Selection of maize hybrids for tolerance to aluminum in minimal solution

C.J. Coelho[1], D. Molin[2], H.A. Wood Joris[1], E.F. Caires[1],
J.R. Gardingo[1] and R.R. Matiello[1,2]

[1]Programa de Pós-Graduação em Agronomia,
Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brasil
[2]Programa de Pós-Graduação em Biologia Evolutiva,
Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brasil

Corresponding author: R.R. Matiello
E-mail: rrmatiel@uepg.br

ABSTRACT. The aim of this study was to determine the Al concentration and the period of exposure of the roots of maize hybrids in minimal solution for efficient selection of genotypes that are Al-tolerant. Two experiments were performed (48 and 96 h of exposure) with increasing doses of Al in minimal solution; the block design was completely randomized in a split-plot design with 3 replications. By assessing differences in root growth (cm) and the percentage of inhibition of the growth of the main root (%), a marked decrease was observed in maize root growth with increasing Al concentration in the solution. Exposure of the roots to 2 mg/L Al for 48 h in minimal solution was the most efficient for selecting sources of tolerance, particularly for the hybrids H 44 and H 38.

Key words: Difference in root growth; Inhibition; Root length;
Sources of tolerance

INTRODUCTION

In many areas of the world, soil acidity limits agricultural yield. In Brazil, it is estimated that more than 500 million hectares of land are composed of acidic soils, comprising approximately two-thirds of the country (Vitorello et al., 2005). Low levels of basic cations, particularly calcium (Ca), as well as the toxicity of aluminum (Al) have the most limiting effect on crop productivity in acid soils in tropical and subtropical regions (Coleman and Thomas, 1967). In general, the adverse effects of Al are reflected in the roots, which grow at a slower rate. Because of the interference of Al in the cell division process, the roots paralyze plant growth and exhibit profound morphological changes. They subsequently thicken and show abnormal branching (Doncheva et al., 2005). Maize is one of the crops most affected by Al toxicity, resulting in yield reductions in excess of 80% (Conceição et al., 2009).

The problems caused by soil acidity, and thus Al toxicity, are commonly corrected by lime application. In areas that are prepared conventionally, topsoil acidity is neutralized by mechanically incorporating lime, inducing reaction between the lime and the soil. In no-till systems, lime cannot be mixed with the soil and is instead applied to the surface (Caires et al., 2008). As the reaction of lime is typically limited to the site of its application in the soil, acidity in the subsurface layers (in the case of toxic levels of Al) can compromise the root growth, affecting plant nutrition and leaving crops susceptible to water stress (Caires et al., 2005). Thus, choosing plants with genotypes that are adapted to acidic conditions along with more efficient nutrient use is important for increasing the sustainability of conservation agriculture (Parentoni et al., 2001).

Selecting for aluminum tolerance in the field is very complex, laborious, and subject to various errors. The natural variability of soils and the difficulty of assessing the root damage result in failures in genotype selection (Mazzocato et al., 2002). Assessing damage to the root system of seedlings in nutrient solution containing Al at toxic concentrations has been effectively adopted for selecting highly tolerant genotypes (Jorge and Arruda, 1997; Mazzocato et al., 2002; Paterniani and Furlani, 2002; Alves et al., 2004; Piñeros et al., 2005).

Clark's complete nutrient solution (Clark, 1975) has become the basis for most studies examining Al tolerance (Magnavaca, 1982; Furlani and Furlani, 1988; Maron et al., 2008). However, there are limitations to using complete nutrient solution, as Al can interact with phosphate and sulfate to form poorly soluble compounds and could decrease Al activity in the solution. Some previous studies have proposed the use of minimal solution containing Ca and Al for the characterization of Al-tolerant genotypes (Mazzocato et al., 2002; Conceição et al., 2009). However, the Al concentrations and time of exposure of the roots in minimal solution for characterization of maize genotypes tolerant to Al have not been thoroughly described. Thus, in this study, we determined the concentration of Al and time of exposure of maize roots in minimal solution for selecting Al-tolerant maize hybrids.

