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Constitutive Changes in Nutrients and Phytochemicals in Kernels of Aluminium-Tolerant Maize (*Zea mays* L.)

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Abstract: Maize (*Zea mays* L.) is among the three most important food crops worldwide. Maize growth is affected by high aluminium content in acid soils, which constitute nearly 50% of the world's cultivable area. Therefore, the cultivation of aluminium-tolerant maize hybrids could be a healthier alternative and an attractive food source in these regions. In this regard, to produce hybrids kernels, 16 inbred lines aluminium-tolerant (Al-T) and aluminium-susceptible (Al-S) maize were screened for their constitutive patterns of selected nutrients and phytochemicals. Proximate analysis, free phenolic acids (FPA) and cell wall-bound phenolic acids (CPA) contents, as well as antioxidant capacity (AOX) were assayed in the anatomical kernel parts (pericarp, endosperm, and germ). Kernels of Al-T maize contained significantly higher germ protein, oil, and fibre (2.9, 3.0, and 0.5%, respectively) than Al-S kernels (1.9, 1.8, and 0.3%, respectively). Importantly, the nutraceutical contents in terms of pericarp FPA and germ CPA were significantly higher in kernels belonging to Al-T maize (92 mg and 140 mg EGA/100 g). The highest AOX was observed in germ CPA of Al-T kernels (9.0 mmol TE/100 g). The results herein indicate that Al-tolerance mechanisms induce positive changes in the nutrients and phytochemicals; this implies that the hybrids generated using Al-T maize inbred lines could emerge as an attractive source of nutrients and phytochemicals in farming regions containing acid soils.

Keywords: *Zea mays*; phenolic acids; aluminium-tolerant; nutrients; phytochemicals

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1. Introduction

Maize (*Zea mays* L.) is among the most important food crops worldwide [1]. Maize, rice, and wheat provide at least 60% calories and 50% protein to consumers in developing countries [2]. Essentially, maize production and yield should increase constantly in order to satisfy the increasing demand for food, given the expected population growth [3]. Unfortunately, maize production is limited in developing countries of America, Asia, and Africa due to the presence of large extensions of acidic soils (pH < 5.5) that represent around 50% of the world's cultivable area [3]. These acidic soils constitute about 30% of the total land in the world and 70% of potentially arable land in the world [4] (Panda et al., 2009).

Aluminium (Al) toxicity is the primary factor limiting plant growth in acidic soils and is mainly responsible for the reduction in crop yields [5,6]. This toxicity reduces root water and nutrient uptake due to both root damage and growth inhibition [7,8]. Previous studies have reported that maize has an important heritable tolerance to acidic soil stress [9,10]. In addition, the use of Al-tolerant (Al-T) maize genotypes could enhance productivity in a sustainable system by minimising root damage in acidic soils [6,11].

The tolerance of maize genotypes to Al is a complex process that involves multiple genes and physiological mechanisms, which are still unclear [12]. The resistance mechanisms are also affected by fundamental plant and kernel composition in terms of macro- and micronutrients. Therefore, the cultivation of aluminium-tolerant maize hybrids could be a healthier alternative and an attractive food source in these regions. This research was conducted to investigate the involvement of constitutive nutrients and phytochemicals

associated with Al-tolerant maize kernel genotypes. In this regard, to produce hybrids kernels, 16 inbred lines aluminium-tolerant (Al-T) and aluminium-susceptible (Al-S) maize were screened for their constitutive patterns of selected nutrients and phytochemicals. Furthermore, a more detailed study was conducted using their dissected kernel tissues (pericarp, endosperm, and germ). These anatomical parts were compared in terms of proximate composition, free phenolic acids (FPA), cell wall-bound phenolic acids (CPA), and antioxidant capacity (AOX).

The practical implication of this research is that Al-tolerant inbred lines may produce hybrids that are capable of overcoming nutrient deficiencies when planted in acid soils. In addition, they could help in reducing the problem of food scarcity in the investigated areas and at the same time provide important nutrients and nutraceuticals for inhabitants of developing countries. As a preliminary study, we expect that in future studies a detailed evaluation of the effect of Al concentration on specific PA and its profile in maize kernels should be the main focus.

