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Aluminum tolerance in castor bean lines[1]

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ABSTRACT

Castor bean plants are susceptible to aluminum (Al) in the soil, requiring adequate management techniques for their cultivation in acid soils containing high Al levels, as it occurs in tropical regions. This study aimed to assess the Al tolerance of castor bean lines. A randomized block design, in a 2 x 9 factorial scheme, with four replicates, was used. The treatments consisted of presence and absence of Al, as well as nine castor bean lines (CRZ H06, CRZ H11, CRZ H12, CRZ H15, CRZ H17, CRZ H18, CRZ H19, CRZ H22 and FCA). Based on a distribution into quartiles, the lines were divided into two groups. The Al-tolerant group contained the CRZ H06, H11 and H17 lines, while the group susceptible to Al was composed of CRZ H12, H15, H18, H19, H22 and FCA. The FCA and CRZ H17 lines showed the highest growth, when cultivated without Al.

KEYWORDS: *Ricinus communis*; root growth; quartiles.

RESUMO

Tolerância de linhagens de mamona a alumínio

Plantas de mamona são suscetíveis à presença de alumínio (Al) no solo. Dessa forma, técnicas de manejo são necessárias para o seu cultivo em solos ácidos e com altos teores de Al, como em regiões tropicais. Objetivou-se avaliar linhagens de mamona, quanto à sua tolerância a Al. O delineamento experimental utilizado foi o de blocos casualizados, em modelo fatorial 2 x 9, com quatro repetições. Os tratamentos consistiram de presença e ausência de Al, bem como de nove linhagens de mamona (CRZ H06, CRZ H11, CRZ H12, CRZ H15, CRZ H17, CRZ H18, CRZ H19, CRZ H22 e FCA). De acordo com a distribuição das linhagens em quartis, foram separados dois grupos. O grupo tolerante a Al foi composto por CRZ H06, H11 e H17; enquanto o grupo suscetível a Al constituiu-se por CRZ H12, H15, H18, H19, H22 e FCA. As linhagens FCA e CRZ H17 apresentaram maior crescimento, quando cultivadas sem Al.

PALAVRAS-CHAVE: *Ricinus communis*; crescimento radicular; quartis.

INTRODUCTION

Castor bean (*Ricinus communis* L.) plants are known for their drought tolerance (Beltrão et al. 2003). On the other hand, they require a high amount of nutrients, with an ideal soil pH between 6.0 and 6.5 and base saturation above 60 % (Severino et al. 2006). Additionally, the crop is susceptible to the presence of trivalent aluminum (Al) in the soil, tolerating a maximum of 10 % of Al saturation (Lima et al. 2007). This drought tolerance and high nutrient demand create confusion (Weiss 1983), leading to the mistaken belief that castor bean plants are hardy, require few nutrients and are resistant to acidity and Al.

Al is toxic to plants and inhibits their growth, with roots being the primary site of Al toxicity (Ryan et al. 2007). The roots of plants under Al stress become atrophied, with fewer fine, rigid branches, thicker cell walls and changes in the membrane that transport proteins (Kochian et al. 2004, Meriga et al. 2010, Motoda et al. 2010, Sun et al. 2010, Garzon et al. 2011, Guo et al. 2012, Freitas et al. 2017a). Thus, the roots are less efficient at absorbing water and nutrients, particularly in deeper soil layers (Wang et al. 2006, Guo et al. 2007, Famoso et al. 2010, Lima et al. 2014, Freitas et al. 2017b).

Lima et al. (2007) studied the effect of Al toxicity on castor bean growth and observed a decrease of 88 % and 84 % in root and shoot dry

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weight, respectively, in plants exposed to Al, when compared to those without toxicity. Passos et al. (2015) found that the main and secondary root length decreased in castor bean cultivars, as a function of increased Al levels in the nutrient solution. Silva et al. (2014) reported a reduction in the relative root elongation of castor bean cultivars, with a rise in the nutrient solution of Al content.

