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Wheat cultivars reared in nutritive solution screening towards aluminum toxicity tolerance

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ABSTRACT

Aluminum present in acidic soils hinder plant development and growth, while impacting grain productivity in susceptible wheat cultivars. The objective of this study was to evaluate aluminum toxicity tolerance in commercial wheat cultivars reared in a nutritive solution. The experimental design was completely randomized with four repetitions. The treatments were arranged following a split-plot, with a total of 12 wheat genotypes reared in a nutritive solution that contained Aluminum and one which was absent of Al. The wheat seedlings were maintained in a climate-controlled chamber, at 23°C ± 1 ºC, with constant photoperiod and aeration. After 96 hours, the following characteristics were evaluated: root number (RN), shoot length (SL), longest root length (R1), second longest root length (R2) and sprout dry mass (DM). Variance analysis was performed on the data and the means were classified utilizing Scott & Knott (5% significance). The results revealed the genetic variation between the cultivars, highlighted was the cultivar ORS Ágile which was sensitive towards aluminum, just as the sensitive witness Anahuac 75. The cultivar BRS Atobá demonstrated greater tolerance, as did BRS Sabiá and TBIO Sossego.

Keywords: soil acidity, toxic aluminum, hydroponics, genotype selection, *Triticum aestivum* L.

INTRODUCTION

During the 2023 harvest, 798.97 million tons of wheat was produced which highlights this grain, as one of the most harvested in the world, along with maize (*Zea mays*) and rice (*Oryza sativa*).[(1)] In the 2024 harvest season, a total of 7.9 million tons of wheat were harvested, and the Brazilian southern region alone was responsible for a 6.7 million tons harvest.[(2)]

Aluminum toxicity is very common in wheat cultivating regions, especially in acidic soils, and in some regions the damage is so intense that it represents “blight” symptoms.[(3)] This phenomenon occurs due to aluminum’s negative influence towards the growth and development of wheat. The set of symptoms observed in the wheat crop range from a violet leaf color to leaf scorching and plant stunting. In greater blight symptom scenarios, sensitive plants do not produce tillers and the ears, when produced, are small.[(4)]

Aluminum toxicity is one of the main cultivating restrictions in acidic soils. Toxic aluminum (Al^{3+}) inhibits root development which compromises water and nutrient absorption, while also causing physiological damage to the plants. This limiting factor is further emphasized by other soil conditions, such as low phosphorus availability and high acidity, both deterrents of wheat productivity.[(5)]

Screenings are routinely conducted to identify acidity and aluminum tolerant genotypes. Hydroponic rearing with a known aluminum concentration has previously resulted in successful access screening for common-bean (*Phaseolus vulgaris*)[(6)] and wheat genotypes.[(7)] Additionally, this screening process could assist in distinguishing the tolerance levels of different wheat cultivars to other elements with toxic concentrations, such as iron and manganese, which are commonly found in acidic soils.[(8)]

One of the main strategies to minimize the effects of aluminum toxicity is through the use of tolerant cultivars. Their identification requires efficient methodologies that can evaluate plant responses under controlled conditions, such as in hydroponic systems. In this context, this study aimed to assess aluminum toxicity tolerance in wheat cultivars, using a hydroponic system to differentiate between tolerant and sensitive genotypes.

MATERIAL AND METHODS

The experiment was conducted at the Instituto de Desenvolvimento Rural do Paraná - IAPAR-EMATER (IDR-Paraná), in Londrina, Paraná State (23°23′S, 50°01′W, altitude of 556 m), Brazil. Twelve wheat cultivars were evaluated for aluminum toxicity tolerance, and the witnesses were the Anahuac 75 cultivar (sensitive) and IAC 5-Maringá (tolerant) (Table 1).