2. Results

2.1. Morphological Parameters and Proximate Composition

Significant changes in morphological of both Al-T and Al-S kernels were observed. In Al-T kernels, a difference of 61% in flotation index was observed in comparison with Al-S kernels (Table 1). In terms of the anatomical parts, an increment of 66% in germ quantity and a decrease of 6% in endosperm quantity were found in Al-T kernels when compared to Al-S kernels. Moreover, the kernel anatomical structures significantly differed between the two lines. The tolerant lines contained 66% more protein, 83% more oil and 67% more fibre in the germ (2.9% vs. 1.9% protein; 3.0% vs. 1.8% oil; 0.5% vs. 0.3% fiber), as well as 7% less starch and 15% less fibre in the endosperm. In contrast, the pericarp structures of both Al-T and Al-S lines showed no significant difference in terms of proximal composition (Table 2). These results clearly indicate that tolerance to Al was related to changes in kernel anatomical proportions and compositions, particularly the nutrients associated with the endosperm and germ.

2.2. Phytochemical Composition

A similar trend was observed when the phenolic compounds and AOX of tolerant and susceptible inbred lines were compared. Significant differences in pericarp and germ FPA and CPA were observed. Total phenolic content and pericarp FPA of Al-T kernels were approximately 90% higher (Figure 1A) than those of Al-S kernels (92 mg vs. 46 mg EGA/100 g); Al-T kernels germ CPA were 26% higher compared with Al-S kernels germ (Figure 1B) (140 mg vs. 122 mg EGA/100 g). The significant differences found in phenolic compounds between Al-T and Al-S lines indicate that the mechanism of tolerance to Al could enhance the nutraceutical composition of the kernel, especially in terms of antioxidants.

2.3. Antioxidant Activity

The AOX of each genotype is depicted in Figure 2. Comparison between average AOX of Al-T and Al-S indicated similar AOX for free compounds in all the anatomical structures investigated with ranges between 1.2 mmol to 5.3 mmol of TE/100 g (Figure 2A). The same trend was observed when cell wall-bound compounds associated with both the pericarp and endosperm were compared (Figure 2B). In contrast, a significant difference in the germ AOX of cell wall-bound compounds (an increase of 23% in Al-T with 9.3 vs. 7.2 mmol TE/100 g of Al-S) was found (Figure 2B). These findings confirm that AOX may positively change due to Al tolerance mechanisms. Nevertheless, this result was only observed at the germ structure.

Table 1. Comparison in main morphological and anatomical characteristics of selected maize inbred lines tolerant and susceptible to aluminum.

