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Tolerance of high-yield corn hybrids to early aluminum toxicity[1]

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ABSTRACT - Aluminum (Al^{3+}) inhibits plant growth and limits agricultural production in acidic soils with pH ≤ 5.5. However, high-yielding corn hybrids have distinct degrees of Al^{3+} tolerance. Exploring this trait may be useful to improve the sustainable use of acidic tropical soils. Fifteen corn hybrids commonly grown in the central-western region of Brazil were assessed for Al^{3+} stress tolerance during the early plant growth stage. Plants were grown under laboratory conditions with solutions containing 1,000 μmol L^{-1} Ca^{2+} (control) or 200 μmol L^{-1} Al^{3+} combined with 1,000 μmol L^{-1} Ca^{2+} (Al stress). Plant emergence and morphological traits were measured to calculate Al^{3+} toxicity tolerance indices. The results showed that the corn hybrids AG 8701 PRO4, DKB 390 PRO4, and B2800 VYHR had a greater tolerance to Al^{3+} toxicity. These findings offer preliminary insights for agricultural cultivation of corn in acidic soil conditions. However, the mechanisms of tolerance to Al^{3+} toxicity of these corn hybrids still remain unknown. Additionally, the greater stability and tolerance of the corn hybrids AG 8701 PRO4 and DKB 390 PRO4 indicate that these genotypes can be used as genitors in breeding programs to obtain offspring with higher tolerance to Al^{3+} toxicity. On the other hand, the corn hybrids AG 8088 PRO2, AS 1868 PRO4 and BP 2201 VIP3 are more sensitive to Al^{3+} toxicity and should not be recommended for cultivation in acidic soils. Therefore, greater attention to soil corrective practices should be given when using these corn hybrids in agricultural areas.

Key words: Acidic soils. Tolerance index. Al^{3+} stress. *Zea mays*.

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INTRODUCTION

Corn (*Zea mays* L.) is one of the world's main cereal crops. Brazil is currently the third largest corn producer in the world with total production estimated at 118.5 million tons for the 2023/2024 growing season (CONAB, 2024). This corn production plays an important role in Brazilian agribusiness, and approximately two thirds of national production has been produced in the tropical soils of the Cerrado region (Vilela *et al*., 2020). However, tropical Cerrado soils are generally acidic and poor in many essential plant nutrients (Cassol *et al*., 2023; Gomes *et al*., 2019), which can limit the development and production of agricultural crops, especially due to aluminum (Al) toxicity (Cabral *et al*., 2022; Freitas *et al*., 2018).

Acidic soils (pH 5.5 or lower) represent approximately 50% of the world's agricultural areas, which hinders the expansion of economic cultivation of crops for food, fiber, and fuel production (Sade *et al*., 2016). Soil acidification can occur due to natural and/or anthropogenic processes (Bojórquez-Quintal *et al*., 2017). Soil acidification in tropical and subtropical regions is a natural process that occurs due to the partial dissociation of atmospheric carbon dioxide in rainwater, the removal and leaching of basic cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) and anions (Cl^-, NO_3^- and SO_4^{2-}) from the soil, biological nitrogen fixation, and due to the mineralization of soil organic compounds (Zhu *et al*., 2024). However, soil acidification can be aggravated by the reaction of some mineral fertilizers in the soil that release H^+ ions, especially nitrogen fertilizers (Tkaczyk *et al*., 2020; Zhang *et al*., 2022).

Exchangeable aluminum (Al^{3+}) in soil solution is the major limiting factor for the development and yield of agricultural crops grown in acidic soils. Exchangeable Al is produced through the weathering process of soil rock minerals, which remains bound to soil colloid particles. In turn, Al^{3+} in the soil solution becomes available to plants and then results in toxic effects on plants (Kopittke *et al*., 2015). Therefore, understanding and identifying species or genotypes that are less susceptible to the negative effects of Al^{3+} toxicity are alternatives to enhance agricultural production of crops in acidic soils (Steiner *et al*., 2021).

The main effect of Al^{3+} toxicity on plants is the inhibition of root growth, especially due to changes in different cellular mechanisms and structures. However, plant species and genotypes exhibit different degrees of tolerance to Al^{3+} toxicity, which in many situations is due to genetic control (Cabral *et al*., 2022; Freitas *et al*., 2018; Santos *et al*., 2018). Al^{3+} affects DNA synthesis and the regulation of proteins that control the cell cycle (Mohan *et al*., 2013). Therefore, Al^{3+} toxicity initially results in a drastic reduction in the rate of root elongation (Liu *et al*., 2022; Puntel *et al*., 2024) and subsequently induces a reduction in shoot growth due to the inhibition of water and nutrient absorption (Ofoe *et al*., 2023). The toxic effects of Al^{3+} on roots include the emergence of a smaller number and length of lateral roots, lower accumulation of dry matter and smaller root exploration area and are often associated with an increase in the average root diameter (thickening) and root volume (Liu *et al*., 2022; Long *et al*., 2024; Sade *et al*., 2016).

The mechanisms involved in differential tolerance between plant species and genotypes are associated with mechanisms of exclusion or mitigation of Al^{3+} absorption by roots. These mechanisms are induced by changes in rhizosphere pH, excretion of chelating molecules, secretion of mucilage, alleviation of Al^{3+} toxicity with auxin, malate, among other elements, and by the presence of protective structures at the root apex (Bojórquez-Quintal *et al*., 2017; Liu *et al*., 2022; Ofoe *et al*., 2023; Rahman *et al*., 2024). Furthermore, plants also have internal tolerance mechanisms, which are promoted by the chelation of Al^{3+} in the cell cytoplasm and modifications of plant metabolism (Ofoe *et al*., 2023; Rahman *et al*., 2024).