Stock nutritional solution

The wheat aluminum tolerance evaluation was described by Baier et al.[(9)] and Voss et al.[(10)] Initially a stock solution was created, individually, with the following pure for analysis reagents and respective quantities: a) 58.80g of $CaCl_22H_2O$ L^{-1}; b) 65.70g of de KNO_3 L^{-1}; c) 50.80g of $MgCl_2.6H_2O$ L^{-1}; d) 1.30g of $(NH_4)2SO_4$ L^{-1}; e) 3.20g of NH_4NO_3 L^{-1}. For the aluminum stock solution, 0.894g of $AlCl^3$ was dissolved in distilled water using a 100mL volumetric flask. The final solution’s concentration was

Table 1. Evaluated wheat cultivars for aluminum tolerance while reared in a nutritive solution

Treatment	Cultivars	Pedigree
1	IAC 5 - Maringá *	II 12300//Lerma Rojo 65/815656 /3/Norteño 67
2	Anahuac 75 *	Frontana / Kenya 58//PG1
3	IPR Potyporã	PF 973518 / LD 975
4	TBIO Ponteiro	Fuste / TBIO Mestre
5	TBIO Sossego	BIO 08400 'S' / Quartzo // Quartzo
6	BRS Atobá	BRS Tangará/BRS 220
7	BRS Jacana	WT 02092/BRS Tangará
8	BRS Sabiá	BRS 210/PF 980583
9	IPR Catuara	LD 975 / IPR 85
10	ORS Ágile	ORL 05831/IOR 09001//Abalone
11	TBIO Audaz	TBIO Toruk / Celebra
12	IPR 144	SERI*3/BUC/5/BOW/3/CAR 853/COC//VEE/4/OC 22

* Witnesses: IAC 5 – Maringá: tolerant; Anahuac 75: sensitive.

1mg of Al mL^{-1}. Every solution was stored in a dark bottle and light-free environment.

The stock solutions had the following final compositions and concentrations: a) 400 mmol L^{-1} of $CaCl_2$; b) 650 mmol L^{-1} of KNO_3; c) 250 mmol L^{-1} of $MgCl_2$; d) 10 mmol L^{-1} of $(NH_4)2SO_4$; e) 40 mmol L^{-1} of NH_4NO_3 (pH 4,0). One mL of each stock solution was then dissolved together in one liter of deionized and distilled water to form the nutritive solution. The pH was adjusted to 4.0 utilizing HCl (0.1 N).

Seed preparation and germination

The seeds were treated to external sanitation with a commercial sodium hypochlorite solution (2%) and distilled water with a 1:3 (v:v) proportion for four minutes. The solution amount was just enough to cover every seed. Then, the seeds were thoroughly washed three times with distilled water. 50 seeds were equidistantly conditioned in transparent plastic containers (11 width x 11 length x 3.5 cm height) over two distilled-water soaked Germitest® papers. Two containers were produced for each cultivar. The seeds were kept in climate chambers for 48 hours at a temperature of 16 ± 1 ºC and constant photoperiod.

Hydroponic essay preparation and execution

Three liters of the nutritive solution was added to a plastic container (40 width x 24 length x 7.5 cm height). Two containers were used, one primed with the aluminum solution (2 mL per liter) and the other absent. Sprouted seeds with 0.5 cm radicle were transferred to a plastic sheet (2 mm mesh) and kept floating inside the plastic containers. The radicles were inserted into the mesh. Each cell was formed by eight pre-sprouted seeds. The containers were stored in a climate-controlled chamber for 96 h, at 23 ºC ± 1 ºC with constant aeration and photoperiod. The solutions were substituted after 24, 48 and 72 h. At every substitution the aluminum solution was also reapplied.

Data collection

After 96 hours the containers were then transferred to a freezer to halt development. A ruler (cm) was used to measure the two longest roots (R1 and R2) and the shoot length (SL) for each seedling, the number of roots (NR) was also noted. A six sprout mean was established for each repetition.

The previously mentioned seedlings were also weighed to determine their dry mass (DM). The cotyledons were removed utilizing a scalpel. Then, the seedlings were stored in a paper bag and transferred to an air circulation chamber (40 °C) for 24 hours. Later, every sample was kept in a dehydrator prior to being weighed. The dry mass is a result of the total repetition weight divided by the seedling count, which results in a mean seedling dry weight (mg).