MATERIAL AND METHODS

Two experiments using 8 commercial hybrids from different companies [H 13 and H 14 (Pioneer Hi-Bred International, Johnston, IA, USA); H 22 and H 30 (Syngenta, Basel, Switzerland); and H 38, H 43, H 44, and H 5 (Dow AgroSciences, Indianapolis, IN, USA)] were performed at the Laboratory of Plant Breeding of the Universidade Estadual de Ponta Grossa in Paraná State, Brazil. The seedling roots from different hybrids were exposed to Al for 48 h in the first experiment and 96 h in the second.

Hybrid seeds were placed on germination paper rolls (Germitest®) in a germination chamber for 4 days at a temperature of 25°C and a photoperiod of 12 h. After pre-germination, the seedlings (main root was 3.5-4.5 cm) were placed in minimal solution containing 40 mg/L Ca (CaCl$_2$·2H$_2$O) and Al concentrations of 0, 2, 4, and 6 mg/L in the form of aluminum chloride (AlCl$_3$·6H$_2$O). Experiments were conducted in a completely randomized block, with a split-plot design with 3 replications. The Al concentrations were used in the plots and the 8 corn hybrids were used in the subplots, using 12 seedlings per replication of each hybrid at the respective concentrations of Al.

The initial length of the main root (IL-cm) was measured before transferring the seedlings to 96-well polystyrene trays (12 x 8). The trays were then deposited in transparent plastic pots with 8 L minimal solution containing the varying Al concentrations. The main root of the seedlings remained in minimal solution (Al + Ca) for 48 h (first experiment) and 96 h (second experiment) with uniform and constant aeration. The pH of all solutions was adjusted from 4.2-4.6 using a solution of 1 M NaOH. After the exposure periods (48 and 96 h), the final length of the main root (FL-cm) of all seedlings was measured again. The difference between IL and FL (FL-IL) was identified as DIF (cm) based on the method proposed by Mazzocato et al. (2002).

DIF variable data for both experiments were subjected to analysis of variance. In the presence of a significant effect of the hybrid x Al concentration interaction, we analyzed the decomposition of Al concentrations for each hybrid studied using regression analysis. The regression equations were adjusted using the square root model. The decomposition of the hybrids within the Al concentration was performed using the Student t-test at 5% for the 2 experiments. In addition, the inhibition of growth of the main root (IGMR, in %) was calculated for the 8 hybrids at concentrations of 2, 4, and 6 mg/L Al, as well as during the 2 exposure times (48 and 96 h) using (Equation 1) proposed by Cambraia and Cambraia (1995). Analyses of variance and tests of means were performed using the statistical program SISVAR, version 5.3 (Ferreira, 2011) and regression analyses was performed using the SAEG program, version 9.1 (Ribeiro and Melo, 2008).

$$IGMR(\%) = \left[\frac{100 - (DIF_{+Al} \times 100)}{DIF_{-Al}} \right] \qquad \text{(Equation 1)}$$

RESULTS AND DISCUSSION

Analysis of variance for both experiments revealed a significant effect of the interaction hybrid x concentration of Al for the DIF variable (Table 1). The effect of Al concentrations on each hybrid maize after 48 h revealed a large reduction in the DIF variable of all hybrids with increasing Al concentrations in solution (Figures 1 and 2). The reduction in the length of the main primary root of hybrids was most pronounced with 2 mg/L Al in the solution; the effect was attenuated from 2-4 mg/L, and relatively stable from 4 mg/L. After exposing the roots to Al for 96 h (Figures 3 and 4), the responses of hybrids were similar to those observed at 48 h. Over the 2 periods of exposure to Al (48 and 96 h), reduced root growth of the maize hybrids was evident at 2 mg/L Al in solution.