The germination and initial plant establishment phases are potentially the most critical stages of the crop cycle. The Al^{3+} toxicity may limit proper plant stand establishment in the field and compromise crop yield potential. Therefore, the identification of corn hybrids with greater tolerance to Al^{3+} toxicity is important to improve agricultural activity and food production in the acidic soils of the Cerrado crop areas. This research was conducted to evaluate the degree of tolerance to Al^{3+} toxicity of 15 commercial corn hybrids (*Zea mays* L.) during the emergence and initial plant growth stages.

MATERIAL AND METHODS

Study site, Plant materials and Stress treatments

The study was conducted at the Plant Ecophysiology Laboratory, State University of Mato Grosso do Sul (UEMS), in Cassilândia, MS, Brazil (19°05'30.0"S and 51°48'55.0"W). Seeds from 15 commercial corn hybrids for the Brazilian Cerrado region were purchased from the agricultural seed market in the municipalities of Chapadão do Sul and Cassilândia, State of Mato Grosso do Sul, Brazil. Before starting the study, the water content, the mass of thousand seeds, and the germination rate were determined as described in the Rules for Seed Analysis (Brasil, 2009). The main characteristics of corn seeds and hybrids are shown in Table 1.

Table 1 - Agronomic characteristics, thousand seed weight (1,000-SW), water content (WC), and germination rate (GR) of the 15 corn hybrids used in this study

N°	Hybrid	Agronomic characteristics				1,000-SW (g)	WC (%)	GR (%)
		Biotechnological Event	Maturation Cycle	Plant Height (cm)	Yield Potential			
1	AG 8088 PRO2	VT PRO 2®	Early	250	High	330	12.1	82
2	AG 8700 PRO4	VT PRO 4®	Early	235	High	356	12.5	97
3	AG 8701 PRO4	VT PRO 4®	Early	242	High	384	12.3	100
4	AS 1868 PRO4	VT PRO 4®	Early	238	High	379	13.0	83
5	B 2800 VYHR	Leptra®	Early	272	High	372	12.1	91
6	BP 2201 VIP3	Agrisure Viptera® 3	Early	265	High	385	11.9	83
7	DKB 360 PRO3	VT PRO 3®	Early	247	High	390	12.4	82
8	DKB 390 PRO4	VT PRO 4®	Early	245	High	388	12.7	95
9	FS 575 PWU	Power Core® Ultra	Early	245	High	340	11.8	99
10	GALO VIP3	Agrisure Viptera® 3	Early	225	High	365	12.6	94
11	GNZ 7757 VIP3	Agrisure Viptera® 3	Early	240	High	355	12.6	96
12	LG 36745 PRO4	VT PRO 4®	Early	240	High	379	12.0	90
13	MG 545 PWU	Power Core® Ultra	Early	233	High	365	11.9	92
14	ONÇA PRO2	VT PRO 2®	Early	230	High	362	12.5	94
15	STINE 9075 VIP3	Agrisure Viptera® 3	Early	266	High	360	12.8	83

The seeds were first sterilized with 2% (w/v) sodium hypochlorite (NaOCl) solution for 8 minutes and washed immediately with distilled water many times. The sterilized seeds were shade-dried at laboratory room temperature for 96 hours. Finally, the seeds were allowed to germinate under non-stressful (control) and stressful conditions (Al^{3+} toxicity).

Aluminum toxicity stress was induced by exposing seeds to solution containing 200 μmol L^{-1} Al^{3+} and 1,000 μmol L^{-1} Ca^{2+} prepared with aluminum sulfate octadecahydrate [$Al_2(SO_4)_3.18H_2O$] and calcium chloride dihydrate ($CaCl_2.2H_2O$), respectively. A solution containing 1,000 μmol L^{-1} Ca^{2+} was used as control treatment. The pH of the solution containing Al^{3+} was adjusted to 4.3 by adding 0.5 mol L^{-1} HCl. The use of solutions containing between 165 and 220 μmol L^{-1} Al^{3+}, combined with 1,000 μmol L^{-1} Ca^{2+}, has been efficient in determining the degree of tolerance of corn hybrids to Al^{3+} toxicity stress (Mazzocato *et al*., 2002; Paterniani; Furlani, 2002).

Plant Growth Condition

Six 40-seeds replicates were sown in plastic boxes (44 × 30 × 7.5 cm) filled with quartz sand, at a depth of 2.0 cm. The sand used as substrate was previously washed, sterilized and sieved through a mesh with a diameter of 0.05 to 0.8 mm. The sand was then moistened with a solution containing 1,000 μmol L^{-1} Ca^{2+} (control) or a solution containing 200 μmol L^{-1} Al^{3+} combined with 1,000 μmol L^{-1} Ca^{2+} (aluminum toxicity) in a proportion of 70% of the substrate's water retention capacity (Brasil, 2009), equivalent to 120 mL of aqueous solution per kilogram of sand.

The plastic boxes were kept in laboratory conditions using artificial light supplementation with red (620-630 nm) and blue (455-475 nm) wavelengths at the ratio of 85% (red) and 15% (blue) from light-emitting diodes (LEDs) at a 300 ± 60 μmol m^{-2} s^{-1} light intensity, temperature of 25.2 °C (± 2.4 °C) and a photoperiod of 12 h/12 h (light/darkness) cycle, for 18 days.

Measurement of emergence, plant growth, and tolerance indexes

At 18 days after the beginning of the abiotic stresses, the emergence rate of corn seedlings was recorded. Subsequently, five plants per replicate were randomly chosen and the plant height (PH), length of the longest root (LR), total plant length (TPL), total root system length (TRL), root volume (RV), leaf area (LA), and dry matter of shoots and roots were determined.