Genotype	Type	Morphological Properties							Anatomical Proportions (%)												Kernel Dimensions (mm)								
		Color	Tex		FI		1000 K		Pericarp			Endosperm			Germ			Tip			Width			Long			Thickness		
CLA161	T	YW	1.2	35	±	5	192.8	± 0.2	5.5	±	0.2	78.3	±	0.1	13.9	±	0.3	2.2	±	0.1	7.9	±	0.5	7.7	±	0.3	4.4	±	0.4
CLA309	T	YW	0.5	0	±	0	195.7	± 1.4	7.6	±	0.2	77.5	±	1.1	11.7	±	0.9	3.1	±	0.4	7.4	±	0.4	8.5	±	0.4	4.2	±	0.4
CLA37	T	YW	2.3	10	±	2	229.0	± 0.3	5.4	±	0.1	79.8	±	0.6	11.7	±	0.3	3.0	±	0.3	7.8	±	0.2	9.4	±	0.6	4.4	±	0.3
CML-483	T	WH	2.7	0	±	0	289.9	± 6.5	4.2	±	0.0	84.6	±	0.3	9.2	±	0.1	2.0	±	0.3	8.8	±	0.2	9.1	±	0.3	4.8	±	0.1
CLA307	T	YW	2.2	20	±	2	229.2	± 8.0	5.7	±	0.0	83.2	±	0.3	9.4	±	0.4	1.7	±	0.3	8.8	±	0.2	8.5	±	0.4	4.8	±	0.4
CLA41	T	YW	2.6	65	±	5	195.2	± 1.9	5.7	±	0.1	82.8	±	0.2	7.9	±	0.3	3.6	±	0.2	9.1	±	0.4	9.0	±	0.4	3.8	±	0.2
CLA44	T	YW	3.0	10	±	0	204.5	± 1.1	5.9	±	0.3	80.4	±	0.7	10.6	±	0.8	3.1	±	0.3	7.3	±	0.3	7.9	±	0.4	5.0	±	0.5
CLA18	T	YW	2.8	20	±	5	203.9	± 3.4	4.0	±	0.1	84.4	±	0.6	8.5	±	0.5	3.1	±	0.2	8.4	±	0.5	9.1	±	0.3	4.7	±	0.2
CLA81	T	YW	2.2	15	±	5	216.6	± 2.5	5.7	±	0.0	82.3	±	0.6	10.1	±	0.8	1.9	±	0.2	8.2	±	0.3	9.4	±	0.2	4.3	±	0.3
CLA84	T	YW	2.2	25	±	5	227.2	± 1.7	5.8	±	0.2	81.4	±	0.6	9.3	±	0.3	3.5	±	0.2	8.9	±	0.3	10.6	±	0.5	4.3	±	0.4
DTPWC9	S	WH	3.4	15	±	5	234.8	± 2.4	3.9	±	0.1	83.5	±	0.1	9.9	±	0.2	2.8	±	0.2	8.1	±	0.3	8.5	±	0.3	5.0	±	0.5
LaPosta	S	WH	2.5	60	±	5	263.7	± 3.5	5.9	±	0.1	84.2	±	0.9	6.6	±	0.4	3.3	±	0.7	8.7	±	0.4	10.6	±	0.2	4.7	±	0.3
CML311B	S	WH	2.7	60	±	6	132.8	± 0.7	4.2	±	0.1	87.2	±	0.8	5.4	±	0.2	3.2	±	0.5	7.7	±	0.4	7.0	±	0.4	4.5	±	0.4
CLA35	S	YW	2.5	55	±	5	157.1	± 2.7	6.2	±	0.2	81.2	±	0.2	7.1	±	0.1	5.4	±	0.5	7.5	±	0.4	8.6	±	0.7	3.8	±	0.4
P390aC3	S	YW	2.1	0	±	0	309.0	± 5.2	5.2	±	0.2	85.4	±	0.4	6.3	±	0.4	3.2	±	1.0	9.0	±	0.2	9.9	±	0.1	4.8	±	0.5
DTPWC9	S	WH	2.3	60	±	5	186.8	± 2.7	4.5	±	0.2	90.1	±	0.6	4.9	±	0.4	0.5	±	0.2	8.1	±	0.7	9.3	±	0.2	3.8	±	0.1
Mean	T		2.2	20	±	3	218.4	± 2.7	5.6	±	0.1	81.5	±	0.5	10.2	±	0.5	2.7	±	0.2	8.3	±	0.3	8.9	±	0.4	4.5	±	0.3
	S		2.6	42	±	5	214.0	± 2.9	5.0	±	0.2	85.2	±	0.5	6.7	±	0.3	3.1	±	0.5	8.2	±	0.4	9.0	±	0.3	4.4	±	0.4
Tukey-Test			ns		*		ns		ns			*			*			ns			ns			ns			ns		

Data represents the average of three replicates ± standard derivation. Abbreviations: T = tolerant to aluminum, S = susceptible to aluminum, Tex = texture of endosperm, FI = Flotation Index, 1000 K = Thousand-kernel (g) weight. * Significant difference at $p < 0.05$; ns = non-significant.

Table 2. Comparison proximal analysis by kernel structure of selected maize inbred lines tolerant and susceptible to aluminum.