Table 1. Summary of analysis of variance for the difference in root growth (DIF) for different Al concentrations in minimal solution and 2 periods of exposure for 8 maize hybrids.

| Source of variation		Mean squares (MS)	
	d.f.	DIF	
		48 h	96 h
Blocks	2	1.03	1.60
Al concentrations (C)	3	248.51**	1179.93**
Error (a)	6	0.27	1.80
Hybrids (H)	7	3.18**	7.61*
C x H	21	0.96**	8.39**
Error (b)	56	0.16	3.40
Decomposition (C x H)			
0	7	3.39**	30.55**
2	7	1.21**	0.81
4	7	0.75**	1.04
6	7	0.70**	0.39
CV_{plot} (%)		12.6	21.2
$CV_{subplot}$ (%)		9.6	29.2
Means		4.11	6.32

*P < 0.05 and **P < 0.01.
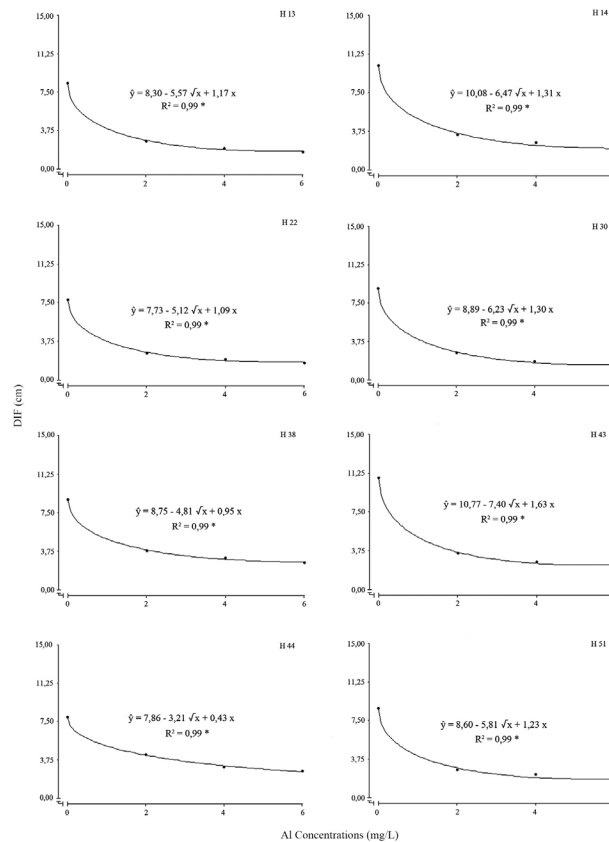
Figure 1. Decomposition effect of Al concentrations (0, 2, 4, and 6 mg/L) for each hybrid using the square root regression model for the DIF variable (48 h exposure). *P < 0.05.
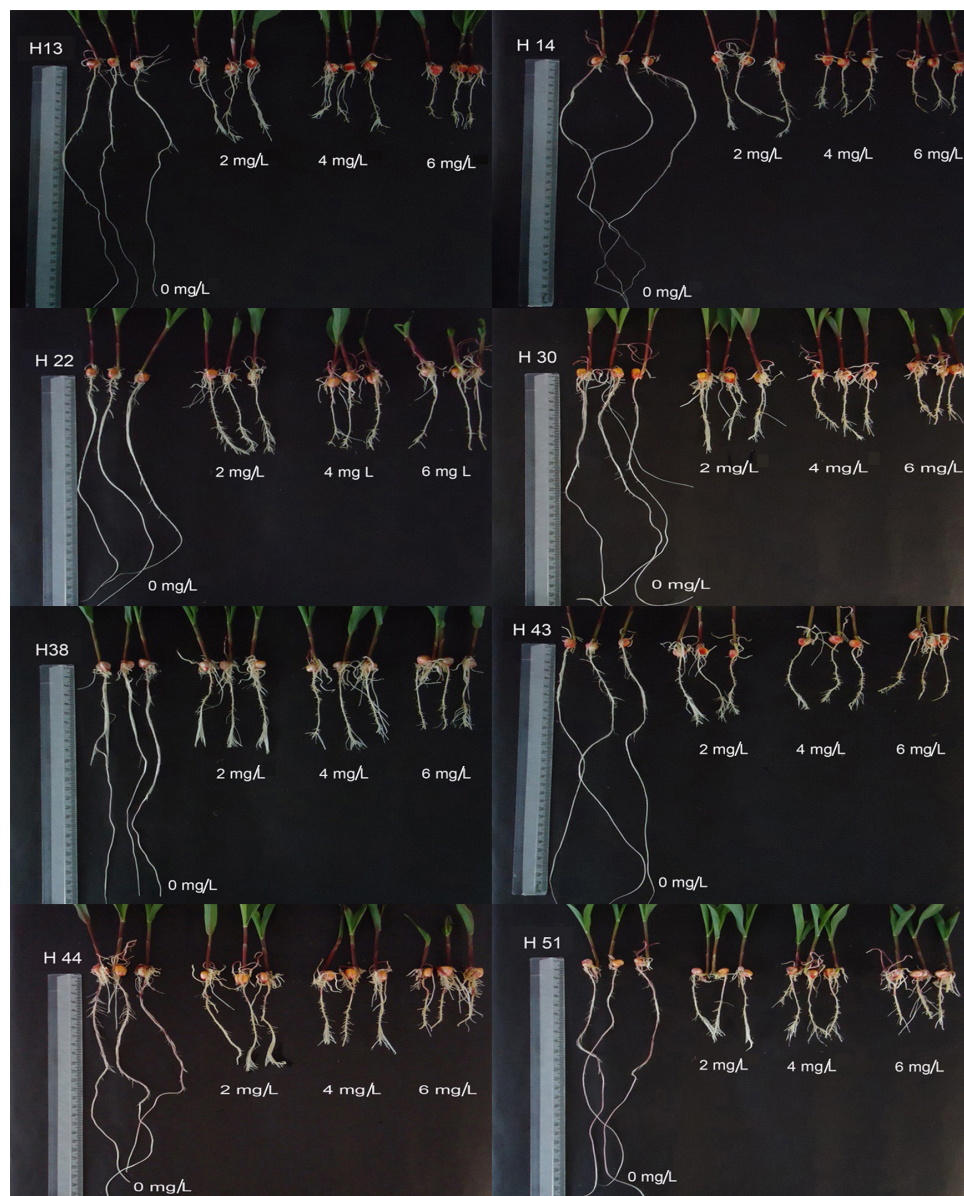
Figure 2. Effect of Al concentrations (0, 2, 4, and 6 mg/L) on the hybrids (H 13, H 14, H 22, H 30, H 38, H 43, H 44, and H 51) after 48 h exposure.

