**Crop Science**

ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

# *ZmMATE1* improves grain yield and yield stability in maize cultivated on acid soil

**Renato C. C. Vasconcellos[1]**  |  **Flávia F. Mendes[2]**  |  **Antônio Carlos de Oliveira[3]**  |
**Lauro J. M. Guimarães[3]**  |  **Paulo E. P. Albuquerque[3]**  |  **Marcos O. Pinto[3]**  |
**Beatriz A. Barros[3]**  |  **Maria Marta Pastina[3]**  |  **Jurandir V. Magalhaes[3]**  |
**Claudia T. Guimaraes[3]**

[1] Dep. de Biologia Geral, Univ. Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627, Belo Horizonte, MG 31270–901, Brazil

[2] Faculdade Ciências da Vida, Av. Prefeito Alberto Moura, 12632, Sete Lagoas, MG 35702-383, Brazil

[3] Embrapa Milho e Sorgo, Rodovia MG 424, km 65, Caixa Postal 151, Sete Lagoas, MG 35701–970, Brazil

**Correspondence**
Claudia T. Guimaraes, Embrapa Milho e Sorgo, Rodovia MG 424, km 65, Caixa Postal 151, Sete Lagoas, MG, 35701–970, Brazil.
Email: claudia.guimaraes@embrapa.br

Assigned to Associate Editor Devinder Sandhu.

**Abstract**

The development of aluminum (Al)-tolerant cultivars is a complementary strategy to overcome the constraints caused by Al toxicity on acid soils and can contribute positively to the food supply for the growing global population. A major Al tolerance quantitative trait locus in maize is controlled by a citrate transporter encoded by *ZmMATE1*. Our goal was to evaluate the impact of the superior allele of *ZmMATE1* on the yield performance of maize lines and hybrids cultivated on acid soils. Near-isogenic lines carrying the superior allele of *ZmMATE1*, and the recurrent Al-sensitive parent were crossed with elite lines, generating near-isogenic hybrids contrasting for these alleles. All maize genotypes carrying this superior allele were more Al tolerant in nutrient solution than their isogenic counterparts having the *ZmMATE1* allele derived from the Al-sensitive parent. These genotypes were cultivated in control and Al stress soils for 2 yr. Aluminum toxicity caused a significant yield reduction of 18.7% for lines and 14.7% for hybrids over the 2 yr. The yield performance of maize genotypes declined in the second year compared with the first year, probably due to water deficiency after the grain-filling stage. The superior allele of *ZmMATE1* in maize hybrids conferred yield gains from 21 to 48% compared with the hybrids harboring the alternative allele in the Al stress soil in the first and second years, respectively. As this superior allele is rare in maize and is likely absent in several elite germplasms, molecular breeding based on *ZmMATE1* can improve maize yield stability on acid soils.

# 1 | INTRODUCTION

Maize (*Zea mays* L.) is the most produced cereal in the world and is widely used as feed and food in several countries (Awika, 2011). Food production must double in the next 25 yr to cope with population growth worldwide (McCouch et al., 2013). This increasing global demand requires yield improvement and the expansion of production systems to marginal soils.

Acid soils occupy close to 50% of the potentially arable land worldwide (von Uexküll & Mutert, 1995), including approximately 68% of American, 38% of Asian, and 27% of African tropical regions, where toxicity caused by aluminum (Al) is a major limiting factor for crop production (Yang et al., 2013). Additionally, agricultural practices such as the intensive use of ammonia-based fertilizers and other anthropogenic interventions accelerate the natural process of soil acidification (Rao et al., 1993). Intensive agricultural systems significantly increased soil acidity in an Oxisol in the south of Brazil (Ciotta et al., 2002), as well as in major Chinese crop production areas in long-term studies (Guo et al., 2010). On acid soils, Al is solubilized as a trivalent cation ($Al^{3+}$) which is highly rhizotoxic and inhibits root growth and function (Kochian et al., 2015). Root systems damaged by Al make Al-sensitive cultivars more vulnerable to drought stress and mineral nutrient deficiency (Tang et al., 2001, 2003). Soil acidity decreased maize yield up to 70% in Guadeloupe, Colombia, and Cameroon (Welcker et al., 2005). To minimize this problem, liming is widely applied to neutralize the soil acidity, which is mainly effective in the arable soil layer (Yang et al., 2013) and is not readily accessible to low-income farmers in developing countries (The et al., 2006; Ouma et al., 2013).

Acid soils cover the main agricultural regions in Brazil (Fageria, 2001), where intensive cultivation occurs in two cropping seasons. The first season happens in spring–summer (Sept.–Nov.) with well-distributed rainfall, whereas the second season (autumn–winter, Jan.–Apr.) is highly affected by intermittent rainfall distribution that typically causes long dry spells, mainly at the end of the cropping cycle. Brazil is the third-largest maize producer in the world, with 73% of the total production in 2019–2020 harvested in the second cropping season (CONAB, 2020), making maize production in Brazil highly dependent on rainfall. Variability in rainfall distribution on tropical acid soils has been an important yield-limiting factor that is increasing severity with global climate change (Yang et al., 2013). Hence, Al tolerance, by allowing for better root development on Al toxic soils, is expected to produce a lasting positive impact on drought tolerance, contributing to sustainable maize production.

Aluminum tolerance in maize is a complex trait involving a few genes (Magnavaca et al., 1987; Pandey et al., 1994; Lima et al., 1995), which was confirmed by the identification of up to nine genomic regions associated with Al toler-

> **Core Ideas**
>
> - High Al toxicity on acid soil causes a significant reduction in maize yield.
> - The superior allele of *ZmMATE1* improves maize yield on acid soil.
> - Yield constraints on acid soil are ameliorated by this *ZmMATE1* allele in maize hybrids.
> - This *ZmMATE1* allele is strategic in maize breeding to increase yield stability on acid soils.