Genotype	Type	Protein (%)				Oil (%)				Starch (%)				Fiber (%)				Ash (%)			
		Per	End	Ger	Tot	Per	End	Ger	Tot	Per	End	Ger	Tot	Per	End	Ger	Tot	Per	End	Ger	Tot
CLA161	T	0.2	7.6	3.8	11.6	0.1	1.9	3.7	5.6	0.1	60.0	0.5	60.5	0.3	0.9	0.7	1.9	0.1	1.0	0.1	1.1
CLA309	T	0.2	8.7	2.6	11.6	0.1	2.0	2.7	4.7	0.1	57.6	2.2	59.8	0.5	1.0	0.4	1.9	0.1	0.9	0.1	1.1
CLA37	T	0.0	9.0	2.4	11.4	0.1	2.0	3.2	5.2	0.1	58.2	2.1	60.2	0.3	0.9	0.3	1.6	0.1	0.9	0.0	1.0
CML-483	T	0.0	9.1	2.5	11.6	0.0	1.8	2.6	4.5	0.0	61.5	0.6	62.2	0.3	0.9	0.5	1.7	0.1	1.0	0.0	1.1
CLA307	T	0.2	9.1	2.9	12.3	0.1	2.2	2.8	5.1	0.1	63.3	1.5	64.8	0.3	1.0	0.4	1.7	0.1	1.2	0.0	1.4
CLA41	T	0.1	11.3	2.3	13.6	0.1	1.9	1.8	3.6	0.1	62.0	1.5	63.4	0.4	1.6	0.4	2.4	0.1	1.3	0.1	1.4
CLA44	T	0.3	10.6	3.7	14.6	0.1	2.2	3.7	5.9	0.1	59.5	0.0	59.5	0.4	1.4	0.5	2.3	0.1	1.1	0.1	1.2
CLA18	T	0.1	9.5	1.9	11.6	0.1	2.0	2.2	4.2	0.1	58.9	1.6	60.5	0.2	1.3	0.3	1.8	0.1	1.0	0.0	1.1
CLA81	T	0.1	11.3	3.2	14.6	0.1	1.9	3.3	5.2	0.1	61.3	0.0	61.2	0.4	1.4	0.6	2.4	0.1	1.1	0.0	1.3
CLA84	T	0.1	10.7	3.2	14.0	0.1	2.8	3.5	6.3	0.1	60.9	0.0	61.0	0.5	1.3	0.6	2.3	0.1	1.1	0.1	1.3
DTPWC9	S	0.1	6.6	2.2	9.0	0.1	1.8	2.6	4.4	0.1	61.5	1.9	63.5	0.2	1.3	0.3	1.7	0.1	1.0	0.1	1.1
LaPosta	S	0.1	10.7	2.1	12.9	0.1	2.3	1.9	4.3	0.1	62.7	0.2	62.9	0.4	1.3	0.4	2.1	0.1	1.2	0.1	1.3
CML311B	S	0.2	11.8	1.5	13.4	0.0	2.1	1.3	3.4	0.0	62.5	1.7	64.2	0.3	1.4	0.3	2.0	0.1	1.2	0.0	1.4
CLA35	S	0.1	10.9	2.0	13.0	0.1	1.9	1.7	3.6	0.1	62.1	1.9	64.1	0.4	1.2	0.3	2.0	0.1	1.1	0.0	1.3
P390am	S	0.1	12.2	1.8	14.2	0.1	2.0	1.6	3.6	0.1	61.6	0.9	62.5	0.4	1.5	0.3	2.1	0.1	1.2	0.0	1.4
DTPWC9	S	0.2	9.1	1.6	10.9	0.1	2.0	1.4	3.4	0.1	70.5	0.3	70.7	0.3	1.4	0.3	2.0	0.1	1.4	0.1	1.5
Mean	T	0.1	9.7	2.9	12.7	0.1	2.1	3.0	5.0	0.1	60.3	1.0	61.3	0.4	1.2	0.5	2.0	0.1	1.1	0.1	1.2
	S	0.1	10.2	1.9	12.2	0.1	2.0	1.8	3.8	0.1	63.5	1.2	64.6	0.3	1.3	0.3	2.0	0.1	1.2	0.1	1.3
Tukey-Test		ns	ns	*	ns	ns	ns	*	*	ns	*	ns	*	ns	*	*	ns	ns	ns	ns	ns