The DIF averages for the 8 hybrids exposed to different Al concentrations for 48 h are shown in Figure 5A. In the absence of Al, there was wide variation in root growth between the hybrids, with the largest DIF found for hybrid H 43 and the lowest for hybrids H 44, H 22, and H 13. This may have resulted from genetic differences between the hybrids in the early growth phase of the seedlings (Figure 5A). Changes in the ranking of the hybrids in the absence of

Al were observed following exposure to increasing Al concentrations. At 2 mg/L Al, greater root growth of hybrids H 44 (4.3 cm) and 38 H (3.8) was observed, whereas the H 13, H 51, H 30, and H 22 hybrids showed the least root growth, ranging from 2.6-2.7 cm, confirming the greater sensitivity of these hybrids to Al (Figure 5A). Exposure for 48 h at a concentration of 4 mg/L Al also showed greatest root growth for the hybrids H 44 (3.0 cm) and H 38 (3.1 cm); however, there was no significant difference from the H 43 and H 14 hybrids. In contrast, hybrids H 22 and H 30 showed DIFs of only 1.9 and 1.8 cm, respectively. Exposure for 48 h at 6 mg/L Al maintained the hybrids H 44, H 38, and H 43 with greater root growth, while the hybrids H 22, H 51, H 13, and H 30 showed the least root growth (Figure 5A). These results indicate the greater tolerance of the hybrids H 44 and H 38 to Al, as well as the higher sensitivity of hybrids H 13, H 22, H 30, and H 51 to Al. Even following exposure to increasing concentrations of Al, the hybrids H 44 and H 38 stood out because their root growth was statistically higher than the other hybrids, confirming that they are sources of Al-tolerance in maize. Other authors (Mazzocato et al., 2002; Conceição et al., 2009) also found significant differences in DIF values for maize genotypes after 48 h of exposure to Al in minimal solution (Ca + Al).
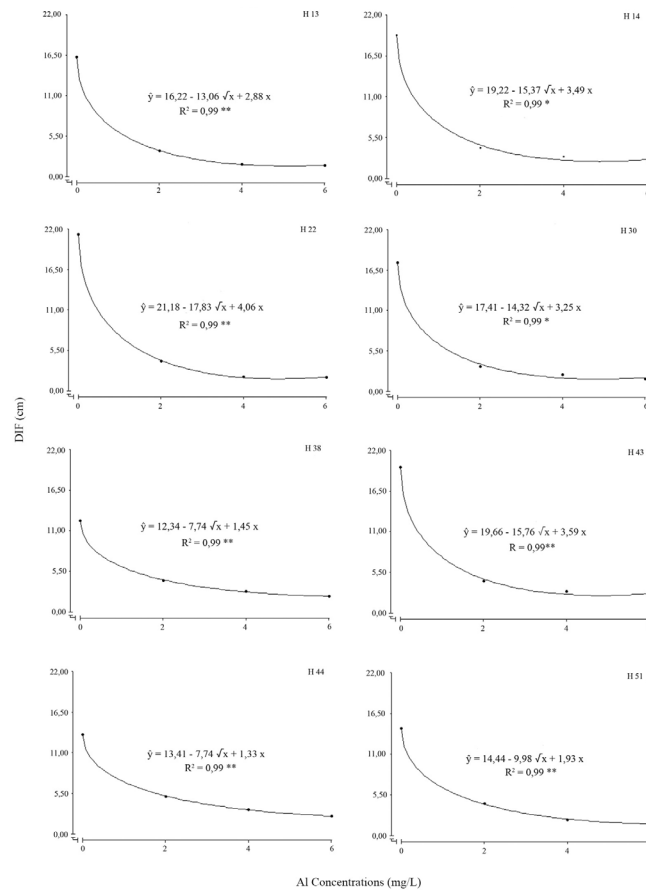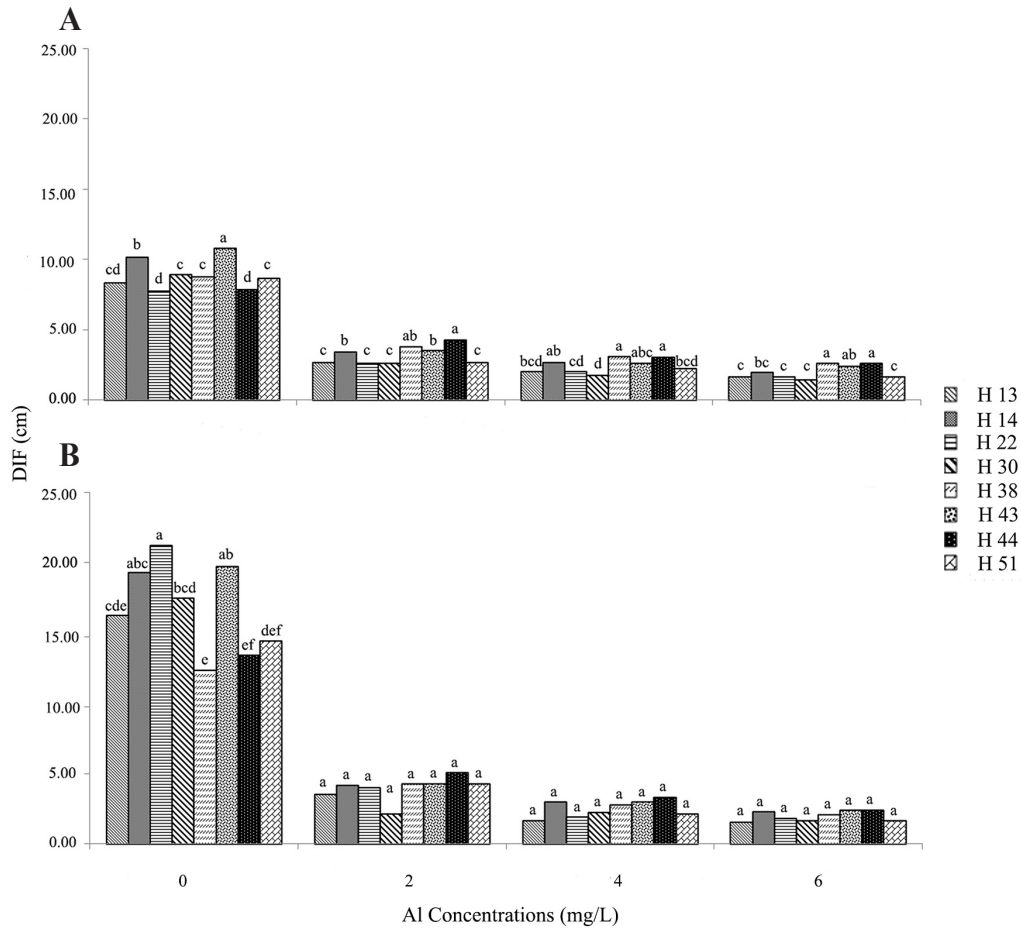