Tropical regions, such as the Brazilian Savannah, show a potential for the expansion of castor bean cultivation areas, using a mechanized system in the second harvest (Rangel et al. 2003). However, these areas typically exhibit acidic soils with Al saturation greater than 10 % (Campos et al. 2011), which can negatively affect the root growth of castor bean plants (Lima et al. 2007). This may also occur in the subsurface layers of no-tillage areas, where lime is applied as topdressing and generally only reduces Al in the surface layer (Costa et al. 2016). This means that root growth is limited to the surface layer, particularly when gypsum is not applied to reduce subsurface Al levels (Soratto & Crusciol 2008). As such, management strategies are needed to overcome these limitations, primarily the adoption of Al-resistant varieties.

Despite the variability of Al tolerance in castor bean crops (Lima et al. 2014, Silva et al. 2014), few studies in the literature investigate the effects of Al toxicity on these plants and the selection of lines, genotypes or cultivars adapted to acidic soils with high Al levels, which would be of significant interest to breeding programs or in scientific research on the crop. Thus, this study aimed to assess the Al tolerance of castor bean lines.

MATERIAL AND METHODS

The experiment was conducted from April to May 2013, in a greenhouse of the Universidade Estadual Paulista, in Botucatu, São Paulo state, Brazil (48º23'W, 22º51'S and altitude of 765 m).

A randomized block design was used, in a 2 x 9 factorial scheme, with four replicates. The treatments consisted of presence (+Al) and absence (-Al) of Al, as well as nine castor bean lines (CRZ H06, CRZ H11, CRZ H12, CRZ H15, CRZ H17, CRZ H18, CRZ H19, CRZ H22 and FCA) grown in a nutrient solution.

The nutrient solution proposed by Furlani & Furlani (1988), originally described for plant selection experiments under nutrient stress, such as Al toxicity, was used. The composition of the nutrient solution was Ca = 3.490 μmol L^{-1}; K = 2.171 μmol L^{-1}; Mg = 0.863 μmol L^{-1}; N-NO_3 = 2.252 μmol L^{-1}; N-NH_4 = 0.997 μmol L^{-1}; P = 0.032 μmol L^{-1}; S = 0.542 μmol L^{-1}; Cl = 0.541 μmol L^{-1}; Fe = 0.513 μmol L^{-1}; Mn = 0.007 μmol L^{-1}; B = 0.020 μmol L^{-1}; Zn = 0.001 μmol L^{-1}; and Cu = 0.0004 μmol L^{-1}. The salt types used were $Ca(NO_3)_2.4H_2O$, NH_4NO_3, KCl, K_2SO_4, KNO_3, $Mg(NO_3)_2.6H_2O$, KH_2PO_4, $FeSO_4.7H_2O$, NaEDTA, $MnCl_2.4H_2O$, H_3BO_3, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $Na_2MoO_2.2H_2O$.

A pre-test was carried out to determine the efficiency of the nutrient solution composition in the nutrition and growth of castor bean plants. Al concentrations of 0.3 mmol L^{-1}, 0.7 mmol L^{-1}, 1.1 mmol L^{-1} and 1.4 mmol L^{-1} were also tested to assess their toxicity. An Al concentration of 1.1 mmol L^{-1} (equivalent to Al activity in the solution of 0.33 mmol L^{-1}, estimated in the Visual Minteq 3.0 software - Gustafsson 2012) was selected for the experiment as the most toxic to castor bean plants under the experimental conditions adopted, while still allowing enough shoot and root growth for analysis. The Al source used was $AlCl_3.6H_2O$.

In order to obtain plants for the experiment, castor bean seeds were treated with carboxin + thiram (400 mL 100 kg^{-1} of seeds). On April 22, 2013, they were distributed across the upper section of a filter paper, spaced 2.0 cm apart, with the embryonic axis lying vertically, and the paper was rolled up and moistened.

The material was placed in a seed germinator at an average temperature of 25 ºC, with 8 h of light. After 72 h, when the roots began to emerge, the rolls were placed vertically into plastic containers filled with 1/5 strength ionic solution at pH 4.0, without Al. The containers were moved to benches in the greenhouse and the rolls remained under these conditions for 14 days.

Next, the plants were selected for growth uniformity and transferred to plastic pots containing 4 L of a half-strength nutrient solution (pH 4.0), for acclimation purposes. The lids used to hold the plants in place were made from perforated Styrofoam sheets, with three plants per pot, fixed using pieces of foam, thereby allowing the roots to come into contact with the nutrient solution and preventing light from reaching them.