Data in percent/structure represents the average of three replicates ± standard derivation. Abbreviations: T = tolerant to aluminum, S = susceptible to aluminum, Per = pericarp, End = endosperm, Ger = germ, Tot = total percent. * Significant difference at $p < 0.05$; ns = non-significant.

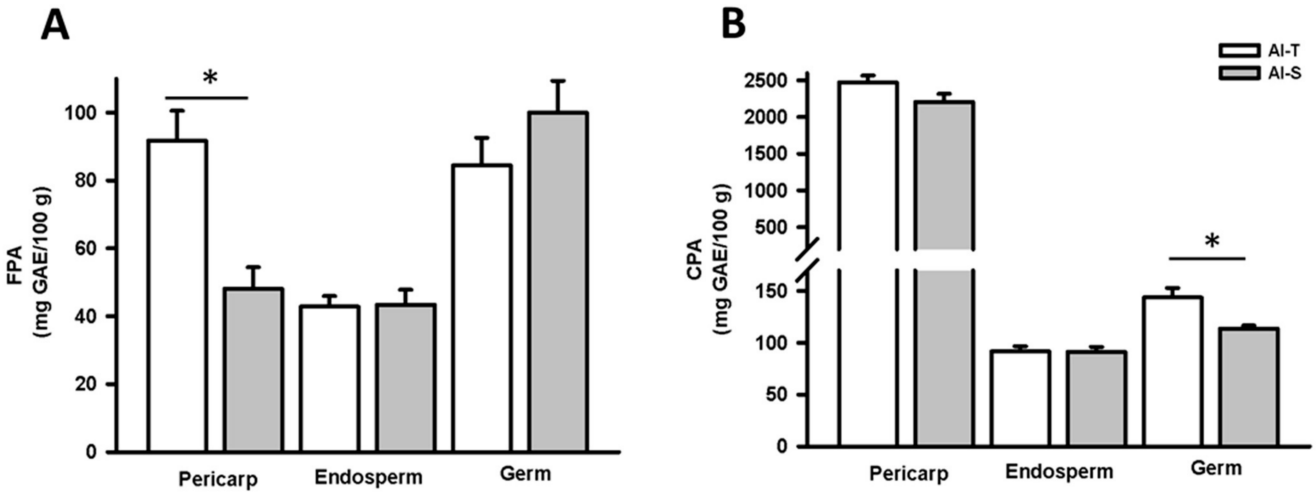

Figure 1. Phenolic acids content found in kernel structures of Al-tolerant (Al-T) and Al-susceptible (Al-S) maize inbred lines. Free phenolic acids (**A**) and cell wall-bound phenolic acids (**B**) content in pericarp, endosperm, and germ from Results are expressed as mg of gallic acid equivalents (GAE) in 100 g. Data represents the mean of six genotypes plus the standard error of the mean. * Significant difference at $p < 0.05$ by Tukey test comparison. Abbreviations: FPA = Free phenolic acids, CPA = Cell wall-bound phenolic acids.