Reduction Index (RI)

The reduction index was performed for every evaluated characteristic using the following equation (1):

$$RI\,(\%) \;=\; 100 - \frac{(CAl * 100)}{AAl} \tag{1}$$

where CAl: evaluated characteristics containing the aluminum solution and AAl: evaluated characteristics absent of the aluminum solution.

Statistical analysis

The experimental design was completely randomized with four repetitions. The treatments were distributed following a split-plot. The treatments were characterized by the stock solution aluminum concentration (zero and 2 mg Al L^{-1}). The subdivided plots were established by the twelve wheat cultivars. Six seedlings composed the experimental unit, and the parcel mean was determined using the following equation (2):

$$Y_{ijk} = m + P_i + \epsilon_{ij} + S_k + (PS)_{ik} + \epsilon_{ijk} \tag{2}$$

where Y_{ijk} = treatment i obtained data, for genotype k, and repetition j; m = essay general mean; P_i = i-th Al treatment data (i = 1, 2); ε_{ij} = random error a; S_k = k-th genotype effect (k = 1, 2, 3 ... 14); $(PS)_{ik}$ = i-th treatment and k-th genotype interaction effect; ε_{ijk} = random error b.

The plant aerial length (PA), the two longest roots (R1 and R2) and dry mass (DM) were variance analyzed (ANOVA), followed by the F test ($p < 0.05$). The means were compared using the Scott & Knott test at 5% probability. The statistical program Genes was used during these analyses.[11]

RESULTS AND DISCUSSION

For all the evaluated characteristics, a significant effect was found for the isolated solution factor. In hydroponic cultivation with aluminum, there was a decrease in the average values for root length (R1, R2, and R1 + R2), shoot length (SL), and seedling dry mass (DM) (Table 2). The average number of roots per seedling (NR) was higher in the presence of aluminum.

The isolated genotype factor was significant for all characteristics, indicating variability among the cultivars. This was expected since cultivars have different genetic compositions, and consequently, their responses to toxic aluminum also varied in magnitude. The effect of the solution × genotype interaction was significant, indicating that each genotype reacts differently to aluminum toxicity, depending on its intrinsic tolerance levels.

Wheat cultivars exposed to aluminum stress showed reduced shoot and root development (Figure 1). The shoot length of the cultivars IAC 5 - Maringá, BRS Atobá, and BRS Sabiá did not show a statistically significant reduction when reared in the presence of aluminum (Figure 1).

The root length sum (R1 + R2) of BRS Atobá was not affected by aluminum, as indicated by the absence of statistical differences in root length between treatments containing and absent of aluminum (Figure 1). The root length sum of IAC 5 - Maringá showed a slight decrease of 9.8% in the presence of aluminum (Table 3). The genotypes most affected by aluminum were Anahuac 75 and ORS Ágile, with a root reduction index exceeding 72% (Table 3). Aluminum inhibits root cell expansion, elongation and prevents cell division.[12] The results confirm the plants' physiological reactions towards aluminum toxicity, given that the site of action occurs at the root apex. Previous studies have identified distinct phenotypic classes for aluminum tolerance based on differences in root development.[13]

It was observed that the number of roots (NR) tended to be higher in the presence of aluminum (Figure 2), leading to negative values in the reduction index's (RI) estimation for NR. It is suggested that aluminum-induced stress stimulated seedlings to produce more roots, despite inhibited development and reduced length.[14] The increase in root number, observed in some cultivars under aluminum stress, may represent a compensatory response of seedlings towards root growth inhibition. Although toxic aluminum restricts root elongation, the formation of additional lateral or adventitious roots can be stimulated as an adaptive mechanism to maintain water and nutrient uptake. Similar responses have been reported in cereal species exposed to abiotic stresses, where changes in root

Table 2. Variance analyses (ANOVA) for longest root (R1) and second longest root (R2), the sum of the longest roots (R1 + R2), number of roots (NR), shoot length (SL) and seedling dry mass (DM), evaluated for twelve wheat cultivars reared in a nutritional solution present and absent of aluminum