After 7 days in contact with the half-strength nutrient solution (acclimation period), it was replaced by a full-strength solution and the plants were left for other 7 days. After this period, the Al treatments were added and the plants were grown under these conditions for 14 days, until analysis.

During the experiment, the pH of the nutrient solution was maintained at 4.0 (± 0.1) in all treatments, using 0.1 mol L^{-1} of NaOH and HCl. The solution in each pot was aerated individually and replaced weekly, and losses due to transpiration were replenished daily, with deionized water. The ambient temperature (22-27 ºC) in the greenhouse was controlled.

The variables assessed were plant height, stem diameter, root and shoot dry weight production, total root length and average root diameter (WinRhizo), shoot and root Al content and accumulation (Malavolta et al. 1997) and the Al susceptibility index.

The castor bean lines were separated into tolerant and susceptible groups, using the root length to calculate the Fisher & Maurer's (1978) susceptibility index, adapted by Guimarães et al. (2006) for Al stress in rice plants (equations bellow), whereby the lower the SI, the less affected to Al stress plants are:

$$SI = \frac{Yi - Ys}{Yi \times D}$$

$$D = \frac{1 - Yms}{Ymi}$$

where *D* is the severity of the stress applied; *Yi* and *Ys* the root length with and without stress, respectively; and *Yms* and *Ymi* the mean root length in the experiment, with and without stress, respectively.

The castor bean lines were distributed into quartiles, according to the average root length, without Al toxicity, plus 75 % of their standard deviations and mean susceptibility index for Al toxicity, less 25 % of their standard deviation. For the remaining variables, the experimental data were submitted to analysis of variance. The Scott-Knott test was used to compare the means of the different lines and the F-test to compare the means with and without Al, both at 5 % of probability.

RESULTS AND DISCUSSION

The castor bean lines were divided into two groups (Al tolerant and susceptible), using the distribution into quartiles, as proposed by Fisher & Maurer (1978) and adapted by Guimarães et al. (2006). The first group consisted of CRZ H06, H11 and H17, whose susceptibility index of root growth to Al was lower than 0.97 (Figure 1). These lines obtained a lower-than-average susceptibility index for Al toxicity, after subtracting 25 % of their standard deviation, and were therefore considered Al tolerant.

The CRZ H06 line stands out among the tolerant varieties, due to its low susceptibility index and high root growth, when cultivated without Al (Figure 1). Additionally, CRZ H17 exhibited a susceptibility index of 0.97 (index used to separate the lines); however, it was considered Al tolerant.

The second group contained the lines CRZ H12, H15, H18, H19, H22 and FCA, which obtained susceptibility index values greater than 0.97 and were, therefore, deemed susceptible to Al. CRZ H18 stands out among the Al susceptible lines, because it displayed the highest susceptibility index.

It is important to note that the quartile distribution resulted in only three lines resistant to Al. As such, in general, it may be concluded that, despite the variability indicated in Figure 1 and reported by Lima et al. (2014), the root growth of castor bean plants was affected by Al under the conditions applied here, corroborating the results obtained by Amorim

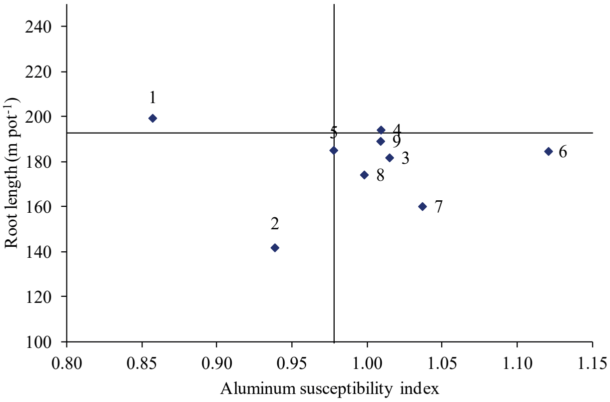

Figure 1. Distribution of lines into quartiles, according to the root length without aluminum in the solution and susceptibility index of root growth to aluminum toxicity, at points determined by the average root length (without aluminum) + 75 % of their standard deviation and average susceptibility index minus 25 % of standard deviation. Lines: 1 - CRZ H06; 2 - CRZ H11; 3 - CRZ H12; 4 - CRZ H15; 5 - CRZ H17; 6 - CRZ H18; 7 - CRZ H19; 8 - CRZ H22; 9 - FCA.