ance (Sibov et al., 1999; Ninamango-Cárdenas et al., 2003; Conceição et al., 2009; Guimaraes et al., 2014; Coelho et al., 2019). A major Al tolerance quantitative trait locus (QTL) mapped on maize chromosome 6 (*qALT6*) was co-localized with *ZmMATE1* (Maron et al., 2010), a maize homolog of *SbMATE* that controls sorghum Al tolerance (Magalhaes et al., 2007). *ZmMATE1* is highly expressed in root tips of the Al-tolerant line Cateto Al237 and encodes a citrate transporter of the multidrug and toxic compound extrusion (MATE) family which is responsible for citrate exudation in the rhizosphere (Maron et al., 2010). The presence of three copies in tandem of *ZmMATE1* in Cateto Al237 was associated with improved Al tolerance in maize (Maron et al., 2013) and the introgression of this three-copy allele doubled Al tolerance in maize near-isogenic lines (NILs) compared with the recurrent line, L53 (Guimaraes et al., 2014). Aluminum tolerance is advantageous for crop production on acid soils, as reported for rice (*Oryza sativa*; Kang et al., 2011), wheat (*Triticum aestivum*) and barley (*Hordeum* L.; Tang et al., 2003), and sorghum (*Sorghum bicolor*; Carvalho et al., 2016). The presence of the Al-tolerance allele of *SbMATE* increased grain yield by 0.5 ton $ha^{-1}$ in sorghum hybrids compared with isogenic hybrids carrying the Al-sensitive allele cultivated on acid soil, confirming that Al tolerance conferred by *SbMATE* is crucial for sorghum production on acid soil (Carvalho et al., 2016).

In the present study, a significant increase in grain yield was conferred by the superior allele of *ZmMATE1* in maize lines and hybrids cultivated in a Brazilian acid soil, along with reduced yield losses caused by Al toxicity. These results can stimulate a broad utilization of *ZmMATE1* in marker-assisted breeding to improve maize yield on acid soil.

# 2 | MATERIALS AND METHODS

## 2.1 | Genetic materials

The genetic stocks were derived from the Al-tolerant maize line, Cateto Al237, the donor of the superior allele of

*ZmMATE1* (+*ZmMATE1*), and the Al-sensitive line, L53, the source of the Al-sensitive allele (-*ZmMATE1*). Three NILs (NIL05, NIL13, and NIL19) carrying the *ZmMATE1* allele from Cateto Al237 (+*ZmMATE1*) were obtained by marker-assisted backcrossing with the Al-sensitive recurrent parent, L53, as previously reported (Guimaraes et al., 2014). Near isogenic lines and L53 were 96% similar (Guimaraes et al., 2014).

In order to evaluate the effect of *ZmMATE1* in hybrid combinations, L53 (-*ZmMATE1*), and three NILs (NIL05, NIL13, and NIL19; +*ZmMATE1*) were crossed with four elite lines (-*ZmMATE1*) belonging to different heterotic groups of the Embrapa maize breeding program: 521529, 262841-1-4-1, L228-3, and L3 (Supplemental Table S1), generating four groups of hybrids with uniform genetic backgrounds and contrasting for favorable and unfavorable *ZmMATE1* alleles.

## 2.2 | Aluminum tolerance in nutrient solution

Aluminum tolerance was evaluated in a growth chamber as described by Guimaraes et al. (2014). Four-d-old seedlings were acclimated for 24 h in nutrient solution (Magnavaca et al., 1987) at pH 4.0 under continuous aeration, and the initial root length (IRL) of the seminal root was measured. The seedlings were transferred to containers with and without {39} μM of $Al^{3+}$ activity supplied as $AlK(SO_4)_2 \cdot 12H_2O$. Value in brackets denotes free $Al^{3+}$ activity estimated with GEOCHEM-EZ software (Shaff et al., 2010) that corresponds to 222 μM of Al. The final root length (FRL) of each seedling was measured after 5 d and net root growth (NRG) was calculated as FRL minus IRL under Al ($NRG_{+Al}$) and control (-Al) conditions ($NRG_{-Al}$). The phenotypic index to evaluate Al tolerance was percent relative net root growth (RNRG), calculated as ($NRG_{+Al}/NRG_{-Al}$) × 100.

The experiment was performed in a randomized complete block design with four replicates and seven plants per plot.

## 2.3 | Field experiments

The experiment was carried out at a phenotyping site for Al toxicity located at Embrapa Maize and Sorghum in Sete Lagoas, MG, Brazil (19°27'57" S, 44°14'49" W and 766 m above sea level), in a dark red Oxisol clay under Cerrado vegetation. The sites were sampled in the topsoil (0–20 cm) and in the subsoil (20–40 cm) on square grids of 10 by 10 and 5 by 5 m for the control and Al stress sites, respectively (Carvalho et al., 2016). Average Al saturation in the control site was approximately 2% in the topsoil and 15% in the subsoil, whereas the Al stress site had 56% Al saturation in the topsoil

and 65% in the subsoil (details in Supplemental Table S2 of Carvalho et al., 2016).

Maize lines and hybrids were evaluated in both soil sites in a randomized complete block design with four replicates for lines and three replicates for hybrids. Each plot consisted of a 4-m row, with 0.8 m between rows and 20 plants per plot. The experiments were fertilized with 300 kg ha$^{-1}$ of 8–28–16 (N–P–K) plus 0.3% Zn at sowing, 50 kg ha$^{-1}$ of urea at the vegetative stage, V4, and 50 kg ha$^{-1}$ of urea at V8. Grain yield (GY in kg ha$^{-1}$) was measured by weighing the grains in each plot with adjustment for 13% moisture content.

Trials were conducted during the summer seasons of 2011–2012 and 2013–2014. The first trial (2011–2012) was sown on 23 Jan 2012 and was sprinkle-irrigated during the entire crop cycle. The second trial (2013–2014) was sown on 11 Oct. 2013 and the rainwater was supplemented by irrigation until the flowering stage. For both trials, the soil was at field capacity up to 40-cm depth at planting. The total water retention in the soil was considered uniform in the area and calculated as 1.0 mm for every 10-mm depth (Albuquerque, 2007). Soil water balance was monitored using the irrigation management system developed by Albuquerque (2007), considering daily rainfall and supplemental irrigation. Estimation of daily reference evapotranspiration (ETo) was calculated using the Penman-Monteith equation (Allen et al., 1998), based on the crop coefficient (Kc) per phase and daily climatic data from the local climatological station of the National Institute of Meteorology (INMET; portal.inmet.gov.br).