Variance Source	**DF**	**Mean square**					
		R1	**R2**	**R1 + R2**	**RN**	**SL**	**DM**
Solution	1	80.722**	69.581**	300.157**	1.122**	69.564**	99.838**
Genotype	11	11.314**	8.021**	37.877**	1.28**	10.448**	13.268**
S x G	11	2.562**	2.669**	10.172**	0.322**	2.189**	2.299**
Error	72	0.061	0.060	0.199	0.088	0.321	0.751
CV (%)		6.51	8.28	6.59	7.11	7.13	9.51
Factor	Treatment	R1	R2	R1 + R2	NR	PA	DM
Solution	Absent of Al	4.71 a	3.81 a	8.53 a	4.08 b	8.80 a	10.13 a
	Containing Al	2.88 b	2.11 b	4.99 b	4.29 a	7.09 b	8.10 b
Genotype	IAC 5 - Maringá	5.9 a	4.9 a	10.8 a	4.9 a	10.1 a	11.6 a
	Anahuac 75	2.6 f	2.0 h	4.7 g	4.3 b	7.2 d	9.3 c
	IPR Potyporã	2.0 h	1.5 i	3.6 i	3.8 c	5.9 e	7.4 d
	TBIO Ponteiro	3.4 e	2.7 e	6.2 e	4.1 c	7.1 d	7.9 d
	TBIO Sossego	3.2 e	2.3 g	5.6 f	3.9 c	7.5 d	7.6 d
	BRS Atobá	4.8 b	4.1 b	8.9 b	3.5 d	7.9 c	9.4 c
	BRS Jacana	5.0 b	3.9 b	9 b	4.4 b	9.7 a	10.6 b
	BRS Sabiá	4.5 c	3.4 c	7.9 c	4 c	8 c	9.6 c
	IPR Catuara	3.7 d	2.8 e	6.6 d	3.8 c	8.2 c	10 c
	ORS Ágile	3.4 e	2.6 f	6 e	4.4 b	7.3 d	7.8 d
	TBIO Audaz	4.7 c	3 d	7.8 c	4.8 a	8.7 b	9.1 c
	IPR 144	2.4 g	1.7 i	4.1 h	4.3 b	7.8 c	9.2 c

DF = Degrees of freedom. CV = Coefficient of variation; ** significance at $p \leq 0.01$, according to F test. Means followed by different lowercase letters in the 'Solution' or 'Genotype' factor column differ from each other according to the F test or Scott & Knott test ($p \leq 0.05$), respectively.

system architecture partially compensate for reductions in primary root growth.[12,13] However, the increased number of roots does not necessarily indicate tolerance, since root functionality and total soil exploration capacity may still be severely reduced.

The DM values showed that dry mass decreased in all genotypes exposed to aluminum, confirming the damage caused by stress (Figure 3). However, this reduction may not be an immediate indicator of aluminum toxicity, as dry mass reflects accumulated plant growth over time.[3] Since aluminum primarily affects roots, changes in the shoot length and DM may only become evident in later stress stages.[7] Therefore, root growth is a more sensitive parameter for distinguishing tolerant and susceptible genotypes in hydroponic systems.[15]