Figure 3. Decomposition effect of Al concentrations (0, 2, 4, and 6 mg/L) for each hybrid using the square root regression model for the DIF variable (96 h exposure). *P < 0.05 and **P < 0.01.

Figure 4. Effect of Al concentrations (0, 2, 4, and 6 mg/L) on the hybrids (H 13, H 14, H 22, H 30, H 38, H 43, H 44, and H 51) after 96 h exposure.

Other studies carried out in complete nutrient solution showed significant differences between maize genotypes, even after long periods of Al exposure (Cambraia and Cambraia, 1995; Jorge and Arruda, 1997; Piñeros et al., 2005). However, in all studies performed using minimal solution (Ca + Al), the maize roots were exposed for a maximum of 48 h. In the present study, we increased the exposure period of the hybrids to Al to 96 h to confirm the genetic potential of Al-tolerance in hybrids that had previously been classified as tolerant (48 h).

Figure 5. Effect of the decomposition of the hybrids (H 13, H 14, H 22, H 30, H 38, H 43, H 44, and H 51) in the respective concentrations of Al (0, 2, 4, and 6 mg/L). **A.** DIF (48 h exposure) and **B.** DIF (96 h exposure). Means followed by the same letter in each Al concentration did not differ statistically from each other according to the Student *t*-test at 5% probability.