Neto et al. (2001), Severino et al. (2006), Lima et al. (2007 and 2014), Passos et al. (2015) and Silva et al. (2014).

The definition of castor bean lines as Al tolerant or susceptible via quartiles, using the method proposed by Fisher & Maurer (1978) and adapted by Guimarães et al. (2006), was also established in upland rice plants, as demonstrated by Guimarães et al. (2006) and Freitas et al. (2016).

Quartile distribution was applied to separate plants into groups, according to their Al tolerance, based on root length. However, the other variables were evaluated individually, in order to assess the growth of castor bean lines under Al toxicity and without Al at a pH 4.0, which is below the recommended pH for castor bean cultivation (Amorim Neto et al. 2001).

CRZ H12, H17 and FCA demonstrated a higher shoot growth, when cultivated without Al (Table 1), whereas CRZ H19 and H22 showed a lower growth with Al. Only CRZ H06 and H11 were able to maintain a similar shoot growth, when cultivation with and without Al were compared. This corroborates the findings obtained in the quartile distribution, since these lines were considered Al tolerant (Figure 1).

In relation to the other lines, only FCA displayed a larger stem diameter, when grown without Al (Table 1). CRZ H19 and H22 showed smaller stem diameters with Al and were classified as sensitive to Al (Figure 1). Except for CRZ H12 and H17, the stem diameter in the remaining lines declined in plants exposed to Al, when compared to those grown without it.

FCA exhibited a higher shoot dry weight production without Al, in relation to the others (Table 1), what may be related to its greater height under these conditions (Table 1). When cultivated with Al, CRZ H06, H15, H17 and FCA were more efficient at producing shoot dry weight.

CRZ H06 and H17 maintained a good shoot growth, even under Al stress, corroborating their selection as Al tolerant (Figure 1). It is important to point out that the selection via quartile distribution is based on root length.

CRZ H12, H15, H17, H18 and FCA obtained higher root dry weight values without Al, when compared to the other lines (Table 1). On the other hand, there was no difference in root dry weight production among the lines, when exposed to Al stress. Nevertheless, there was a difference between lines, in terms of shoot dry weight production, what may suggest that plants with the best results for this variable have a mechanism capable of ensuring a good shoot growth, despite the root exposure to Al stress (Kochian et al. 2004).

The shoot and root dry weight (Table 1) of all the castor bean lines decreased, on average, by 44 % and 50 %, respectively, when grown with Al. This demonstrates the toxic effect of Al to castor bean plants, even in lines considered as Al resistant. Lima et al. (2014) also observed a decline in shoot and root growth for the Lyra, BRS Nordestina and BRS Paraguaçu genotypes.

Since root length was used to separate the lines into groups via quartile distribution (Figure 1), analyzing this variable individually could provide a better understanding of the result. Root length was

Table 1. Average plant height, stem diameter, shoot and root dry weight of castor bean plants grown with (+Al) and without (-Al) aluminum.

Line	Plant height (cm)		Stem diameter (mm)		Shoot dry weight (g pot^{-1})		Root dry weight (g pot^{-1})	
	-Al	+Al	-Al	+Al	-Al	+Al	-Al	+Al
CRZ H06	9.18 bA*	8.00 aA	6.74 bA	5.90 aB	4.7 bA	3.1 aB	2.6 bA	1.4 aB
CRZ H11	8.66 bA	8.31 aA	7.07 bA	5.80 aB	4.3 bA	2.7 bB	2.5 bA	1.3 aB
CRZ H12	10.68 aA	8.00 aB	6.78 bA	6.09 aA	4.9 bA	2.6 bB	2.8 aA	1.3 aB
CRZ H15	9.93 bA	8.18 aB	6.90 bA	5.82 aB	5.0 bA	3.1 aB	2.7 aA	1.5 aB
CRZ H17	10.50 aA	8.93 aB	6.90 bA	6.25 aA	5.5 bA	3.3 aB	3.0 aA	1.5 aB
CRZ H18	9.75 bA	8.06 aB	6.63 bA	5.62 aB	5.0 bA	2.8 bB	2.8 aA	1.2 aB
CRZ H19	9.12 bA	7.00 bB	6.69 bA	5.27 bB	4.8 bA	2.4 bB	2.4 bA	1.1 aB
CRZ H22	8.93 bA	6.93 bB	6.62 bA	5.02 bB	4.9 bA	2.4 bB	2.4 bA	1.1 aB
FCA	11.18 aA	9.43 aB	7.76 aA	6.16 aB	6.4 aA	3.1 aB	2.9 aA	1.3 aB
CV (%)	10.0		2.6		9.2		10.7	