## 2.4 | Statistical analyses

Analyses of variance (ANOVA) were performed for Al tolerance in nutrient solution and for field experiments for each soil condition (control and Al stress) and year, following a randomized complete block, according to the statistical model:

$$y_{ij} = m + g_i + b_j + e_{ij}$$

where $y_{ij}$ is the observation for the genotype $i$ in block $j$; $m$ is the general mean; $g_i$ is the effect of genotype $i$ ($i = 1, \ldots, 9$ for lines; $i = 1, \ldots, 16$ for hybrids); $b_j$ is the effect of blocks $j$ ($j = 1, \ldots, 4$, for lines, or $j = 1, \ldots, 3$, for hybrids); and $e_{ij}$ is the experimental error.

Combined analyses were performed for the field experiments to test for genotype × year interactions. The least significant difference (LSD) test ($P < .05$) was used to compare the differences between genotypic means. Additionally, $F$ tests were used to contrast the maize genotypes carrying the superior allele of *ZmMATE1* (+*ZmMATE1*) with those carrying the *ZmMATE1* allele derived from the Al-sensitive parent (-*ZmMATE1*). These analyses were carried out using the
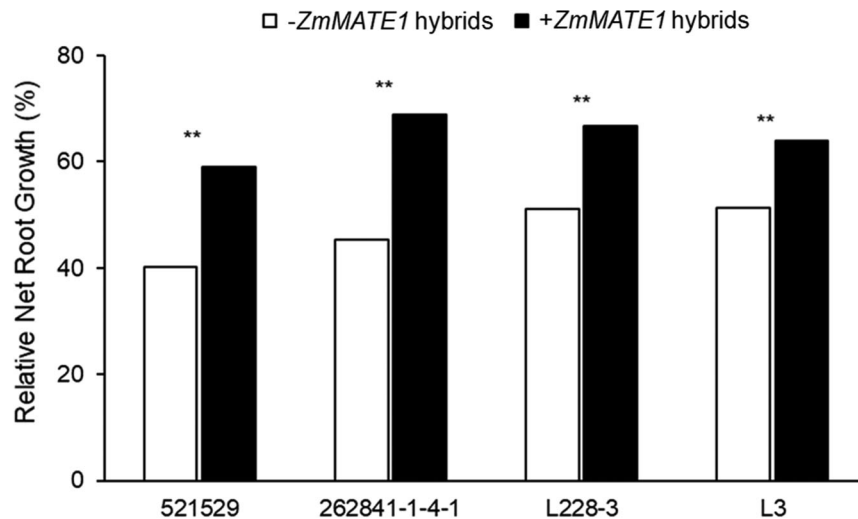
**FIGURE 1** Aluminum (Al) tolerance of maize hybrids contrasting for the *ZmMATE1* alleles. Aluminum tolerance was measured as relative net root growth (%) in nutrient solution with 39 μM of Al. Labels in the *x*-axis depict the common elite lines 521239, 262841-1-4-1, L228-3, and L3 that generated the groups of hybrids without (-*ZmMATE1* hybrids) or with the superior allele of *ZmMATE1* (+*ZmMATE1* hybrids). The -*ZmMATE1* hybrids (white bars) were generated by crosses with L53 (-*ZmMATE1*) whereas +*ZmMATE1* hybrids (black bars) were derived by crosses with three NILs (NIL05, NIL13, and NIL19). **Significant difference for the hybrids contrasting for the *ZmMATE1* alleles by *F* test at *P* < .01

general linear model procedure in the SAS (SAS Institute). The ANOVA and pairwise comparisons of genotypic means were performed separately for lines and hybrids.

# 3 | RESULTS

## 3.1 | *ZmMATE1* improves Al tolerance of maize hybrids in nutrient solution

Aluminum tolerance of 40 maize lines, based on RNRG, was reported by Guimaraes et al. (2014). Aluminum tolerance results of the seven lines used to generate all hybrids evaluated here are shown in Supplemental Figure 1. Briefly, L53 is the most Al-sensitive line and the NILs showed a two-fold increase in Al tolerance compared with L53, due to the introgression of the *ZmMATE1* allele from Cateto Al237, the most Al-tolerant line. Elite lines 262841-1-4-1, L228-3, and L3 showed intermediate Al tolerance similar to the NILs, whereas 521529 was more Al-sensitive than the other elite lines.

The effect of *ZmMATE1* on Al tolerance was evaluated in groups of hybrids either lacking (-*ZmMATE1* hybrids) or carrying the superior allele *ZmMATE1* (+*ZmMATE1* hybrid, Figure 1). The -*ZmMATE1* hybrids were generated by crossing four elite lines (521239, 262841-1-4-1, L228-3, and L3) with L53 (-*ZmMATE1*), whereas +*ZmMATE1* hybrids were derived by crossing the same elite lines with NILs (NIL05, NIL13, and NIL19) having the superior allele of *ZmMATE1*.

The average RNRG of +*ZmMATE1* hybrids was 20 to 34% higher than that of the respective -*ZmMATE1* hybrids (Figure 1). The hybrids derived from elite line 521529 were slightly less tolerant than those derived from the other elite lines (Figure 1), probably due to reduced Al tolerance of the common line 521529, as presented in Supplemental Figure 1.

## 3.2 | Phenotyping sites and environmental characterization

Two phenotyping sites highly contrasting for Al toxicity (control and Al stress) were used to assess the *ZmMATE1* effect on maize grain yield over 2 yr. In the first year (2011–2012), rainfall was supplemented by irrigation, ensuring a sufficient soil water supply throughout the cropping cycle (Figure 2a). In the second year (2013–2014), irrigation was carried out until 60 d after sowing that coincided with the end of flowering time, after which rainfall remained the only water source until the end of the cycle (Figure 2b). A dry spell of 15 d occurred after the grain-filling stage, causing a strong water depletion in the soil between 90 and 112 d after planting (Figure 2b). The mean temperature during the cropping cycle was similar in both years (~20 °C, Supplemental Figure S2), minimizing its influence on plant development between years. Thus, water availability in the soil can be considered a major contrasting environmental component between the 2 yr.

## 3.3 | *ZmMATE1* confers maize yield stability on acid soil

Analysis of variance revealed significant genetic variability among lines and hybrids for grain yield, as well as the significant effects of year and genotype × year interaction under the control and Al stress soils (Table 1). Aluminum toxicity caused a significant maize yield reduction compared with the control site, which was 18.7% for lines and 14.7% for hybrids, on average for both years.

