypes without limiting the growth of tolerant genotypes is the main problem evidenced in methods to select plants with tolerance to Al (Macedo *et al.*, 1997; Braccini *et al.*, 2000; Macedo *et al.*, 2008 Macedo & Lopes, 2008; Rosado *et al.*, 2012).

Macedo & Lopes (2008) and Braccini *et al.* (2000), for coffee, and Konzak *et al.* (1976), for soybeans, also found values close to those found in this study. These authors reported that a dose of 45 mg L^{-1} of Al was suitable to distinguish tolerance among individuals of the same species.

An exponential decrease in root length were observed with the increase of the Al concentration (Figures 1). The behavior of the genotypes when

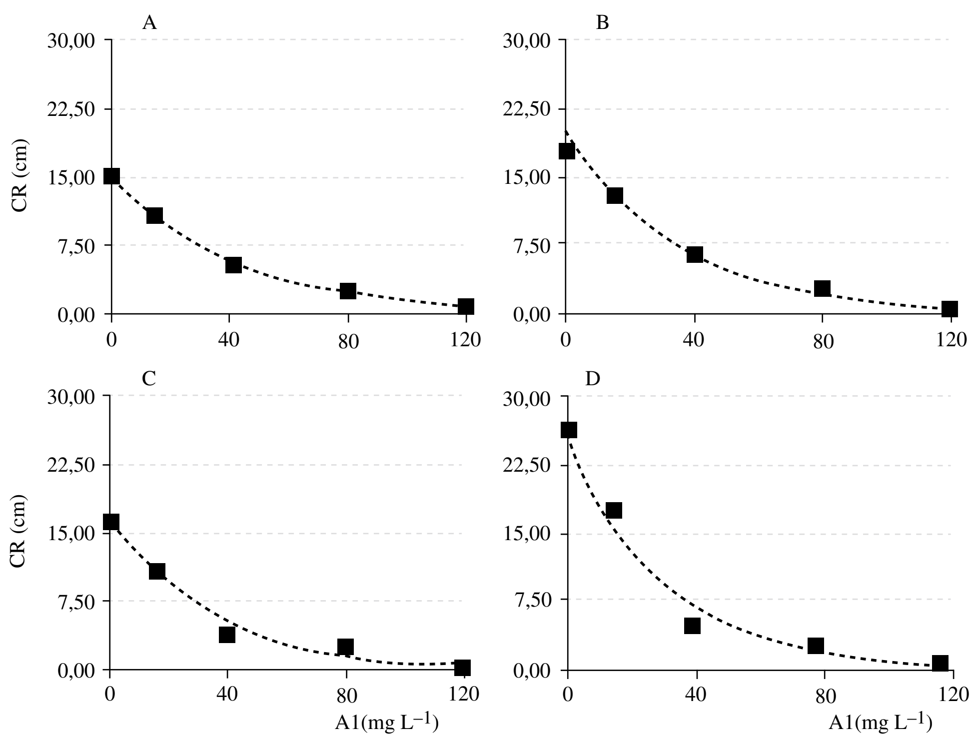

Figure 1. Root length of seedlings of *Jatropha* as a function of Al concentrations in solution (0, 15, 40, 80 and 120 mg L^{-1}), using the solution-paper method, for different genotypes: (A) CNPAE-248, (B) CNPAE-301, (C) CNPAE-308 and (D) CNPAE-144.

subjected to increasing concentrations of aluminum was adjusted to the regression curves with coefficients above 0.95 (Table 2). Compared to the control, the mean value of root length suffered a 70% reduction when subjected to 40 mg L^{-1} of Al in the solution, and above this concentration, the root lengths tended to very small values, showing 99% reduction in the concentration of 120 mg L^{-1} of Al (Table 2).

The noticeable reduction in root growth may be related to mechanisms responsible for tolerance based on morphological, physiological, biochemical or any combination there of (Purcino *et al.*, 2001). The growth restriction is due to reaction of aluminum with polygalacturonic acid chains in the walls of young cells, resulting in loss of elasticity, not only inhibiting cell division but also cell elongation (Custodio *et al.* 2002). It is also known that inhibition of cell division is probably related to the binding of Al to nuclear DNA resulting in a decrease of the activity of DNA (Matsumoto et al, 1976), leading to the paralyzation of the cell division process. Contrarily, the possible tolerance shown by some genotypes, such as CNPAE-301, can be explained by the existence of routes to eliminate Al from the cell by compartmentalization in the vacuole, as previously reported for other species (Abichequer *et al.* 2003).