The plants were separated into shoots (leaves and stem) and roots, oven-dried at 85 °C for three days and then shoot dry matter (SDM), root dry matter (RDM), and total plant dry matter (TDM) were recorded in an analytical balance (±0.0001 g). The PH, LR, and TPL were measured using a ruler. The LA was determined using an automatic leaf area meter (Li-Cor®, model LI-3100, Lincoln, Nebraska, USA). For the determination of TRL and RV, the plant roots were scanned using an optical scanner (Scanjet 4C/T, HP) at 300 dpi resolution, and the digitized images were analyzed with WinRhizo program version 3.8-b (Regent Instrument Inc., Quebec, Canada).

The original data of emergence and morphological traits of corn plants were used to calculate the Al^{3+} stress tolerance indexes (STI) according to Equation 1 used by Zuffo *et al.* (2020):

$$\Delta STI = \left[\left(\Delta \text{ under Stressful conditions} / \Delta \text{ under control conditions} \right) \times 100 \right] \quad (1)$$

where, Δ are the dependent variables measured in corn plants [i.e., E, PH, LA, LR, TPL, TRL, RV, SDM, RDM, and TDM] under stressful conditions (Al^{3+} toxicity) and non-stressful (control).

Experimental design and Statistical analysis

The experimental bioassay was arranged in a completely randomized design (CRD) in a 2 × 15 factorial scheme: two stress treatments [control and aluminum toxicity) and 15 commercial corn hybrids, with six replicates of 40 seeds.

The data were submitted to analysis of variance (ANOVA) and means of stress tolerance indexes were compared by the Scott-Knott test ($\alpha = 0.05$). The analyses were performed using Rbio® software version 140 for Windows (Rbio Software, UFV, Viçosa, MG, BRA).

The identification of tolerant or susceptible hybrids to Al^{3+} toxicity was performed based on all stress tolerance indexes (STI), using three multivariate analysis methods [ranking method, hierarchical clustering analysis (HCA) and principal component analysis (PCA)].

The ranking method was used as proposed by Zuffo *et al*. (2020) and Cabral *et al*. (2022). In this method, a hybrid with the highest value for each STI of plant morphological traits received a ranking score equal to 1, while the hybrid with the lowest value for each stress tolerance index received a ranking score equal to 15. The mean rank ($\bar{R}$) and standard deviation of ranks (SD_R) of all stress tolerance criteria were then calculated. The discrimination of corn hybrids regarding their tolerance degree to Al^{3+} toxicity was performed based on the mean rank score of each genotype, considering the values of the quartiles that divide the 15 possible positions (i.e., 15 corn hybrids) into four equal parts.

A hybrid with a mean ranking ($\bar{R}$) lower than the value of the first quartile (<4.5 points) is classified as tolerant (T); a hybrid with an $\bar{R}$ between the first and second quartiles (4.6 to 8.0 points) is classified as moderately tolerant (MT); a hybrid with an $\bar{R}$ between the value of the second and third quartiles (8.6 to 11.5 points) is classified as moderately susceptible (MS); and the group of Al^{3+}-susceptible hybrids (S) is represented by genotypes with an $\bar{R}$ higher than the value of the third quartile (> 11.6 points).

Hierarchical clustering analysis (HCA) of 15 corn hybrids based on the Euclidean distance method and unweighted pair group method with arithmetic mean (UPGMA) was performed using the Rbio® software version 140 for Windows (Bhering, 2017). The optimal number of Al^{3+} toxicity tolerance groups formed in the dendrogram was defined by Mojena's criterion (Mojena, 1977). This criteria is based on computing the highest amplitude between clusters through the following formula: $\alpha_j > \breve{\alpha} + \omega S_\alpha$, where j = (1, 2, …, n) is the number of clusters; α_j is the correspondence joint point to n − j + 1 clusters; $\breve{\alpha}$ and S_α are the mean and the standard deviation of α'_j s, respectively, and ω is a constant equal to 1.25, as suggested by Milligan and Cooper (1985). Principal component analysis (PCA) based on the correlation matrix of all Al^{3+} toxicity tolerance indices and Biplot analysis were also performed using the Rbio® software version 140 for Windows (Bhering, 2017).

RESULTS AND DISCUSSION

The seeds of the corn hybrids used in this study had an initial water content between 11.8 and 13.0%. Corn hybrids had thousand seed weights ranging from 330 to 390 g. The germination rate was greater than 80% for all corn hybrids (Table 1). The minimum germination value required for the commercialization of corn seeds in Brazil.

The coefficient of variation (CV) values obtained in this experimental study were less than 10% for all morphological traits of corn plants (Table 2). This value is considered low for laboratory experiments with agricultural plants. According to Pimentel-Gomes (2009), the CV is a measure of dispersion or variability that allows conclusions to be drawn about the experimental precision of scientific research, and when the values are less than 10%, it indicates that the results of the experimental bioassay have excellent precision.

The stress tolerance index for plant emergence (E) ranged from 81% to 100%, allowing the differentiation of corn hybrids into four groups. The hybrids AG 8700 PRO4, AG 8701 PRO4, DKB 390 PRO4, FS 575 PWU, GALO VIP3 and GNZ 7757 VIP3 represented the group with the highest values of the Al^{3+} toxicity tolerance index. The hybrids AS 1868 PRO4, BP 2201 VIP3 and LG 36745 PRO4 represented the group with the lowest values of the Al^{3+} toxicity tolerance index (Table 2). Seed germination potential is a key factor for growing plants under adverse soil conditions, such as Al^{3+} toxicity (Ofoe *et al*., 2023). Therefore, in acidic soils with high levels of toxic Al^{3+}, the use of hybrids belonging to the group with the highest values of Al^{3+} stress tolerance indexes can be an excellent alternative to enhance food production and crop profitability in several tropical regions of the world.