The genotype × year interaction for grain yield was significant for both the control and Al stress sites, whose effects were decomposed in each year. In general, Al toxicity caused yield reduction in maize lines in both years, but induced a stronger

**FIGURE 2** Soil water supply based on rainfall and irrigation applied during the maize cropping cycle in 2 yr. White bars represent rainfall in mm, black bars represent irrigation in mm, black line with dots represents soil water supply in %, and horizontal fine black line represents crop phase. The *x*-axis shows the number of days after planting. The first-year trial (2011–2012) was irrigated during the entire cycle (a) and the second-year trial (2013–2014) was irrigated until the flowering stage, which led to a water depletion after the grain-filling stage (b)
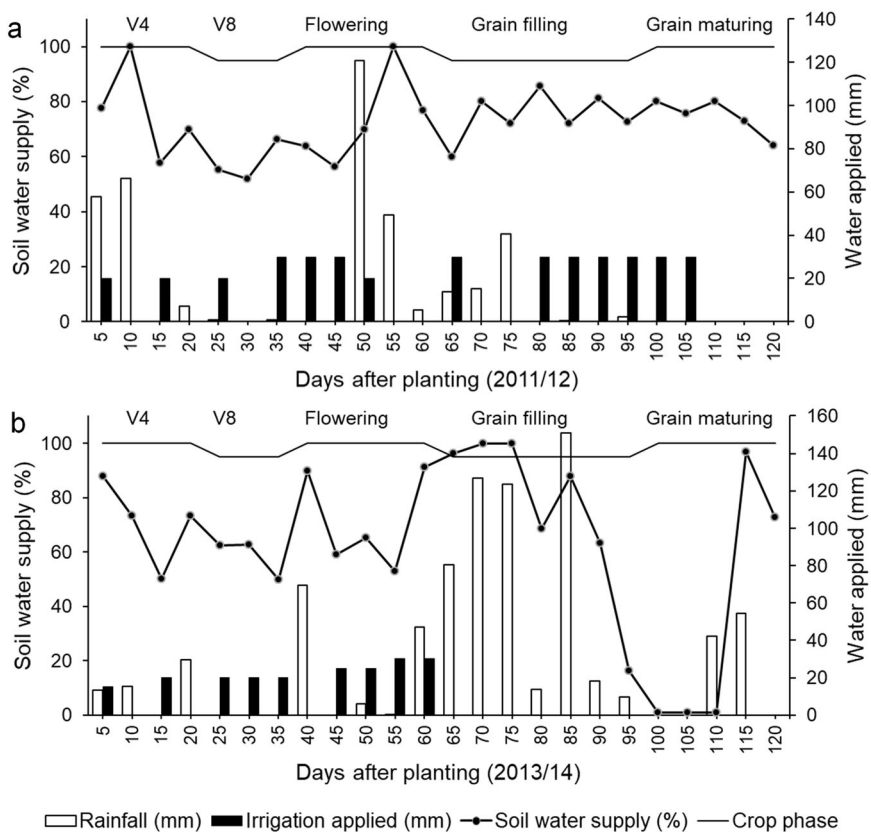


**TABLE 1** Analysis of variance for grain yield of maize lines and hybrids cultivated in control and aluminum (Al) stress soils in 2011–2012 and 2013–2014

| Source of variation | Lines | | Hybrids | |
|---|---|---|---|---|
| | Control | Al stress | Control | Al stress |
| Years | 14.2** | 47.3** | 208.2** | 47.3** |
| Genotype | 206.2** | 31.3** | 4.9** | 4.1** |
| Genotype × year | 6.9** | 2.5† | 3.6** | 1.7 |
| Mean | 3,123A | 2,538B | 8,257a | 7,043b |
| CV, % | 8.04 | 21.21 | 8.93 | 18.26 |

*Note*: Means of grain yield (kg ha$^{-1}$) not sharing a common letter are significantly different at $P < .05$ according to $F$ test. Capital letters were used for lines and lowercase letters were used for hybrids.

†Significant at the .10 probability level.

**Significant at the .01 probability level.

effect and higher yield loss in the second year than in the first (Table 2). Among maize lines, Cateto Al237, the source of the superior *ZmMATE1* allele, maintained a very low yield independent of Al saturation in both years, with minor yield loss conferred by Al (Table 2). L53, the most Al-sensitive line, substantially reduced grain yield under high Al saturation compared with the control site in both years, whereas the yield performance of the +*ZmMATE1* NILs was significantly less affected by Al toxicity than L53 in both years (Table 2).

Yield performance of all maize hybrids and the general mean were smaller in the second year than in the first year

(Table 3). However, the yield loss caused by Al toxicity was slightly lower in the second year (16%) than in the first year (20%; Table 3), probably due to the general yield in the second year being already reduced, lessening the differences caused by Al toxicity. Under high Al saturation, most of the maize hybrids carrying the superior allele of *ZmMATE1* (+*ZmMATE1*) presented higher yield and lower yield losses caused by Al than their counterparts with the *ZmMATE1* allele derived from the Al-sensitive parent (-*ZmMATE1*) in both years (Table 3). In the control site, the majority of maize hybrids contrasting for *ZmMATE1* alleles did not differ for

**TABLE 2** Grain yield and yield loss of maize inbred lines cultivated in control and aluminum (Al) stress soils in 2011–2012 and 2013–2014

| Lines | 2011–2012 | | | 2013–2014 | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Control | Al stress | Yield loss[a] | Control | Al stress | Yield loss[a] |
| | ———kg ha$^{-1}$——— | | % | ———kg ha$^{-1}$——— | | % |
| Cateto Al237 | 1,510a | 1,527a | −1 | 1,467a | 1,290a | 12 |
| L53 | 2,370bc | 1,509a | 36 | 1,950ab | 1,062a | 46 |
| NIL05 | 2,850cd | 2,561bc | 10 | 1,749ab | 1,482a | 15 |
| NIL13 | 3,268d | 2,981c | 9 | 3,151c | 2,594b | 18 |
| NIL19 | 2,046b | 2,192b | −7 | 2,014ab | 1,713a | 15 |
| 521529 | 2,893d | 2,584bc | 11 | 2,294b | 1,363a | 41 |
| 262841-1-4-1 | 4,548e | 4,196d | 8 | 4,663d | 2,399c | 49 |
| L228-3 | 4,529e | 4,053d | 11 | 5,118d | 3,560c | 30 |
| L3 | 5,139f | 5,182e | −1 | 4,663d | 3,432c | 26 |
| Mean | 3,239 | 2,976 | 8 | 3,008 | 2,099 | 28 |

*Note*: For each year and soil combination, means not sharing a common letter are statistically different at $P < .05$ according to least significant difference test.