The partition of the interaction of the hybrids within each Al concentration after 96 h of exposure showed no significant differences in DIF values between the hybrids at 2, 4, and 6 mg/L Al (Figure 5B). Significant changes (P < 0.01) only occurred in the absence of Al, potentially because of genotypic differences among the hybrids in the early growth phase of the seedlings. Under this condition, hybrid H 22 showed a DIF of 21.2 cm, but this value did not differ significantly from those of hybrids H 43, H 14, and H 30 (Figure 5B). In this experiment, no significant differences in DIF values at different Al concentrations were observed among the hybrids. The 96 h exposure to Al was very long and may have caused large effects on the rate of cell division and elongation of the principal root. This fact prevented the detection of possible differences in tolerance/sensitivity among the studied hybrids. Thus, exposure of maize seedlings to Al for longer periods should only be performed when using complete

nutrient solution because of the reduced nutrient reserves contained in the seeds. Several studies have found significant differences among genotypes for Al tolerance when exposed to complete nutrient solution for a period of more than 96 h (Jorge and Arruda, 1997; Paterniani and Furlani, 2002; Alves et al., 2004; Piñeros et al., 2005).

The reduction in root growth of the maize hybrids increased with increased Al concentrations in the minimal solution, and this effect was observed for both 48 and 96 h of exposure. Cambraia et al. (1991) observed similar results when evaluating 2 sorghum cultivars in complete nutrient solution with 2, 4, 6, 8, and 10 mg/L Al. These authors found significant differences among the cultivars up to 4 mg/L, above which a level of stability was reached; the largest differences in root growth were detected with 2 mg/L. Cambraia and Cambraia (1995) studied Al concentrations (0, 5, 10, and 20 mg/L) in complete nutrient solution for the hybrids BR 126 and BR 136 and suggested 5 mg/L for selecting Al-tolerant hybrids because higher concentrations generally eliminated differences between hybrids.

The IGMR values for the main root of the hybrids showed significant reductions in main root growth, both by increasing Al concentrations as well as increasing the root exposure period (Table 2). The mean IGMR observed for 48 h exposure to Al was 71.2%, with a range of 63.3 (2 mg/L) to 77.4% (6 mg/L). The greatest effect in average IGMR was demonstrated at 96 h exposure (82.4%), ranging from 74.0 (2 mg/L) to 87.8% (6 mg/L) (Table 2).

Table 2. Effect of Al concentrations (2, 4, and 6 mg/L) and exposure period (48 and 96 h) on inhibition of growth of the main root (IGMR) in 8 maize hybrids.

Hybrids	IGMR (%) - 48 h			
	2 mg/L	4 mg/L	6 mg/L	Means
H 30	70.7[a]	80.2[a]	84.2[a]	78.4
H 51	68.9[a]	74.0[b]	80.9[ab]	74.6
H 13	67.8[a]	75.8[ab]	80.5[ab]	74.7
H 43	67.6[a]	76.0[ab]	78.0[b]	73.9
H 14	66.8[a]	74.0[b]	80.8[ab]	73.9
H 22	66.4[a]	74.3[ab]	78.6[ab]	73.1
H 38	57.0[b]	64.8[c]	70.1[c]	63.8
H 44	45.6[c]	61.0[c]	66.2[c]	57.6
Means	63.3	72.5	77.4	71.4
	IGMR (%) - 96 h			
H 30	80.6[a]	87.2[a]	90.6[a]	86.1
H 22	79.1[a]	90.0[a]	90.7[a]	86.6
H 14	78.5[a]	84.6[a]	88.3[ab]	83.8
H 13	78.4[a]	89.9[a]	90.7[a]	86.3
H 43	78.4[a]	85.0[a]	87.9[abc]	86.1
H 51	70.6[b]	85.4[a]	88.8[ab]	81.6
H 38	64.7[c]	77.4[b]	83.4[bc]	75.2
H 44	61.6[c]	75.3[b]	82.3[c]	73.1
Means	74.0	84.4	87.8	82.4

Means followed by the same letter on columns do not differ significantly by the Student *t*-test at P = 0.05.