Table 2 - Stress tolerance indices for emergence and plant growth traits of the 15 commercial corn (*Zea mays* L.) hybrids exposed to aluminum toxicity stress

Corn hybrid	Stress tolerance index (%)									
	E	PH	LA	LR	TPL	TRL	RV	SDM	RDM	TDM
AG 8088 PRO2	88 c	82 d	57 d	64 e	72 c	62 c	112 b	37 g	59 d	47 g
AG 8700 PRO4	99 a	100 b	84 b	69 e	83 c	63 c	104 c	102 b	68 c	85 c
AG 8701 PRO4	100 a	101 b	98 a	114 a	107 a	60 c	113 b	101 b	74 b	85 c
AS 1868 PRO4	82 d	90 c	50 e	72 d	82 c	47 e	68 f	66 e	31 g	47 g
B 2800 VYHR	96 b	100 b	86 b	92 b	97 b	54 d	77 e	104 b	91 a	97 b
BP 2201 VIP3	81 d	116 a	34 g	83 c	97 b	76 a	88 d	58 f	51 e	54 f
DKB 360 PRO3	86 c	83 d	68 d	76 d	80 c	45 e	68 f	145 a	45 f	78 d
DKB 390 PRO4	99 a	98 b	101 a	87 b	92 b	48 e	67 f	90 c	96 a	98 b
FS 575 PWU	99 a	97 b	80 c	80 c	88 c	62 c	116 b	93 c	76 b	85 c
GALO VIP3	99 a	83 d	43 f	83 c	83 c	50 d	100 c	80 d	69 c	75 d
GNZ 7757 VIP3	99 a	94 c	72 c	88 b	91 b	59 c	83 d	100 b	61 d	83 c
LG 36745 PRO4	82 d	92 c	77 c	68 e	80 c	44 e	74 e	130 a	74 b	104 a
MG 545 PWU	88 c	93 c	91 b	74 d	83 c	70 b	134 a	103 b	67 c	84 c
ONÇA PRO2	97 b	103 b	63 d	90 b	96 b	80 a	104 c	98 b	55 e	73 d
STINE 9075 VIP3	93 b	88 c	84 b	91 b	89 b	55 d	74 e	84 d	44 f	64 e
Mean	92	94	72	82	88	58	92	93	64	77
Number of groups	4	4	6	5	3	5	6	7	7	7
CV (%)	3.79	7.72	5.36	8.78	8.73	7.33	5.40	4.73	8.08	4.45

Mean followed by distinct letters on the column for the corn hybrids show significant differences by the Scott-Knott test at the 0.05 level of confidence. CV: coefficient of variation. **Abbreviations:** E: emergence; PH: plant height; LA: leaf area; LR: length of longest root; TPL: total plant length; TRL: total root system length; RV: root volume; SDM: shoot dry matter; RDM: root dry matter; TDM: total plant dry matter

The stress tolerance index lower than 100% for plant emergence indicates that Al^{3+} toxicity inhibited the seed germination process, resulting in the lowest plant emergence rate in most corn hybrids. The lower germination rate of seeds exposed to Al^{3+} toxicity has been reported in other plant species. Long *et al.* (2024) showed that Al^{3+} stress ($\geq$ 0.5 $mmol \cdot L^{-1}$) significantly inhibited the germination rate and vigor index of sophora seeds [*Sophora davidii* (Franch.) Skeels]. Choudhury and Sharma (2014) also reported that Al^{3+} stress inhibited the germination process of chickpea seeds (*Cicer arietinum* L.). Similarly, Puntel *et al.* (2024) showed that Al^{3+} stress ($\geq$ 1.5 mmol L^{-1}) significantly inhibited the germination rate of white oat seeds (*Avena sativa* L.). However, Alves *et al.* (2022) reported that Al^{3+} toxicity did not impair the germination process of purple corn. Although the exact mechanisms of Al-induced inhibition of seed germination still remain unknown. Samad *et al.* (2017) showed that ionic stress caused by the accumulation of K^{+}, Cl^{-} and Al^{3+} ions in the plumule and radicle of plants under Al^{3+} stress conditions are mainly responsible for the inhibition of seed germination process.

For the stress tolerance indices of plant height (PH) and leaf area (LA), four and six groups of corn hybrids were separated, respectively. The hybrids AG 8700 PRO4, AG 8701 PRO4, B 2800 VYHR, BP 2201 VIP3, DKB 390 PRO4, FS 575 PWU and ONÇA PRO2 represented the two superior groups with the highest values of the Al^{3+} stress tolerance index for plant height. The hybrids AG 8700 PRO4, AG 8701 PRO4, B 2800 VYHR, DKB 390 PRO4, MG 545 PWU and STINE 9075 VIP3 represented the two superior groups with the highest values of the Al^{3+} stress tolerance index for plant leaf area (Table 2). Some corn hybrids exposed to Al^{3+} stress expressed typical symptoms of Al^{3+} toxicity in the leaves, which were small, chlorotic, with small necrotic spots on the border, and with the typical appearance of winding.

Considering the stress tolerance indexes of length of the longest roots (LR) and the total root system length (TRL), five groups of corn hybrids were separated. The hybrids AG 8701 PRO4, B 2800 VYHR, DKB 390 PRO4, GNZ 7757 VIP3, ONÇA PRO2 and STINE 9075 VIP3 represented the two superior groups with the highest values of the Al^{3+} stress tolerance index for the length of the longest root. The hybrids BP 2201 VIP3, MG 545 PWU and ONÇA PRO2 represented the two superior groups with the highest values of the Al^{3+} stress tolerance index for the total length of the root system (Table 2). In turn, the stress tolerance index of the total plant length (TPL) ranged from 72% to 107%, allowing the differentiation of corn hybrids into three groups. The hybrid AG 8701 PRO4 represented the group with the highest Al^{3+} stress tolerance index of the total plant length (Table 2).