[a]Yield loss was calculated as the difference between yield in control minus yield in Al stress divided by yield in control soil times 100. Negative values indicated yield gain under Al stress compared with yield in control soil.

**TABLE 3** Grain yield and yield loss of maize hybrids cultivated in control and aluminum (Al) stress soils in 2011–2012 and 2013–2014

| Hybrids[a] | *ZmMATE1* allele | 2011–2012 | | | 2013–2014 | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | Control | Al stress | Yield loss[b] | Control | Al stress | Yield loss[b] |
| | | ———kg ha$^{-1}$——— | | % | ———kg ha$^{-1}$——— | | % |
| 521529 × L53 | - | 9,500a | 6,578a | 31 | 4,764b | 4,285b | 10 |
| 521529 × NILs | + | 7,516b | 6,854a | 9 | 6,577a | 6,024a | 8 |
| 262841-1-4-1 × L53 | - | 8,736a | 7,211b | 17 | 6,232a | 3,940b | 36 |
| 262841-1-4-1 × NILs | + | 9,534a | 8,476a | 11 | 6,991a | 5,710a | 11 |
| L228-3 × L53 | - | 9,546a | 6,581b | 31 | 5,701b | 4,980b | 13 |
| L228-3 × NILs | + | 9,292a | 7,857a | 15 | 7,865a | 7,734a | 2 |
| L3 × L53 | - | 9,413a | 6,412b | 32 | 6,829a | 4,638b | 32 |
| L3 × NILs | + | 10,488a | 9,140a | 13 | 7,456a | 6,971a | 7 |
| Mean | | 9,253 | 7,388 | 20 | 6,552 | 5,539 | 16 |

*Note*: For each year and soil combination, means not sharing a common letter are statistically different at $P < .05$ according to least significant difference test.

[a]Groups of near-isogenic hybrids share uniform genetic background and contrast for *ZmMATE1* alleles derived either from the Al-sensitive parent L53 (-) or the Al-tolerant parent (+), as crosses with three near isogenic lines (NILs; NIL05, NIL13, and NIL19).

[b]Yield loss was calculated as the yield difference between yield in control minus yield in Al stress divided by yield in control soil times 100.

grain yield in the first year, whereas half of the +*ZmMATE1* hybrids performed better than the -*ZmMATE1* hybrids in the second year. An exception was for the hybrids derived from 521529, where the presence of the superior *ZmMATE1* allele significantly reduced grain yield in the first year and conferred similar yield in the second year, compared with -*ZmMATE1* hybrid (Table 3).

## 3.4 | Mean effect of *ZmMATE1* on maize hybrids

The mean effect of *ZmMATE1* on maize yield was assessed using the means of all hybrids contrasting for the *ZmMATE1* alleles (- and +*ZmMATE1*) in each environment, which was a combination of year and Al saturation in the soil. In the first year (2011–2012) in the control site, +*ZmMATE1* and -*ZmMATE1* hybrids presented similar yields greater than 9,000 kg ha$^{-1}$, with a nonsignificant reduction for +*ZmMATE1* hybrids compared with -*ZmMATE1* hybrids (Figure 3). In the first year under Al stress, +*ZmMATE1* hybrids showed 21% higher yield than the -*ZmMATE1* hybrids. In the second year (2013–2014) in the control site, maize yield was approximately 6,000 kg ha$^{-1}$ for -*ZmMATE1* hybrids and 23% higher for +*ZmMATE1* hybrids. In the second year Al-stress site, hybrids harboring the superior allele of *ZmMATE1* presented a yield gain of 48% compared with -*ZmMATE1* hybrids (Figure 3).
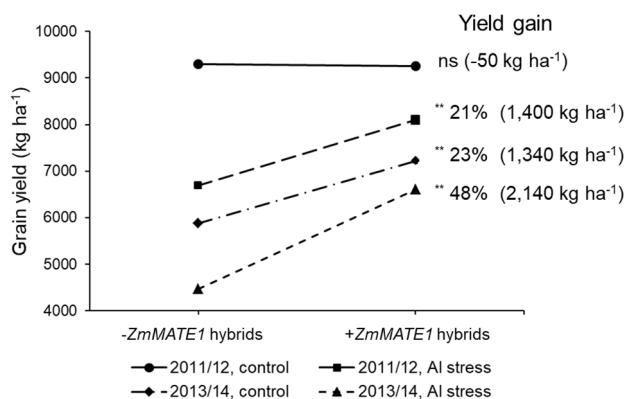
**FIGURE 3** Grain yield and yield gain of maize hybrids contrasting for *ZmMATE1* alleles in each combination of year and aluminum (Al) toxicity. *-ZmMATE1* hybrids represent four hybrids with the allele derived from the Al-sensitive parent, whereas *+ZmMATE1* hybrids represent 12 hybrids carrying the *ZmMATE1* allele derived from the Al-tolerant parent. Each line indicates a combination of year (2011–2012 or 2013–2014) and Al saturation in the soil (control or Al stress). Yield gain was calculated as the yield difference between *+ZmMATE1* hybrids minus *-ZmMATE1* hybrids divided by yield of *-ZmMATE1* hybrids times 100. ns, nonsignificant; ** Significant at the .01 probability level for grain yield of hybrids contrasting for the *ZmMATE1* allele by *F* test

## 4 | DISCUSSION

A suitable combination of genetic stocks, phenotyping sites, and environmental control was used to investigate the contribution of Al tolerance conferred by *ZmMATE1* to maize yield performance on an acid soil. Maize NILs carrying the introgressed region of *qALT6*, where *ZmMATE1* is located, shared approximately 96% of the L53 recurrent genome (Guimaraes et al., 2014). Thus, the differences detected between NILs and L53, as well as their derived hybrids, are likely to be caused by the different *ZmMATE1* alleles. The phenotyping sites contrasted for Al saturation in the topsoil (0–20 cm) and subsoil (20–40 cm), while maintaining similar compositions of other minerals with a small spatial variability (Carvalho et al., 2016). Rainfall and irrigation were monitored daily to calculate the water availability in the soil during the entire cropping cycle in both years. These controlled and well-monitored environmental conditions contributed to the high quality of the phenotypic data and low coefficient of variation.