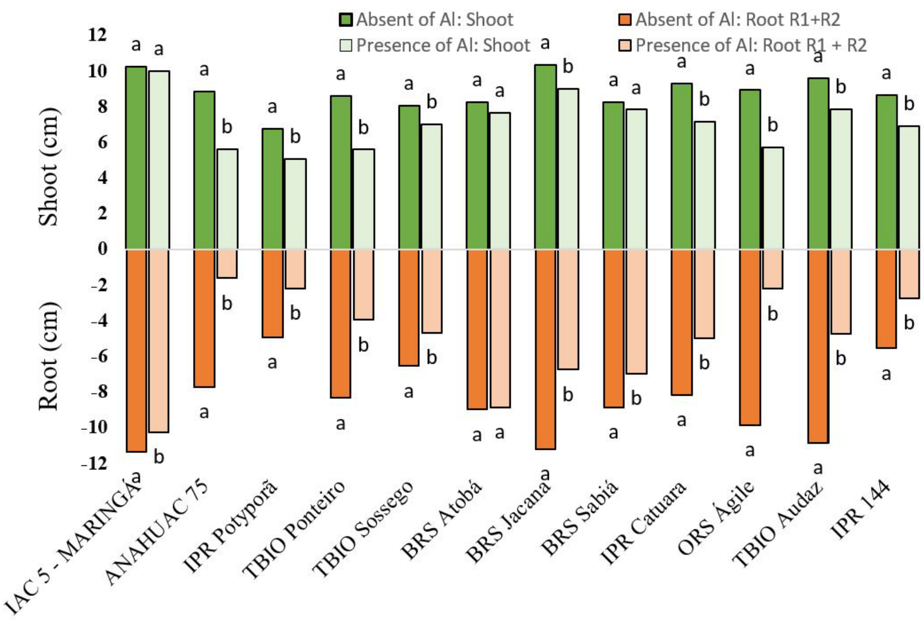

Figure 1. Shoot and the sum of the 1st and 2nd largest root length (cm) of twelve wheat cultivars seedlings evaluated in hydroponic solution in the absence and presence of Al. Mean values compared in the presence and absence of Al followed by different letters, differ from each other according to the F test (analysis of variance – ANOVA).

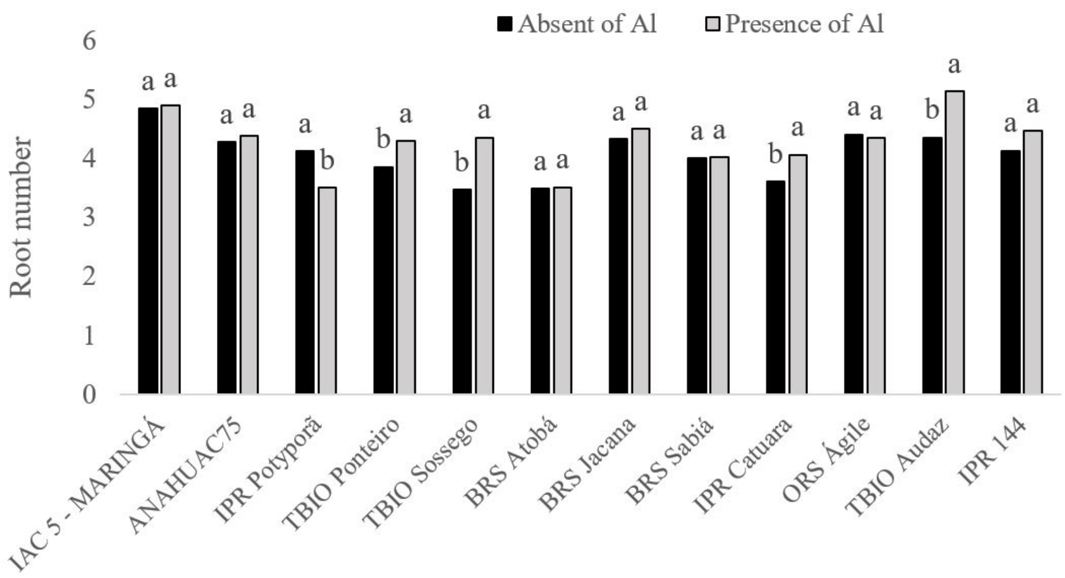

Figure 2. Number of roots evaluated in twelve wheat cultivars seedlings reared in hydroponic solutions in the absence and presence of aluminum.