Plant height under Al^{3+} toxicity conditions represented, on average, 94% of the plant height of the control treatment. The length of the longest root and the total root system length under Al^{3+} stress conditions represented, on average, 82% and 58% of the length of the longest root and the total root system length of the control treatment, respectively (Table 2). These results indicate that the root growth of corn plants was more affected by Al^{3+} toxicity when compared to the shoot growth of the plants. Alves *et al*. (2022) also reported that root growth of purple corn plants was more drastically reduced under Al^{3+} toxicity conditions. In general, the deleterious effects of Al^{3+} from the soil solution have been more evident on the roots than on the shoots, which can be attributed to the low mobility and transport of this element to the plant shoots, as reported by Steiner *et al*. (2012).

High levels of Al^{3+} in the soil solution can cause severe phytotoxic effects and metabolic and cytological changes in most plant species, which inhibits root growth and limits the uptake of water and essential nutrients, limiting plant biomass production (Bojórquez-Quintal *et al*., 2017; Kochian *et al*., 2015; Kopittke *et al*., 2015; Rahman *et al*., 2024). Therefore, the main visual symptom of Al^{3+} toxicity on plants is reduced root growth, which is caused by inhibition of cell elongation and division (Choudhury; Sharma, 2014; Liu *et al*., 2022; Mohan *et al*., 2013). In this context, the tolerance of species or genotypes to Al^{3+} toxicity has been attributed to the ability of plants to maintain root system growth and maintain appropriate levels of essential nutrients in their roots or shoots (Bojórquez-Quintal *et al*., 2017; Rahman *et al*., 2024).

The root volume stress tolerance index ranged from 67% to 134%, allowing the separation of corn hybrids into six groups. The hybrids AG 8088 PRO2, AG 8701 PRO4, FS 575 PWU and MG 545 PWU represented the two superior groups with the highest values of the Al^{3+} stress tolerance index for root volume (Table 2). Stress tolerance index values greater than 100% for root volume of many corn hybrids may be due to the phytotoxic effects of Al^{3+} on root tip cell division and elongation, which results in thickening of the root cell wall and an increase in mean root diameter and root volume (Hartwig *et al*., 2007; Ofoe *et al*., 2023). Root thickening caused by Al^{3+} toxicity has been frequently reported in other plant species. Zhu *et al*. (2019) showed that Al toxicity inhibits root cell elongation and induces root thickening in rice (*Oryza sativa* L.) plants. Sousa-Junior *et al*. (2024) also reported that Al toxicity modified the growth and morphoanatomy of sugarcane (*Saccharum* spp.) roots, increasing root thickness. In roots, the primary symptoms of Al toxicity are manifested by delayed or inhibited elongation of the main axis, thickening of root tips, and reduced number of lateral roots (Ofoe *et al*., 2023; Taiz *et al*., 2017).

Seven groups of corn hybrids were formed for the stress tolerance indexes of shoot dry matter (SDM), roots dry matter (RDM) and total dry matter (TDM). The hybrids AG 8700 PRO4, AG 8701 PRO4, B 2800 VYHR, DKB 360 PRO3, GNZ 7757 VIP3, LG 36745 PRO4, MG 545 PWU and ONÇA PRO2 formed the two superior groups with the highest values of the Al^{3+} stress tolerance index for shoot dry matter, whereas the hybrids AG 8701 PRO4, B 2800 VYHR, DKB 390 PRO4 and LG 36745 PRO4 represented the two superior groups with the highest values of the stress tolerance index for root dry matter (Table 2).

For total dry matter, the hybrids B 2800 VYHR, DKB 390 PRO4 and LG 36745 PRO4 represented the two superior groups with the highest Al^{3+} stress tolerance index values (Table 2). The shoot dry matter accumulation under Al^{3+} toxicity conditions represented, on average, 93% of the maximum shoot dry matter accumulation of plants grown under control conditions. In turn, root dry matter accumulation under Al^{3+} stress conditions represented, on average, 64% of the maximum root dry matter accumulation of plants grown under control conditions (Table 2). This is in line with the findings of Steiner *et al*. (2021) in soybean plants, who highlighted that Al^{3+} toxicity has a greater negative impact on the root system than on the shoots.

The identification of corn hybrids tolerant or sensitive to Al^{3+} toxicity based on only a single stress tolerance index can be contradictory (Table 2). For example, based on the stress tolerance index of leaf area (LA) and longest root length (RL), the hybrid AG 8701 PRO4 was considered tolerant to Al^{3+} stress; however, based on the stress tolerance index of shoot dry matter (SDM), the hybrids DKB 360 PRO3 and LG 36745 PRO4 were considered as tolerant to Al^{3+} toxicity. Therefore, the differentiation of hybrids into different degrees of tolerance to Al^{3+} toxicity should be performed considering all stress tolerance indices through the use of multivariate analysis techniques.

Considering all Al^{3+} stress tolerance indices, the corn hybrid AG 8701 PRO4 expressed the best average classification of the ranking method and, therefore, this hybrid was classified as tolerant to Al^{3+} toxicity. In turn, the hybrids AG 8088 PRO2 and AS 1868 PRO4 received the highest scores in the ranking method and were then classified as sensitive to Al^{3+} stress (Table 3).

Multivariate hierarchical clustering analysis of the 15 corn hybrids based on all stress tolerance indices classified the hybrids into four groups (Figure 1). The first and second groups represented hybrids with intermediate values of Al^{3+} stress tolerance indices, and therefore, corn hybrids belonging to these groups were classified as moderately sensitive and moderately tolerant to Al^{3+} toxicity, respectively. The third group was represented by the corn hybrids with the highest stress tolerance indices and, therefore, was considered the most tolerant group to Al^{3+} toxicity. In turn, the fourth group was represented by the corn hybrid with the lowest stress tolerance indices and, therefore, was considered the group most sensitive to the negative effects of drought stress. Given the above, the corn hybrids AG 8701 PRO4, B 2800VYHR and DKB 390 PRO4 were identified as the most tolerant to Al^{3+} toxicity stress, while the hybrids BP 2201 VIP3, AG 8088 PRO2 and AS 1868 PRO4 were classified as the most sensitive to Al^{3+} toxicity (Figure 2).