High Al saturation in the soil caused a significant yield reduction in maize lines and hybrids compared with the control soil, confirming that Al toxicity limits maize yields. The grain yield of maize genotypes was much lower in the second year than in the first year, as shown by the significant effects of year and genotype × year interaction. The water deficit after grain-filling in the second year differed substantially from the fully irrigated trial conducted in the first year, suggesting that

water availability in the soil may have contributed to different yield responses between years.

In general, maize lines and hybrids carrying the superior allele of *ZmMATE1* (*+ZmMATE1*) showed higher grain yield and lower yield losses caused by Al toxicity than their isogenic counterparts with the Al-sensitive allele of *ZmMATE1* (*-ZmMATE1*). The superior allele of *ZmMATE1* encodes a citrate transporter activated by Al in the root tips of Al-tolerant maize lines (Maron et al., 2010) that chelates $Al^{3+}$ in the rhizosphere, reducing its toxicity to the roots. Citrate exudation mediated by MATE transporters has been characterized as an important mechanism of Al tolerance in several crops, such as sorghum (Magalhaes et al., 2007), wheat (Ryan et al., 2009; Garcia-Oliveira et al., 2014), and rice (Yokosho et al., 2011).

In the control soil site, *+ZmMATE1* hybrids presented similar yield performance to *-ZmMATE1* hybrids in the first year, where water availability in the soil was supplemented by irrigation throughout the cycle. Sorghum isogenic hybrids for the *SbMATE* alleles contrasting for Al tolerance also showed similar yield performance at the same soil site (Carvalho et al., 2016). *SbMATE* and *ZmMATE1* are homologs that encode MATE transporters responsible for Al tolerance in sorghum (Magalhaes et al., 2007) and maize (Maron et al., 2010), respectively, suggesting that Al-activated citrate release by these transporters has no potential yield penalty under nonstress conditions. However, one group of *+ZmMATE1* hybrids showed lower yield than its isogenic *-ZmMATE1* hybrid under the control environment, indicating a possible interaction of *ZmMATE1* with maize genetic background that should be considered in maize breeding programs.

In the second year, when water deficiency occurred at the end of the grain-filling stage, *+ZmMATE1* hybrids cultivated in the control site showed a 23% higher grain yield than *-ZmMATE1* hybrids. The control site consisted of 2% Al saturation in the soil surface and 15% in the subsoil, but without characterization in the deeper layers which may remain acid, as shown in Australian limed acid soils (Scott et al., 1997). Yield advantage of maize cultivars tolerant to acid soil was also reported in corrected acid soils of Cameroon (The et al., 2006), indicating that a combination of soil amendments and Al-tolerant cultivars is a good strategy to increase grain yield on acid soils.

In the Al stress site, *+ZmMATE1* hybrids showed 21% greater grain yield compared with *-ZmMATE1* hybrids in the first year, which was fully irrigated, and 48% yield gain in the second year, where water deficiency stress occurred. The superior allele of *ZmMATE1* probably protected the root system of maize *+ZmMATE1* hybrids cultivated under high Al toxicity. Thus, it can be inferred that $Al^{3+}$ in the soil solution activated the ZmMATE1 transporter, allowing the deeper root system to explore a greater extent of the soil, which contributed for an effective yield gain. An Al-tolerant wheat

line produced greater shoot biomass and grain yield than the Al-sensitive isogenic line in a Western Australian acid soil, probably because of the differences in root length in acid subsoil layers (Tang et al., 2003). Aluminum tolerance also conferred yield advantage on acid soil in isogenic sorghum hybrids with the Al-tolerance *SbMATE* allele that increased yields by 0.5 ton ha$^{-1}$ compared with hybrids carrying the Al-sensitive *SbMATE* allele under high Al toxicity (Carvalho et al., 2016). Additionally, sorghum recombinant inbred lines with the superior allele of *SbMATE* presented a yield advantage of 26% compared with recombinant inbred lines carrying the Al-sensitive allele at the same field site (Carvalho et al., 2016), showing the importance of MATE transporters to improve grain yield on acid soil.

It is important to highlight that the yield gain of the +*ZmMATE1* hybrids was magnified under drought stress compared with sufficient water supply in both soil sites. Drought exacerbated the negative effects caused by Al toxicity in two barley cultivars contrasting for Al tolerance and may account for a significant portion of yield reduction commonly observed on acid soils (Krizek & Foy, 1988). In Brazil, postflowering drought stress is frequent in the second cropping season (when most maize is produced) indicating that drought stress on acid soil can severely limit overall Brazilian maize production.

The superior allele of *ZmMATE1* is rare in maize germplasm and the few sources identified so far are restricted to the South American lines: Cateto Al237, Cat100-6, and Il677, two former inbred lines from Brazil and the latter from Bolivia (Maron et al., 2013). *ZmMATE1* is also absent in highly Al-tolerant maize genotypes from Kenya (Matonyei et al., 2014) and was not responsible for Al tolerance in a Kenyan maize population derived from 203B-14 × SCH3 (Matonyei et al., 2020). Thus, this superior *ZmMATE1* allele is likely absent for most maize breeding programs worldwide, requiring a marker-assisted strategy to efficiently introduce this allele into elite germplasms. This superior allele of *ZmMATE1* can improve maize yield stability on acid soils, mainly helping low-input farmers in developing countries.

## AUTHOR CONTRIBUTIONS
C.T.G.: Conceptualization, Supervision, Writing-review & editing. J.V.M.: Conceptualization, Writing-review & editing. L.J.M.G.: Conceptualization, Writing-original. R.C.C.V.: Data curation, Formal analysis, Writing-original draft. B.A.B., M.O.P., and P.E.P.A.: Methodology. A.C.O. and M.M.P.: Formal analysis. F.F.M.: formal analysis, writing-review & editing. All authors read and approved the final manuscript.