For both exposure periods (48 and 96 h), it the hybrids H 44 and H 38 consistently showed the lowest percentage of IGMR, regardless of Al concentration (Table 2). The mean growth inhibition of the main root of these hybrids was 57.6 and 63.8% (48 h) and 73.1 and 75.2% (96 h), respectively. These results confirm that the hybrids H 44 and H 38 are sources of Al tolerance in maize because although aluminum affects normal root development, the magnitude of this effect was lower compared to the values for other hybrids. In contrast, the most

pronounced action of Al on IGMR (48 h) was observed for hybrids H 30 (70.7-84.2%), H 13 (67.8-80.5%), and H 22 (66.4-78.6%), confirming the greater sensitivity of these genotypes to Al. At 96 h exposure (Table 2), the greatest IGMR was for the hybrids H 30 (80.6-90.6%), H 22 (79.1-90.7%), H 14 (78.5-90.7%), H 13 (78.4-90.7%), and H 43 (78.4-87.9%), confirming the negative effect of this Al exposure period on the elongation and cell division rate of the main root, as well as the greater sensitivity of these maize hybrids to Al.

During the 2 periods of exposure, strong inhibition of root growth was observed up to 4 mg/L, indicating the potential of the concentrations of 2 and 4 mg/L of Al in minimal solution for improving the discrimination of maize genotypes to Al tolerance (Figure 5). Prioli et al. (2002) used 4.5 mg/L Al in complete solution to evaluate the root growth of 2 inbred maize lines (L922, tolerant and Ast214, sensitive) and their progenies (F$_1$, F$_2$, and backcrosses). After 10 days of exposure, marked differences in root growth were observed between tolerant and sensitive strains. Boni et al. (2009) subsequently confirmed the phenotypic differences in Al tolerance between the parental lines at this same concentration.

Discrepancies found in the literature regarding the most appropriate concentration of Al for identifying sources of Al tolerance in cereal crops may be linked to the genetic basis of the tested genotypes, composition of the nutrient solution, and exposure time and pH of the solution. Several authors have used concentrations of 4.5 mg/L Al (Machado and Pereira, 1990; Paterniani and Furlani, 2002; Prioli et al., 2002; Boni et al., 2009) or 6 mg/L Al (Martins et al., 1999; Sibov et al., 1999; Mazzocato et al., 2002) for this purpose. Other studies, based on complete nutrient solution, adjusted the activity (effective concentration) of Al (27-50 mM) based on the genotypes assessed (Doncheva et al., 2005; Piñeros et al., 2005; Caniato et al., 2007; Maron et al., 2008, 2010).

Most studies that have tested periods of exposure of roots to Al found decreased root growth after 24-96 h (Piñeros et al., 2005). Doncheva et al. (2005) reported a reduced rate of cell elongation of the main root in a variety of maize that was Al-sensitive after only a 45-min exposure to 50 mM Al. Maron et al. (2008) found that in the presence of highly contrasting genotypes, only 24 h exposure to Al was sufficient for detecting differences in root growth. However, when longer periods of exposure (96 h) were tested, depending on the Al concentration, the difference between genotypes of contrasting characters is difficult to detect.

Although the reduced root growth increased proportionally with increased Al concentrations, it was not possible to detect significant differences between the hybrids after 96 h exposure. However, this was presumed to have occurred because the long period of exposure to Al inhibited the root growth of the studied hybrids with the same intensity at each Al concentration, considering the cumulative effect of Al in the root cells. Maron et al. (2008) studied the accumulation of Al in the roots of 2 maize genotypes (tolerant and sensitive) exposed to 39 mM Al and found differences between the genotypes regarding the amount of Al present in the roots, as well as a growing accumulation of Al with an increasing exposure period. In the present study, in the experiment with 48 h of exposure, in addition to the deleterious effect of Al on root growth, we detected differences between the hybrids at the studied concentrations, which agreed with the results of Mazzocato et al. (2002) and Conceição et al. (2009).

In our study, the period of 48 h exposure of maize roots to a solution containing 2 mg/L Al enabled us to confirm the suitability of using minimal solution as a fast and efficient approach for selecting genotypes, as well as for identifying sources of Al-tolerance in the hybrids H 44 and H 38.

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