Principal component analysis is a versatile statistical method in which the principal components represent the linear combinations of the original variables that explain the maximum of the variance of all dependent variables (Saed-Moucheshi *et al*., 2013). However, to allow accurate interpretation, the percentage of variances retained in the first two principal components must be greater than 80%. In this study, the accumulated variance in the first two principal components was 82.1%, that is, the first and second principal components explained, respectively, 56.8% and 25.3% of the total variance of the dependent variables (Figure 2).

The first principal component can be interpreted as an index of the overall performance of corn hybrids' tolerance to Al^{3+} toxicity. Since the eigenvector values are positive, the highest values of the stress tolerance indexes result in the highest scores of this principal component and indicate the best overall tolerance index of the corn hybrids. Therefore, a higher score in the first principal component indicates that the stress tolerance index of the hybrid is better. The best overall performance indices were recorded for the hybrids AG 8701 PRO4 and DKB 390 PRO4, and therefore, these corn hybrids were classified as tolerant to Al^{3+} toxicity. In turn, the worst overall performance indices were recorded for the corn hybrids AG 8088 PRO2 and AS 1868 PRO4, and these hybrids were classified as sensitive to Al^{3+} toxicity (Figure 2).

Table 3 - Rank, rank mean ($\bar{R}$), and standard deviation of ranks (SDR) of aluminum stress tolerance indices of 15 commercial corn (*Zea mays* L.) hybrids

Corn hybrid	Stress tolerance index										$\bar{R}$ ± SDR	Tolerance level†
	E	PH	LA	LR	TPL	TRL	RV	SDM	RDM	TDM		
AG 8088 PRO2	10	15	12	15	15	6	4	15	10	14	11.6 ± 3.3	S
AG 8700 PRO4	3	5	5	13	9	4	5	5	7	6	6.2 ± 2.1	MT
AG 8701 PRO4	1	3	2	1	1	7	3	6	5	5	3.4 ± 1.9	T
AS 1868 PRO4	14	11	13	12	12	13	13	13	15	15	13.1 ± 0.9	S
B 2800 VYHR	8	4	4	2	3	10	10	3	2	3	4.9 ± 2.7	MT
BP 2201 VIP3	15	1	15	8	2	2	8	14	12	13	9.0 ± 4.8	MS
DKB 360 PRO3	12	14	10	10	13	14	14	1	13	9	11.0 ± 2.8	MS
DKB 390 PRO4	4	6	1	6	5	12	15	10	1	2	6.2 ± 3.7	MT
FS 575 PWU	2	7	7	9	8	5	2	9	3	4	5.6 ± 2.4	MT
GALO VIP3	6	13	14	7	11	11	7	12	6	10	9.7 ± 2.6	MS
GNZ 7757 VIP3	5	8	9	5	6	8	9	7	9	8	7.4 ± 1.3	MT
LG 36745 PRO4	13	10	8	14	14	15	11	2	4	1	9.2 ± 4.4	MS
MG 545 PWU	11	9	3	11	10	3	1	4	8	7	6.7 ± 3.2	MT
ONÇA PRO2	7	2	11	4	4	1	6	8	11	11	6.5 ± 3.1	MT
STINE 9075 VIP3	9	12	6	3	7	9	12	11	14	12	9.5 ± 2.7	MS

† T, refers to a drought-tolerant hybrid, having mean rank ($\bar{R}$) score of 1 to 4.5; MT, moderately tolerant hybrid with mean rank score of 4.6 to 8.0; MS, moderately sensitive hybrid with mean rank score of 8.1 to 11.5; and S, drought-sensitive hybrid with mean rank score of 11.6 to 15. **Abbreviations:** E: emergence; PH: plant height; LA: leaf area; LR: length of longest root; TPL: total plant length; TRL: total root system length; RV: root volume; SDM: shoot dry matter; RDM: root dry matter; TDM: total plant dry matter

Figure 1 - Dendrogram of the hierarchical cluster analysis of the 15 corn hybrids (*Zea mays* L) using the Euclidean distance and the unweighted pair group method with arithmetic mean (UPGMA) based on the aluminum toxicity tolerance indices of the emergence and plant morphological traits