## ORCID
*Maria Marta Pastina* https://orcid.org/0000-0002-9112-3457

## REFERENCES
Albuquerque, P. E. P. (2007). *Planilha eletrônica para programação da irrigação em sistemas de aspersão convencional, pivô central e sulcos* (Circular Técnica 97). Embrapa Milho e Sorgo.

Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). *Crop evapotranspiration: guidelines for computing crop water requirements*. Food and Agriculture Organization of the United Nations.

Awika, J. M. (2011). Major cereal grains production and use around the world. In J. M. Awika, V. Piironen, & S. Bean (Eds.), *Advances in Cereal science: implications to food processing and health promotion* (Vol. 1089, pp. 1–13) American Chemical Society. https://doi.org/10.1021/bk-2011-1089.ch001

Carvalho, G., Schaffert, R. E., Malosetti, M., Viana, J. H. M., Menezes, C. B., Silva, L. A., Guimaraes, C. T., Coelho, A. M., Kochian, L. V., Eeuwijk, F. A. van., & Magalhaes, J. V. (2016). Back to acid soil fields: The citrate transporter SbMATE is a major asset for sustainable grain yield for sorghum cultivated on acid soils. *G3 Genes, Genomes, Genetics*, 6(2), 475–484. https://doi.org/10.1534/g3.115.025791

Ciotta, M. N., Bayer, C., Ernani, P. R., Fontoura, S. M. V., Albuquerque, J. A., & Wobeto, C. (2002). Acidification of a Oxisol under no-tillage. (In Portuguese, with English abstract.) *Revista Brasileira de Ciência do Solo*, 26(4), 1055–1064. https://doi.org/10.1590/s0100-06832002000400023

Coelho, C. J., Gardingo, J. R., Almeida, M. C., & Matiello, R. R. (2019). Mapping of QTL for aluminum tolerance in tropical maize. *Crop Breeding and Applied Biotechnology*, 19, 86-94. https://doi.org/10.1590/1984-70332019v19n1a12

Companhia Nacional de Abastecimento (2020). *Acompanhamento de safra brasileira de grãos, Safra 2019/20 - Décimo segundo levantamento*. CONAB.

Conceição, L. D. H. C. S., Tessele, C., & Barbosa Neto, J. F. (2009). Diallel analysis and mapping of aluminum tolerance in corn inbred lines. *Maydica*, 54(1), 55–61.

Fageria, N. K. (2001). Effect of liming on rice, beans, corn and soybean production in cerrado soil. (In Portuguese, with English abstract.) *Pesquisa Agropecuária Brasileira*, 36(11), 1419–1424. https://doi.org/10.1590/s0100-204x2001001100013

Garcia-Oliveira, A. L., Martins-Lopes, P., Tolrá, R., Poschenrieder, C., Tarquis, M., Guedes-Pinto, H., & Benito, C. (2014). Molecular characterization of the citrate transporter gene TaMATE1 and expression analysis of upstream genes involved in organic acid transport under Al stress in bread wheat (*Triticum aestivum*). *Physiologia Plantarum*, 152(3), 441–452. https://doi.org/10.1111/ppl.12179

Guimaraes, C. T., Simoes, C. C., Pastina, M. M., Maron, L. G., Magalhaes, J. V, Vasconcellos, R. C. C., Guimaraes, L. J. M., Lana, U. G. P.,

Tinoco, C. F. S., Noda, R. W., Jardim-Belicuas, S. N., Kochian, L. V, Alves, V. M. C., & Parentoni, S. N. (2014). Genetic dissection of Al tolerance QTLs in the maize genome by high density SNP scan. *BMC Genomics*, *15*(1), 153. https://doi.org/10.1186/1471-2164-15-153

Guo, J. H., Liu, X. J., Zhang, Y., Shen, J. L., Han, W. X., Zhang, W. F., Christie, P., Goulding, K. W. T., Vitousek, P. M., & Zhang, F. S. (2010). Significant acidification in major Chinese croplands. *Science*, *327*(5968), 1008–1010. https://doi.org/10.1126/science.1182570

Kang, D. J., Seo, Y. J., Futakuchi, K., Vijarnsorn, P., & Ishii, R. (2011). Effect of aluminum toxicity on flowering time and grain yield on rice genotypes differing in Al-tolerance. *Journal of Crop Science and Biotechnology*, *14*(4), 305–309. https://doi.org/10.1007/s12892-011-0056-9.

Kochian, L. V., Piñeros, M. A., Liu, J., & Magalhaes, J. V. (2015). Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annual Review of Plant Biology*, *66*, 571-598. https://doi.org/10.1146/annurev-arplant-043014-114822

Krizek, D. T., & Foy, C. D. (1988). Role of water stress in differential aluminum tolerance of two barley cultivars grown in an acid soil. *Journal of Plant Nutrition*, *11*, 351–367. https://doi.org/10.1080/01904168809363808

Lima, M., Miranda Filho, J. B., & Furlani, P. R. (1995). Diallel cross among inbred lines of maize differing in aluminum tolerance. *Brazilian Journal of Genetics*, *4*, 579–584.

Magalhaes, J. V., Liu, J., Guimarães, C. T., Lana, U. G. P., Alves, V. M. C., Wang, Y. H., Schaffert, R. E., Hoekenga, O. A., Piñeros, M. A., Shaff, J. E., Klein, P. E., Carneiro, N. P., Coelho, C. M., Trick, H. N., & Kochian, L. V. (2007). A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nature Genetics*, *39*(9), 1156–1161. https://doi.org/10.1038/ng2074

Magnavaca, R., Gardner, C.O., & Clark, R.B. (1987). Inheritance of aluminum tolerance in maize. In W. H. Gabelman, & B. C. Loughman (Eds.), *Genetic aspects of plant mineral nutrition* (pp. 201–212). Martinus Nijhoff.