* Means followed by different uppercase letters in the rows and lowercase letters in the columns are statistically different at 5 % of probability, according to the Scott-Knott test.

greater in CRZ H06, H12, H15, H17, H18, H22 and FCA, without the presence of Al (Table 2). However, when grown with Al, only CRZ H06 achieved a greater root length, corroborating its classification as Al tolerant. The high root growth of CRZ H06 under Al toxicity may indicate the presence of internal or external Al tolerance mechanisms (Kochian et al. 2004).

Additionally, CRZ H18 exhibited shorter roots under Al toxicity (Table 2), demonstrating its susceptibility to Al. Roots are the primary site of Al toxicity, which stunts their growth (Ryan et al. 2007, Freitas et al. 2017a).

As observed for the shoot and root dry weight production (Table 1), root length was negatively affect by Al toxicity in all the lines, when compared to the cultivation without Al (Table 2). This confirms the susceptibility of castor bean plants to Al toxicity, what may also occur in lines classified as Al tolerant, albeit to a lesser degree.

Although CRZ H06 showed the greater root growth under Al stress (Table 2), possibly due to its smaller root diameter (Table 2), its root dry weight values were not higher than those recorded for the other lines (Table 1). This contradicts the findings of Kochian et al. (2004), who reported that Al caused root thickening, a characteristic symptom of Al toxicity.

Moreover, although the root dry weight production did not differ under Al stress (Table 1), a difference in root length was observed among the lines (Table 2). As the primary target of Al (Ryan et al. 2007), roots are the focal point for differentiating between the lines, in terms of their tolerance to it.

Table 2. Average root length and diameter of castor bean lines grown with (+Al) and without (-Al) aluminum.

Line	Root length (m pot^{-1})		Average diameter (mm)	
	-Al	+Al	-Al	+Al
CRZ H06	199.5 aA*	83.7 aB	0.582 aA	0.502 bB
CRZ H11	142.0 cA	54.4 bB	0.665 aA	0.610 aA
CRZ H12	181.9 aA	58.0 bB	0.620 aA	0.587 aA
CRZ H15	194.2 aA	57.7 bB	0.605 aA	0.620 aA
CRZ H17	185.2 aA	59.5 bB	0.592 aA	0.595 aA
CRZ H18	184.7 aA	43.6 bB	0.612 aA	0.625 aA
CRZ H19	160.3 bA	46.6 bB	0.572 aA	0.525 bA
CRZ H22	174.3 aA	54.4 bB	0.575 aA	0.505 bB
FCA	189.2 aA	57.3 bB	0.610 aA	0.555 bA
CV(%)	9.3		7.4	

* Means followed by different uppercase letters in the rows and lowercase letters in the columns are statistically different at 5 % of probability, according to the Scott-Knott test.

Thus, it can be inferred that the methodology of selecting lines based on their root length may be more efficient than the selection on the basis of dry weight yield. In the past, the root dry weight production was the most widely used variable to determine the Al tolerance of different cultivars (Ma et al. 2004, Doncheva et al. 2005).

There was no difference in the average root diameter among the castor bean lines, when grown without Al (Table 2). However, CRZ H11, H12, H15, H17 and H18 exhibited larger average diameters, in relation to the other lines, when exposed to Al toxicity. This may indicate a more pronounced toxic effect in these lines, since an increase in root diameter is one of the main symptoms of Al toxicity (Kochian et al. 2004).