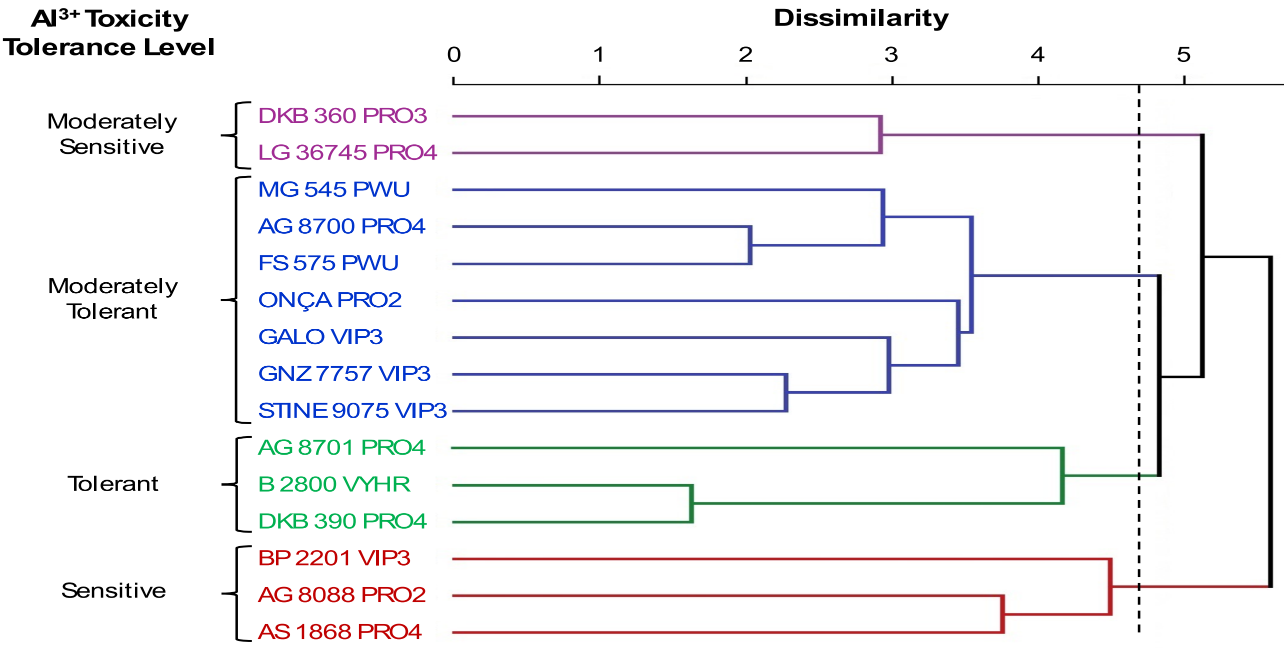
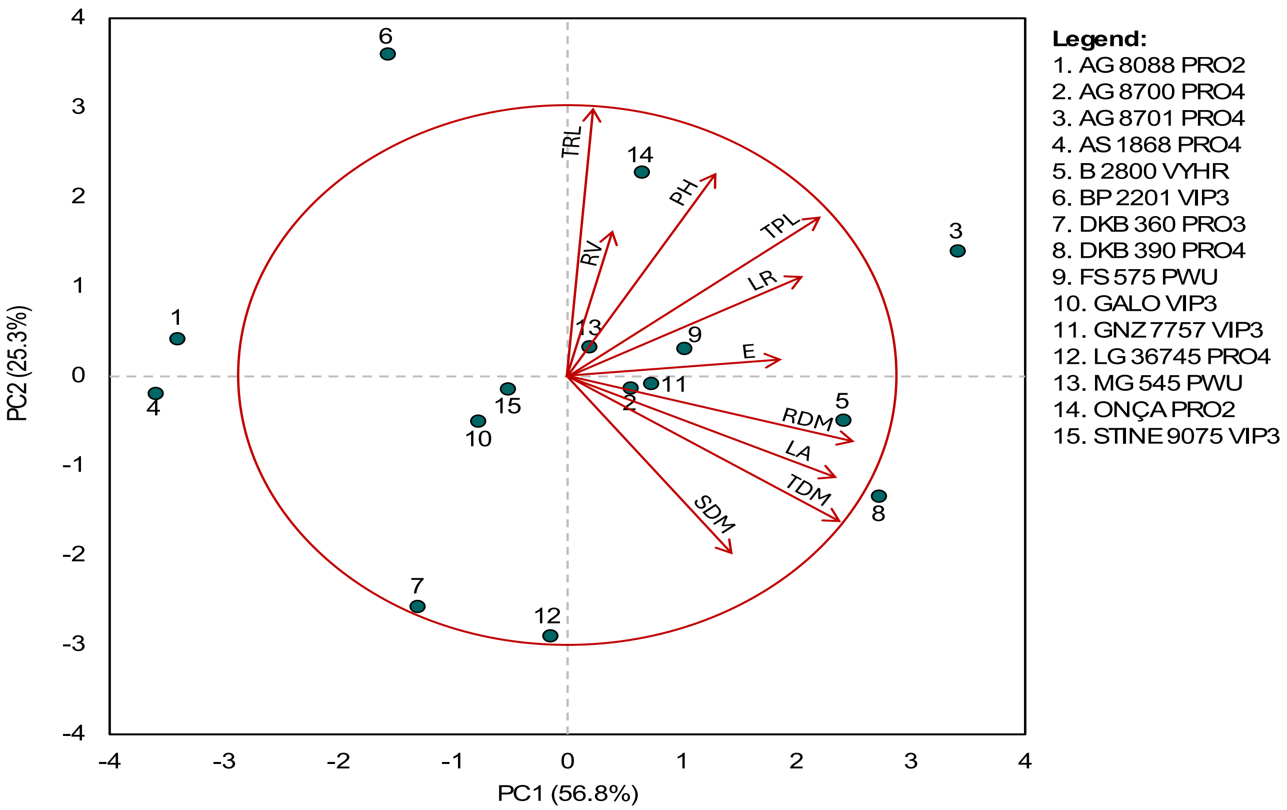


Figure 2 - Biplot diagram based on the first and second principal components (PC) of all aluminum toxicity tolerance indices of morphological traits of the 15 corn hybrids (*Zea mays* L)



Abbreviations: E: emergence; PH: plant height; LA: leaf area; LR: length of longest root; TPL: total plant length; TRL: total root system length; RV: root volume; SDM: shoot dry matter; RDM: root dry matter; TDM: total plant dry matter