Maron, L. G., Guimarães, C. T., Kirst, M., Albert, P. S., Birchler, J. A, Bradbury, P. J., Buckler, E. S., Coluccio, A. E., Danilova, T. V, Kudrna, D., Magalhaes, J. V, Piñeros, M. A, Schatz, M. C., Wing, R. A., & Kochian, L. V. (2013). Aluminum tolerance in maize is associated with higher MATE1 gene copy number. *Proceedings of the National Academy of Science USA*, *110*(13), 5241–5246. https://doi.org/10.1073/pnas.1220766110

Maron, L. G., Piñeros, M. A., Guimarães, C.T., Magalhaes, J. V., Pleiman, J. K., Mao, C., Shaff, J., Belicuas, S. N. J., & Kochian, L. V. (2010). Two functionally distinct members of the MATE (multidrug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. *Plant Journal*, *61*(5), 728–740. https://doi.org/10.1111/j.1365-313X.2009.04103.x

Matonyei, T. K., Barros, B. A., Guimaraes, R. G. N., Ouma, E. O., Cheprot, R. K., Apolinário, L. C., Ligeyo, D. O., Costa, M. B. R., Were, B. A., Kisinyo, P. O., Onkware, A. O., Noda, R. W., Gudu, S. O., Magalhaes, J. V., & Guimaraes, C. T. (2020). Aluminum tolerance mechanisms in Kenyan maize germplasm are independent from the citrate transporter ZmMATE1. *Scientific Reports*, *10*(1), 7320. https://doi.org/10.1038/s41598-020-64107-z

Matonyei, T. K., Cheprot, R. K., Liu, J., Piñeros, M. A., Shaff, J. E., Gudu, S., Were, B., Magalhaes, J. V., & Kochian, L. V. (2014). Physiological and molecular analysis of aluminum tolerance in selected Kenyan maize lines. *Plant and Soil*, *377*, 357–367. https://doi.org/10.1007/s11104-013-1976-6

McCouch, S., Baute, G. J., Bradeen, J., Bramel, P., Bretting, P. K., Buckler, E., Burke, J. M., Charest, D., Cloutier, S., Cole, G., Dempewolf, H., Dingkuhn, M., Feuillet, C., Gepts, P., Grattapaglia, D., Guarino, L., Jackson, S., Knapp, S., Langridge, P., … Zamir, D. (2013). Feeding the future. *Nature*, *499*, 23–24.

Ninamango-Cárdenas, F. E., Guimarães, C. T., Martins, P. R., Parentoni, S. N., Carneiro, N. P., Lopes, M. A., Moro, J. R., & Paiva, E. (2003). Mapping QTLs for aluminum tolerance in maize. *Euphytica*, *130*(2), 223–232. https://doi.org/10.1023/A:1022867416513

Ouma, E., Ligeyo, D., Matonyei, T., Agalo, J., Were, B., Too, E., Onkware, A., Gudu, S., Kisinyo, P., & Nyangweso, P. (2013). Enhancing maize grain yield in acid soils of western Kenya using aluminium tolerant germplasm. *Journal of Agricultural Science and Technology*, *3*, 33–46.

Pandey, S., Magnavaca, R., Bahia Filho, A. F. C., Dugue-Varges, J., & Vinasco, L. E. (1994). Genetics of tolerance to soil acidity in tropical maize. *Crop Science*, *34*, 1511–1514. https://doi.org/10.2135/cropsci1994.0011183x003400060018x

Rao, I. M., Zeigler, R. S., Vera, R., & Sarkarung, S. (1993). Selection and breeding for acid-soil tolerance in crops: upland rice and tropical forages as case studies. *Bioscience*, *43*(7), 454–465. https://doi.org/10.2307/1311905

Ryan, P. R., Raman, H., Gupta, S., Horst, W. J., & Delhaize, E. (2009). A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. *Plant Physiology*, *149*(1), 340–351. https://doi.org/10.1104/pp.108.129155

Scott, B. J., Conyers, M. K., Poile, G. J., & Cullis, B. R. (1997). Subsurface acidity and liming affect yield of cereals. *Australian Journal of Agricultural Research*, *48*(6), 843–854. https://doi.org/10.1071/A96140

Shaff, J. E., Schultz, B. A., Craft, E. J., Clark, R. T., & Kochian, L. V. (2010). GEOCHEM-EZ: A chemical speciation program with greater power and flexibility. *Plant and Soil*, *330*(1), 207–214. https://doi.org/10.1007/s11104-009-0193-9

Sibov, S. T., Gaspar, M., Silva, M. J., Ottoboni, L. M. M., Arruda, P., & Souza, A. P. (1999). Two genes control aluminum tolerance in maize: Genetic and molecular mapping analyses. *Genome*, *42*(3), 475–482. https://doi.org/10.1139/gen-42-3-475

Tang, C., Diatloff, E., Rengel, Z., & Mcgann, B. (2001). Growth response to subsurface soil acidity of wheat genotypes differing in aluminium tolerance. *Plant and Soil*, *236*(1), 1–10. https://doi.org/10.1023/A:1011930205505

Tang, C., Rengel, Z., Diatloff, E., & Gazey, C. (2003). Responses of wheat and barley to liming on a sandy soil with subsoil acidity. *Field Crops Research*, *80*(3), 235–244. https://doi.org/10.1016/S0378-4290(02)00192-2

The, C., Calba, H., Zonkeng, C., Ngonkeu, E. L. M., Adetimirin, V. O., Mafouasson, H. A., Meka, S. S., & Horst, W. J. (2006). Responses of maize grain yield to changes in acid soil characteristics after soil amendments. *Plant and Soil*, *284*, 45–57. https://doi.org/10.1007/s11104-006-0029-9

von Uexküll, H. R., & Mutert, E. (1995). Global extent, development and economic impact of acid soils. *Plant and Soil*, *171*(1), 1–15. https://doi.org/10.1007/BF00009558

Welcker, C., The, C., Andréau, B., De Leon, C., Parentoni, S. N., Bernal, J., Félicité, J., Zonkeng, C., Salazar, F., Narro, L., Charcosset, A., & Horst, W. J. (2005). Heterosis and combining ability for maize adaptation to tropical acid soils: implications for future breeding strategies. *Crop Science*, *45*(6), 2405–2413. https://doi.org/10.2135/cropsci2004.0606

Yang, Z. B., Rao, I. M. & Horst, W. J. (2013). Interaction of aluminium and drought stress on root growth and crop yield on acid soils. *Plant and Soil*, *372*, 3–25. https://doi.org/10.1007/s11104-012-1580-1

Yokosho, K., Yamaji, N., & Ma, J. F. (2011). An Al-inducible MATE gene is involved in external detoxification of Al in rice. *Plant Journal*, *68*(6), 1061–1069. https://doi.org/10.1111/j.1365-313X.2011.04757.x

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.