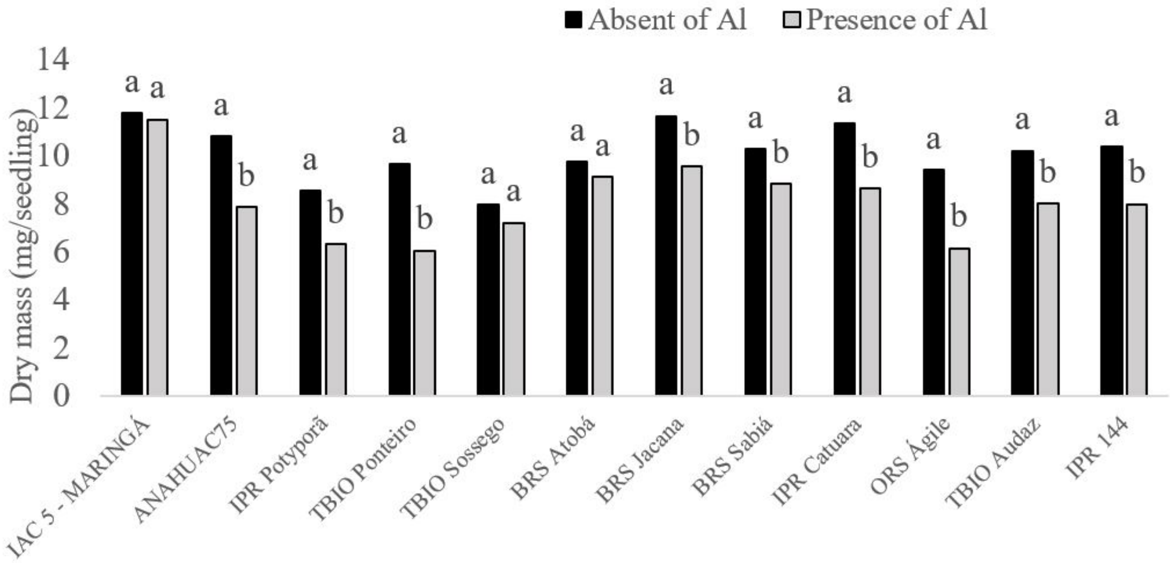

Figure 3. Dry mass evaluated in twelve wheat cultivars seedlings reared in hydroponic solutions in the absence and presence of aluminum.

To identify genotypes with tolerance to toxic aluminum, the reduction index (RI) was used as a criterion. The results allowed grouping genotypes according to their tolerance level, using the reference cultivars IAC 5 – Maringá (tolerant) and Anahuac 75 (sensitive). Considering the reduction index estimates for root development characteristics (R1, R2, and R1 + R2), the most tolerant cultivar was BRS Atobá, along with the witness IAC 5 – Maringá. The cultivars BRS Sabiá and TBIO Sossego were classified as moderately tolerant, as their roots were less affected.

ORS Ágile was the most negatively affected cultivar, classified in the same group as the susceptible witness Anahuac 75, as it showed the highest RI for root characteristics (R1, R2, and R1 + R2), shoot length, and dry mass. It is clear that aluminum impaired root system development in these seedlings, which is consistent with studies by Degenhardt et al.,[16] who found that root growth inhibition is the first visible symptom, potentially leading to other damage such as impaired water and mineral absorption.

Rapid observation of physiological responses to aluminum stress in seedling development can be observed, while using the hydroponic rearing methodology, when compared to field-level methods, where early injuries may only become apparent later, when crop establishment is affected.[17] At the cellular level, aluminum triggers a series of injuries that compromise essential root structures, including damage to the cell wall, limiting cell expansion; alterations in the plasma membrane,[18] affecting integrity and ion transport selectivity; and cytoskeletal dysfunctions, impacting cell organization and structural support.[19] Additionally, aluminum can reach the cell nucleus, interfering with cell division and gene expression, worsening toxicity effects.[20]

These observed afflictions highlight the effectiveness of the methodology in capturing the impacts of aluminum over plant roots, providing insights for identifying more tolerant genotypes. When a plant has genetic characteristics for aluminum tolerance, negative interference does not occur due to the exclusion of aluminum via organic acid exudation triggered by aluminum presence at the root apex.

The contrasting responses observed among cultivars may be associated with differences in the expression of genes involved in aluminum tolerance. In wheat, tolerance is frequently associated with the ALMT1 and TaMATE gene families, which promote the release of malate and citrate from root apices, reducing aluminum toxicity in the rhizosphere.[21,22] The reduced root growth inhibition observed in tolerant cultivars suggests that these physiological mechanisms may be more effective in preventing aluminum accumulation in sensitive root tissues.

Similarly, Pereira[7] evaluated and classified aluminum tolerance in 20 wheat cultivars based on the root development.