CRZ H06 demonstrated a low shoot Al content and accumulation (Table 3) and obtained the highest root Al concentration and accumulation among the tested lines (Table 3), possibly due to its greater shoot growth (Table 1). This may have occurred because of the plant's internal Al tolerance mechanism (Kochian et al. 2004), which compartmentalizes nontoxic Al forms in the vacuole, thereby reducing the Al transport to the shoots and contributing to a greater growth in this part of the plant. On the other hand, although CRZ H06 showed the highest root Al content and accumulation, it also exhibited the highest root length among the studied lines (Table 2), suggesting the presence of an internal tolerance mechanism.

Among the studied lines, FCA obtained the highest shoot and root Al content (Table 3), and produced the largest amount of shoot dry weight (Table 1).

By contrast, low Al concentrations and accumulation were observed in the roots of CRZ H18 and H22, classified as susceptible to Al, according to the quartile distribution (Figure 1), meaning that the plants were unable to tolerate even low levels of Al. Despite their greater sensitivity to Al, when compared to the other lines, they may exhibit Al tolerance mechanisms based on preventing its entry into the root tip (Al exclusion mechanism) (Simões et al. 2012), with Al complexation occurring externally, in the mucilage, as described by Silva et al. (2014), in castor bean plants.

These results demonstrate that the Al content and accumulation were higher in the roots, when compared to the shoots, since the former are directly affected by Al (Ryan et al. 2007).

Table 3. Average aluminum content and accumulation in the shoots and roots of castor bean plants grown with (+Al) and without (-Al) aluminum.

Line	Shoot				Root			
	Content (g kg^{-1})		Accumulation (mg pot^{-1})		Content (g kg^{-1})		Accumulation (mg pot^{-1})	
	-Al	+Al	-Al	+Al	-Al	+Al	-Al	+Al
CRZ H06	0.042 bB*	0.073 dA	0.199 bA	0.230 bA	0.197 aB	9.48 bA	0.639 aB	13.57 aA
CRZ H11	0.037 bB	0.086 cA	0.160 cB	0.242 bA	0.149 aB	9.70 bA	0.434 aB	12.69 aA
CRZ H12	0.053 aB	0.124 aA	0.266 aB	0.324 aA	0.171 aB	9.66 bA	0.574 aB	13.34 aA
CRZ H15	0.056 aB	0.080 dA	0.288 aA	0.250 bA	0.232 aB	9.19 bA	0.635 aB	13.95 aA
CRZ H17	0.048 aB	0.084 dA	0.265 aA	0.284 aA	0.203 aB	8.66 cA	0.740 aB	13.63 aA
CRZ H18	0.053 aB	0.090 cA	0.272 aA	0.253 bA	0.215 aB	8.89 cA	0.617 aB	11.39 bA
CRZ H19	0.045 bB	0.094 cA	0.220 bA	0.228 bA	0.199 aB	9.70 bA	0.596 aB	11.21 bA
CRZ H22	0.049 aB	0.083 dA	0.241 aA	0.204 bA	0.241 aB	8.30 cA	0.624 aB	9.70 bA
FCA	0.049 aB	0.108 bA	0.319 aA	0.340 aA	0.143 aB	11.26 aA	0.416 aB	14.88 aA
CV (%)	9.2		13.1		13.1		21.0	

* Means followed by different uppercase letters in the rows and lowercase letters in the columns are statistically different at 5 % of probability, according to the Scott-Knott test.

In general, CRZ H06, H12, H15, H17, H18 and FCA showed a better growth without Al toxicity (pH 4.0), primarily in light of the root dry weight (Table 1) and root length values observed (Table 2).

With respect to the most efficient lines under Al stress, CRZ H06, H15, H17 and FCA stood out for their shoot dry weight production (Table 1), particularly CRZ H06, in terms of root length (Table 2). CRZ H06 is the most indicated for use in plant breeding programs aimed at obtaining Al-tolerant castor bean plants. However, in general, the plants are susceptible to Al under the conditions used in this study, what corroborates the findings of Lima et al. (2007 and 2014), Silva et al. (2014) and Passos et al. (2015).

CONCLUSIONS

1. Based on the distribution into quartiles, the lines were divided into two groups. The Al-tolerant group contained CRZ H06, H11 and H17, and the group susceptible to Al was composed of CRZ H12, H15, H18, H19, H22 and FCA;
2. FCA and CRZ H17 showed the highest growth, when cultivated without Al toxicity, whereas CRZ H06 and H17 were the most efficient in the presence of Al.

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