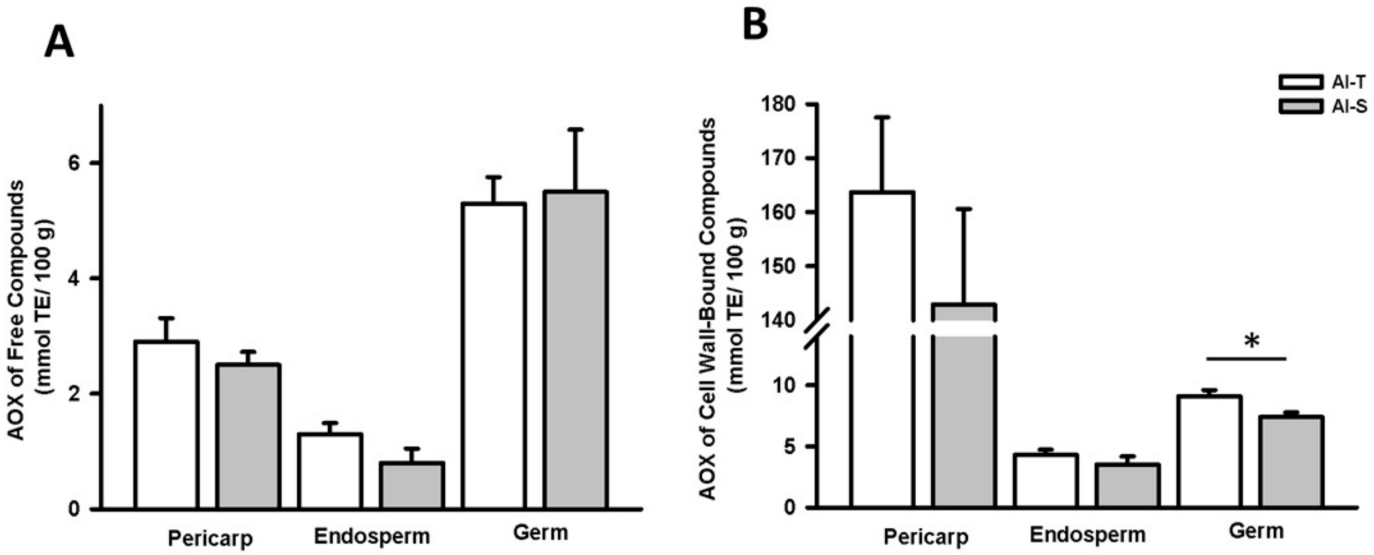

Figure 2. Antioxidant capacity of phenolic acids in kernel structures of Al-tolerant (Al-T) and Al-susceptible (Al-S) maize inbred lines. AOX of free (**A**) and cell wall-bound phenolic compounds (**B**) found in pericarp, endosperm and germ. Data represents the mean of six genotypes plus the standard error of the mean. * Significant difference at $p < 0.05$ by Tukey test comparison. Abbreviations: AOX = Antioxidant capacity, TE = trolox equivalents.

3. Discussion

This study established a relationship between the Al tolerance of maize inbred lines and their nutrient and phytochemicals kernel composition. The different anatomical parts of Al-T kernels presented significant differences in these compounds when compared to their susceptible counterparts.

Total tissue analysis showed that the highest percent of kernel macronutrients, such as protein, oil, starch, and fibre, were found in the endosperm of both groups as this

reserve tissue was the major fraction found in the kernel (83%) [13]. The endosperm mainly contains starch (87.6%), followed by protein and fibre [14]. However, it was observed that the endosperms of Al-tolerant kernels contained lower amounts of starch and fibre. In contrast, a previously reported individual analysis of kernel structures revealed that the germ of Al-tolerant kernels contained higher amounts of macronutrients [13,15]. The Al-T germ contained higher amounts of protein, oil, and fibre than that of the Al-S germ. The maize germ is principally used for oil extraction, and the partially defatted germ meal is mainly used as animal feed [2]. Furthermore, the germ is the maize kernel structure with the best amino acid profile and protein content [14]. Therefore, Al-T lines to produce hybrids could represent a good alternative for the production of human foods with higher protein content and quality.