Tabela 3. Reduction index (%) for longest root length (R1), second longest root length (R2) and the sum of the longest roots (R1 + R2), number of roots (RN), shoot length (SL) dry mass (DM), evaluated in twelve wheat cultivars seedlings reared in hydroponic solutions in the absence and presence of aluminum

Treat.	Genotype	Reduction index (%)					
		R1	R2	R1 + R2	NR	SL	DM
1	IAC 5 - Maringá	9.3 f	9.9 e	9.8 e	-1.5 a	2.4 c	1.6 b
2	Anahuac 75	78.5 a	79.6 a	79.0 a	-2.6 a	36.6 a	26.9 a
3	IPR Potyporã	55.9 b	52.2 b	54.5 b	14.8 a	24.5 b	25.4 a
4	TBIO Ponteiro	45.7 c	59.8 b	52.3 b	-12.9 b	35 a	37 a
5	TBIO Sossego	31.1 d	24.1 d	28.3 d	-26.1 b	12.8 c	8.7 b
6	BRS Atobá	1.6 f	0.5 e	1.0 e	-1.0 a	7.2 c	4.8 b
7	BRS Jacana	39.7 c	39.9 c	39.8 c	-4.0 a	12.9 c	17.1 b
8	BRS Sabiá	20.1 e	22.6 d	21.0 d	-2.5 a	4.7 c	13.5 b
9	IPR Catuara	36.5 c	41.2 c	38.8 c	-13.5 b	22.4 b	23.5 a
10	ORS Ágile	72.8 a	82.7 a	77.5 a	1.0 a	35.9 a	34.4 a
11	TBIO Audaz	45.8 c	69.9 a	56.5 b	-17.9 b	17.9 c	21.3 a
12	IPR 144	43.5 c	58.4 b	50.3 b	-8.3 a	20 c	23.1 a

Means followed by different lowercase letters differ from each other according to Scott & Knott test ($p \leq 0.05$).

These results reinforce the importance of using hydroponic systems as an efficient methodology for assessing aluminum tolerance in wheat cultivars. This approach clearly distinguishes tolerant and sensitive genotypes, while highlighting the genetic variability among evaluated cultivars. From a breeding perspective, the identification of commercial cultivars with greater aluminum tolerance is particularly relevant because these materials may serve as parents in crossing programs aimed at developing cultivars adapted to acidic soils. The superior performance observed in BRS Atobá suggests the presence of favorable alleles associated with aluminum tolerance mechanisms, such as organic acid exudation and aluminum exclusion from root tissues. These cultivars may therefore contribute to increasing the frequency of tolerance alleles in segregating populations and accelerate the development of genotypes suitable for low-input production systems.

Although hydroponic screening is an efficient and widely adopted method for identifying wheat genotypes with contrasting responses to aluminum toxicity, some limitations should be considered. The controlled conditions used in hydroponic systems do not fully reproduce the complexity of field environments, where aluminum toxicity interacts with additional factors such as soil physical properties, phosphorus availability, water deficit, soil microbiota, and genotype × environment interactions. Therefore, the classification obtained in hydroponic conditions should be considered an important preliminary screening step and, whenever possible, complemented by field evaluations in acidic soils to confirm cultivar performance under commercial growing conditions.

CONCLUSIONS

The hydroponic methodology efficiently differentiated wheat cultivars regarding aluminum toxicity tolerance and demonstrated substantial genetic variability among the evaluated genotypes. ORS Ágile exhibited a response similar to the susceptible control Anahuac 75, confirming its sensitivity to toxic aluminum. In contrast, BRS Atobá showed the highest level of tolerance, followed by BRS Sabiá and TBIO Sossego, which displayed moderate tolerance. These findings provide valuable information for wheat breeding programs by identifying promising sources of aluminum tolerance that may be used in parent selection and in the development of cultivars better adapted to acidic soils.

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DATA AVAILABILITY STATEMENT

The entire data set supporting the results of this study was used in this article.

AUTHOR CONTRIBUTIONS

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Writing – review & editing: Christiane dos Santos Silva, Luciano Mendes de Oliveira, Juliana Sawada Buratto.

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