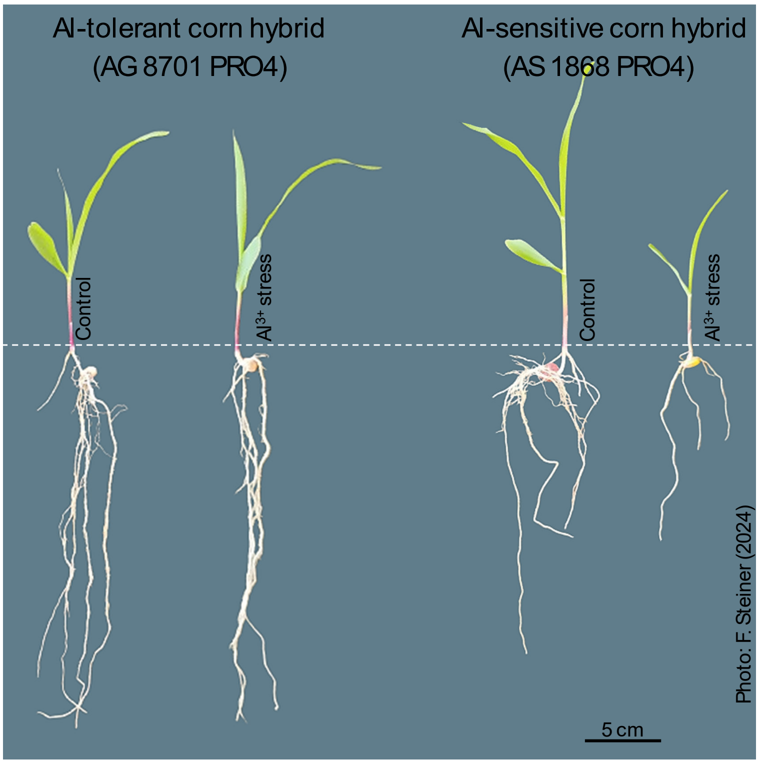
The highest scores of the first principal component (Figure 2) obtained in the stress tolerance indices for total dry matter (TDM), root dry matter (RDM), leaf area (LA) and total plant length (TPL) indicate that these plant morphological traits are more sensitive and appropriate for identifying corn hybrids tolerant to Al^{3+} toxicity. In soybean plants, Cabral *et al*. (2022) showed that root length and total plant length are the most sensitive and suitable morphological traits for selecting cultivars tolerant to Al^{3+} toxicity.

In summary, the three multivariate analysis methods (ranking method, hierarchical cluster analysis, and principal component analysis) were responsible for classifying the corn hybrid AG 8701 PRO4 as tolerant to Al^{3+} toxicity (Table 2, Figures 1 and 2), while the hybrid DKB 390 PRO4 was classified as tolerant to Al^{3+} stress by the hierarchical cluster and principal component analyses (Figures 1 and 2). In turn, the hierarchical cluster analysis also classified the hybrid B2800 VYHR as tolerant to Al^{3+} toxicity. Among these three corn hybrids, we showed that AG 8701 PRO4 and DKB 390 PRO4 are the most stable and tolerant genotypes to Al^{3+} toxicity among the 15 tested hybrids. These two corn hybrids were classified as tolerant to Al^{3+} stress by two or more multivariate analysis techniques, and therefore, AG 8701 PRO4 and DKB 390 PRO4 are the most suitable hybrids to be recommended for cultivation in acidic soils with pH ≤ 5.5. Additionally, from the point of view of plant breeding, these genotypes can be used as parent in corn crossing blocks aiming at obtaining genotypes tolerant to Al^{3+} stress.

Considering that adequate plant emergence is the first step to achieve a satisfactory grain yield, the hybrids selected here show promise for cultivation under Al^{3+} toxicity conditions (Figure 3). However, the mechanisms of tolerance to Al toxicity of these corn hybrids still remain unknown. Therefore, new studies must be carried out to investigate the exact tolerance mechanisms of these corn hybrids. One of the main mechanisms of tolerance to Al toxicity is associated with the ability of corn roots to release organic acids, especially citrate (Alves *et al*., 2022). The exudation of citrate, a tricarboxylic acid trianion ($C_6H_5O_7^{3-}$), by the roots has been effective in mitigating Al^{3+} toxicity by promoting the complexation of apoplastic Al^{3+} and forming stable compounds (chelates) with Al^{3+} ions (Hartwig *et al*., 2007), which reduces the toxic effects of this metal on the root system and maintains root growth and the absorption of water and nutrients.

Our results indicate that among the possible mechanisms of tolerance to Al^{3+} toxicity of the tolerant

Figure 3 - Plants of corn hybrids tolerant (AG 8701 PRO4) and sensitive (AS 1868 PRO4) to aluminum toxicity grown for 18 days under non-stressful (control) and Al^{3+} stress conditions



hybrids obtained in this study, especially the corn hybrids AG 8701 PRO4 and DKB 390 PRO4, is the complexation of Al^{3+} induced by the exudation of organic acids by the roots, which mitigated the harmful effects of Al^{3+} stress and maintained the growth of the roots and shoots of the plants, as can be seen in Figure 3. In turn, for hybrids sensitive to Al^{3+} toxicity, such as the hybrid AG 8088 PRO2, our results showed that these genotypes do not have the capacity to maintain root growth under Al^{3+} stress conditions, which limits water absorption and the shoot growth of the plants (Figure 3).

The ranking method, hierarchical cluster analysis and principal component analysis grouped, respectively, 2, 3 and 2 corn hybrids as sensitive to Al^{3+} toxicity (Table 2, Figures 1 and 2). Corn hybrids AG 8088 PRO2 and AS 1868 PRO4 were classified as sensitive to Al^{3+} stress by the three multivariate analysis methods. In turn, hierarchical cluster analysis also classified corn hybrid BP 2201 VIP3 as sensitive to Al^{3+} toxicity (Figure 1). Therefore, when corn sowing is carried out in acidic soils with high Al^{3+} concentration in the soil solution, these corn hybrids should not be recommended. On the other hand, greater attention to soil corrective practices should be given when using these corn hybrids in agricultural areas.

CONCLUSIONS

1. The corn hybrids AG 8701 PRO4, DKB 390 PRO4, and B2800 VYHR have greater tolerance to Al^{3+} toxicity and are the most suitable genotypes for cultivation in acidic soils;
2. The corn hybrids AG 8088 PRO2, AS 1868 PRO4 and BP 2201 VIP3 are more sensitive to Al^{3+} toxicity and should not be recommended for cultivation in acidic soils.

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