In general, increasing levels of Al in solution causes symptoms of Al toxicity, characterized by inhibition of the root growth and of lateral roots development, appearance of thick roots with ends showing burnt appearance, later evolving into chlorotic spots, with total necrosis of radicle and leading to the seedling death (Figure 2).

The symptoms of Al toxicity observed in genotypes of *Jatropha* were similar to those observed by Rosado *et al.* (2012), coinciding also with reports of other crops such as oats (Silva *et al.*, 2007; Crestani *et al.*, 2009), coffee (Braccini *et al.*, 2000; Macedo *et al.* 2011), maize (Martins *et al.* 1999) and soybean (Custódio *et al.* 2002).

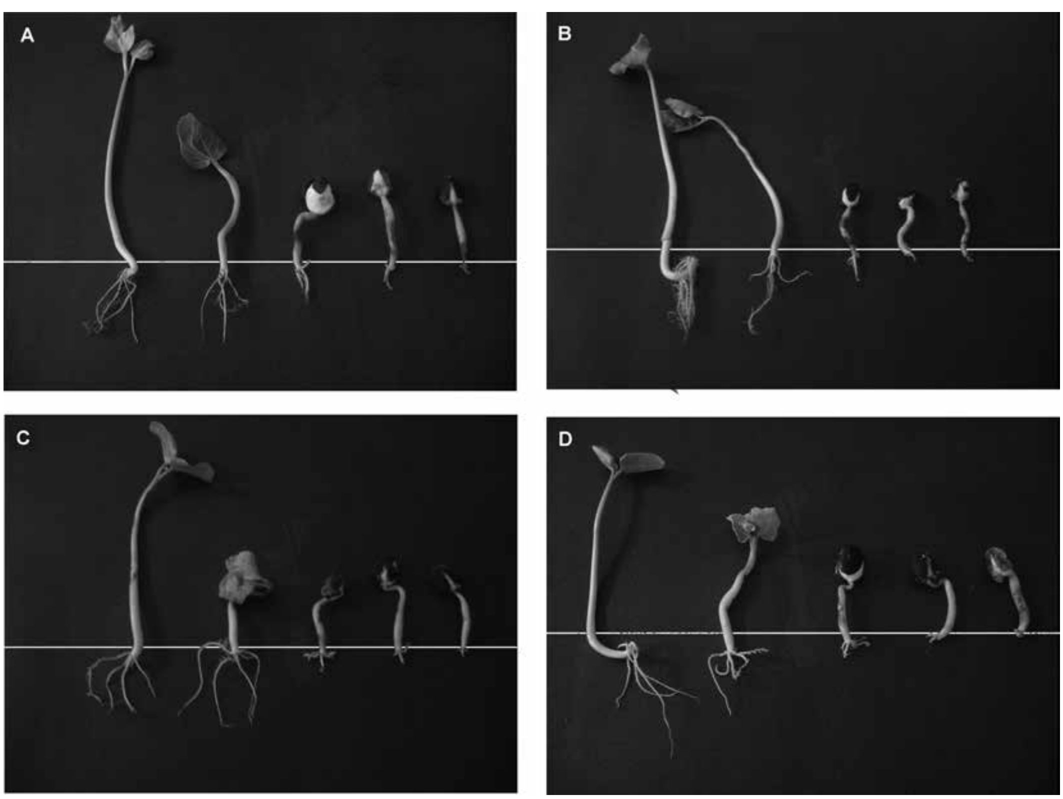

Figure 2. Images of seedlings of *Jatropha* in response to increasing concentrations of Al, applied in solution-paper method (0, 15, 40, 80 and 120 mg L^{-1}, respectively, from left to right in the photographs), for different genotypes: (A) CNPAE-248, (B) CNPAE-301, (C) CNPAE-308 and (D) CNPAE-144.

Conclusions

There is different behavior of genotypes of *Jatropha* in relation to aluminum tolerance. The root length decreases exponentially in function to the increasing concentrations of Al. The solution-paper method allows the selection of genotypes of *Jatropha* in relation to aluminum tolerance and the concentration of 40 mg L^{-1} of Al is recommended to discriminate the tolerance of genotypes to this element.

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