A comparison of phenolic acid (PA) content of Al-T kernels and their susceptible counterparts indicated that the mechanism of resistance resulted in an enhancement of the phytochemicals of the kernel. The most notorious difference in PA between the two groups was found for pericarp FPA, where a 2-fold increment in concentration was observed in the Al-T kernels. In contrast, while a major PA content was observed in the CPA of both Al-T and Al-S kernels, only a significant difference in germ for CPA was observed. It has been shown that some Al-T plants can modify their production of PA [16,17]. This effect has principally been investigated in root tips exudates, where Al-binding compounds play an important role in Al detoxification [18]. In this context, an increase in flavonoid-type phenolic content of root exudates was attributed to Al tolerance in maize [19]. In other Al-T species, such as Polygonaceae, caffeic acid, catechol, and catechin are the principal PAs implicated in root Al detoxification [17]. Nevertheless, to the best of our knowledge, this is the first study in which significant differences in total PA due to the Al tolerance mechanism were found in mature maize kernels [12,20]. At the same time, the higher PA content can contribute to enhancing the nutraceutical potential, especially in terms of AOX and the prevention of oxidative stress.

The highest AOX was observed in the pericarp CPA of both Al-T and Al-S kernels. This higher AOX also coincided with the high CPA observed in both groups. Surprisingly, only the germ of Al-T kernels had a significant difference in the AOX of CPA. This finding corroborates the higher CPA content in the germ of Al-T kernels in comparison with their susceptible counterparts and establishes the Al tolerance-induced changes in the phytochemicals profile of kernels. It is known that Al stress induces the production of reactive oxygen species (ROS), which is efficiently controlled by the antioxidant defence systems of tolerant plants [20–22]. The first defense line against ROS is constituted by the enzymes superoxide dismutase and peroxidase, which use phenolic co-substrates [21]. At the same time, specific genes, such as ZmAT6, are activated in Al-T maize [23]. Recent proteomics studies of Al-T maize have shown the induction of complex transcriptome changes in the transcript levels for several genes, which were primarily related to cell wall structure and metabolism, oxidative stress response, membrane transporters, and organic acid metabolism [24]. Therefore, the increment in AOX and PA compounds in Al-T maize lines may be related to this mechanism. Although our study does not include enzymatic activity quantification or determination of other antioxidant species other than PA as previously reported in Al-susceptible maize lines [25], the novel AOX variation found in maize kernels due to Al tolerance has not been previously reported.

The results presented herein provide the basis for taking advantage of Al tolerance in maize lines to enhance the nutrient and phytochemical content of kernels. These findings would allow the exploitation of this tolerance mechanism in maize kernels lines not only to solve the agricultural problem of reduction in maize grain yield due to Al soil toxicity but also to improve the nutrient and phytochemical contents in novel hybrids maize [26]. To the best of our knowledge, this is the first study that compared nutritional and nutraceutical kernel compositions based on tolerance to Al [12]. Although previous studies have emphasized the quantification of phenolic acids content in plant roots [27,28], our study is mainly focused on the kernel phenolic content. In future studies, a precise evaluation

of the effect of Al concentration on PA profile in maize kernels should be the main focus. Furthermore, the impact of different acidic soils on the phytochemicals of Al-T kernels and the pathways involved should be investigated.

4. Materials and Methods

4.1. Chemical and Reagents

NaOH (Cat. 221465), HCl (Cat. 320331), ethyl acetate (Cat. 270989), H_2O_2 (Cat. 216763), ethanol (Cat. 32221), 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH) (Cat. 440914) and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Cat. 238813) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gibco® phosphate buffer (PBS), pH 7.4 (Cat. 10010) was procured from ThermoFisher Scientific (Waltham, MA, USA).

4.2. Maize Germplasm

Sixteen inbred lines were used in this study in order to test possible parents to produce hybrids. Kernels of Al-T and Al-susceptible (Al-S) maize (*Zea mays* L.) were kindly provided by CIMMYT Maize Program. The Al-T maize inbred lines used were CLA161, CLA309, CLA37, CLA307, CLA44, and CLA84, whereas DTPWC9, LaPostaSeq, CML311-B, CLA35, P390am, and DTPWC9 were selected as Al-S maize inbred lines. The lines were previously screened and selected under aluminum soils conditions (Al toxicity, 60 to 300 µg per liter of water in soil) in Cali Colombia where a 'hot spot' is located for soil acidity [29]. All analyses were performed by triplicate in two independent experiments.

4.3. Determination of Morphological Parameters

Thousand-kernel weight was determined by weighing 1000 kernels at 13% of grain moisture. Endosperm vitreousness was measured by estimating the relative proportion of vitreous endosperm area. Kernel hardness was determined on 100 g samples using the floaters test. The pericarp, endosperm, germ, and tip cap were manually dissected as described by [30], while kernel dimensions (thickness, width, and length) were obtained using a digital micrometer (Mitutoyo IP-65, Osaka, Japan).

4.4. Proximate Analysis

The dissected and separated anatomical parts (pericarp, endosperm, and germ) were assayed for proximate analysis. Crude protein, total starch, oil, crude fibre, and ash contents were determined according to the methods provided by the American Association of Cereal Chemists, including methods 44-15A, 46-12.01, 76-11, 32-10, and 08-12 [31].

4.5. Extraction of Free and Bound Phenolic Acids

Phenolics were extracted according to the method described by [30]. In brief, 1 g of finely ground maize kernels was mixed with 10 mL of ethanol (80% *v/v*) for 10 min at 50 rpm in a shaker. Afterwards, the mixtures were centrifuged at 2500 rpm and 18 °C for 10 min. The resulting supernatants containing FPA were concentrated. The pellets were digested in 10 mL of 2 M NaOH at 23 °C for 1 h with agitation under N_2 gas. Next, the mixtures were neutralised with 0.5 N HCl. The final solution was evaporated to dryness and was further dissolved in 10 mL of water. Phenolic extracts were stored at −20 °C until further analysis.

4.6. Determination of Total Phenolics

Total phenolic acids were determined according to the method of Folin–Ciocalteu [30]. In brief, 500 µL of FPA or CPA extracts were treated with 300 µL of 1.5 M H_2O_2 and assayed using the Folin–Ciocalteu assay. The samples were quantified by spectrophotometry at 765 nm using a micro-plate reader (Epoch, BioTek Instruments, Inc., Winooski, VT, USA). The concentrations of total phenolic acids were expressed as mg of gallic acid equivalents (GAE) per 100 g (GAE/100 g of dry sample weight).

4.7. Antioxidant Capacity

Antioxidant capacity (AOX) was determined by an oxygen radical absorbance capacity (ORAC) assay [30]. Extracts were evaluated against a standard of Trolox using Fluorescein as a probe. Peroxyl radicals were generated by 2,2′-azobis (2-amidinopropane) dihydrochloride and fluorescent loss was monitored in a spectrophotometer micro-plate reader (SynergyTM HT Multi-Detection, BioTek, Inc., Winooski, VT, USA). The absorbance of excitation and emission was set at 485 and 538 nm, respectively. Data were expressed as Trolox equivalents (TEg-1 of dry sample weight).

4.8. Statistical Analysis

All analyses were performed by triplicate in two independent experiments. Data were presented as mean ± standard error of mean and subjected to unpaired Student's *t*-test, considering $p < 0.05$ as the level of significance. All statistical analysis was performed using Statistix v.8 (Analytical Soft, Tallahassee, FL, USA).

5. Conclusions

In summary, the nutritional and phytochemicals of the anatomical constituents of Al-T maize kernels were compared with their susceptible counterparts. An association between the Al tolerance mechanism and the increase in the contents of macronutrients and PAs was principally found in the germ structure. In addition, an increase in AOX was found in the Al-T kernels, which was also related to a higher concentration of PA in cell wall-bound extracts. The evidence provided herein establishes the possibility of utilizing Al-T maize lines to produce hybrids that could grow in acidic soils. In future studies, a precise evaluation of the impact of different acidic soils on the nutraceutical composition of Al-T kernels and the pathways involved should be investigated.

Finally, the results herein this imply that the hybrids generated using Al-T maize inbred lines are an attractive source of nutrients and phytochemicals in farming regions containing acid soils.

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