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Early Criterion to Screen Maize Varieties for Their Tolerance to Aluminium Toxic Soil

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Abstract Tolerance to aluminum toxicity in maize is usually determined after harvesting. This screening process takes place rather too late in the growth stage of the plant and is not economical. In order to speed up the screening of maize varieties for their tolerance to aluminum toxicity in Cameroon, a parameter that could be easily detected early in the plant growth stage was investigated. Thirteen (13) maize varieties were evaluated in pots containing aluminum toxic soil and amended soil (less acidic). There were two experimental blocks and the maize varieties were arranged in a completely randomized block design with three replications. Young plants were carefully off rooted twenty-one (21) days after planting and soluble phenolic compounds quantified. The results suggested that, phenolic compound production varied with maize variety and the soil type. The amount of phenolic compounds produced on aluminum toxic soil was higher (10.44 x 103µg) than that produced on amended soil with high organic content (6.60 x 103µg) (P< 0.0005). Using LSDs of phenolic compound secretion, the 13 varieties were classified into three groups (tolerant, fairly tolerant and sensitive). Ten (10) varieties were tolerant, two were fairly tolerant and one was susceptible. Varieties 91105, 87036, CLA 18, CML 254, CML 247 that were previously unclassified were classified as tolerant to Al toxicity. Variety Exp1 24 that was previously classified as sensitive using using the yield, the interval between anthers and the silk and length of seminal roots was also classified as tolerant using "intact" plants growing under natural conditions confirming that discrepancies may occur when only one method is used for screening. The quantity of phenolic compounds produced in the presence of aluminum could be effectively used to classify maize varieties as tolerant, fairly tolerant and susceptible to soil aluminum toxicity.

Keywords Aluminum Toxicity, Cameroon, Early Selection, Phenolic Compounds, Zea Mays

1. Introduction

Maize is the third widely cultivated cereal in the world[1]. The improvement of food security which implicates an increase of production is constrained by low soil fertility, poor climatic adaptability of the varieties, irregularity and unpredictability of rains, diseases[2], soil acidity and in particular aluminum toxicity[3].

Acidic soils occupy about 30% of the earth's surface[4]. In Cameroon, 75% of soils are acidic[5], where it is responsible for about 67% reduction in maize yields[6].

Liming, the application of organic and mineral fertilizers are measures that have been used to successfully manage soil acidity in croplands ([7],[8]). Bio fertilizers such as mycorrhizae that help to improve crop tolerance to diseases, adaptability to adverse environmental conditions and yields are also used to counteract soil acidity[9].

The screening and use of maize varieties tolerant to soil acidity constitute an efficient and permanent alternative to increase yields on acidic soils, avoiding enormous losses often observed with sensitive varieties[3].

Some early indicators such as callose (1,3-β-glucan), appear to be an excellent physiological marker for the identification of maize varieties sensitive to aluminum toxicity ([8],[3],[10],[4][11]. The accumulation of aluminum in the distal part of root tip is another marker to select susceptible maize varieties[12],[13]. Organic acids such as malic acid, citric acid, oxalic acid and tartric acid ([14],[15]) exuded by maize varieties tolerant to aluminum toxicity play an important role in the detoxification of aluminum. In this case, chelates can be made between organic acids and aluminium, making it non-toxic to plants[14]. The extraction and quantification of these compounds permit the selection of maize varieties tolerant to aluminum toxicity.

However, the extraction and quantification of organic

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acids need very expensive infrastructures that are not always available in poor countries. Furthermore, soluble phenolic compounds are also used to screen maize genotypes tolerant to aluminum toxicity because their extraction and quantification is less expensive. Phenolic compounds play a role in various defense reactions to protect against abiotic stresses like aluminum toxicity[16]. Investigations on the role of the exudation of phenolic compounds as a potential mechanism for the tolerance to aluminum toxicity have been performed by[17]. They analysed phenolic compounds from the root tips of pre-germinated maize in Petri dishes with 50 μM aluminium and they found that the exudation of phenolics was enhanced with increasing Al concentration.

The identification of soluble phenolic compounds as a criterion to screen maize varieties tolerant to aluminum toxicity, which are usually done under laboratory conditions using artificial growth media may show some discrepancies in the results when such experiments are carried out under natural field conditions. These discrepancies may be due to the fact that in the laboratory plants are in a controlled milieu, meanwhile it is not the case on the field. It is thus important to screen maize varieties for their tolerance/sensitivity to aluminum toxicity by evaluating their phenolic compound production using 'intact' plants growing under field conditions.[18] had classified the maize varieties using the yield, the interval between anthers and the silk and length of seminal roots. These parameters are recorded late in the plant growth stage, expensive and this classification has not yet been confirmed using phenolic compound production..

2. Materials and Methods

2.1. Experimental site

This study was done at the Institute of Agricultural Research for Development (IRAD) Regional Center at Nkolbisson (11° 36 'E; 3° 44 'N) in a 'screenhouse' with natural conditions (short dry season with mean daily rainfall and temperature of 4 mm and 30 ^{o}C respectively).

(i) Plant material: Thirteen maize varieties were used and their presumed level of tolerance or sensitivity to aluminum acidity[18] is presented in Table 4.

(ii) Soils used: Two types of soils were used in this experiment. An oxisol sampled from Nkoemvone (South Cameroon), where the soil is acidic and high in aluminum content. The second type was a garden soil collected from Nkolbisson, rich in organic matter. The chemical properties of the soil samples were analyzed at the laboratory of soil analysis at IRAD Nkolbisson-Yaoundé using methods described by[19].

2.2. Experimental design

There were two experimental blocks and the maize varieties were arranged in a completely randomized block design with 3 replications. The main variable was soil type (1. acidic soil with high aluminum content, and 2. amended garden soil with high organic matter and the secondary variable was maize variety). The treatments were applied in a randomized block design with 3 replications. The maize varieties were planted in 5-litre pots and each pot had 4 grains of maize, each in a hole. Watering was done every two days.

2.3. Extraction and quantification of phenolic compounds

All the plants were off-rooted twenty-one (21) days after planting and the roots washed with distilled water. The roots were oven-dried at 40 ^{o}C till constant weight was recorded. The phenolic compounds were extracted from 0.5 g dry matter of each maize variety according to the method described by[20].

2.4. Statistical analysis

The General Linear Model (GLM) of the Statistical Package for Social Sciences (SPSS) version 10.0 was used to analyse the variance between the various treatments. The least significant difference (LSD) of the means of phenolic compounds from different classes of maize varieties grown separately on aluminum toxic and amended soil with high organic content was compared. LSD were used to confirm the classification made earlier by[18] and to classify unclassified maize varieties used in the present study.

3. Results

3.1. Chemical analysis of soil samples

Aluminum toxic soil, of pH (water) 3.40 was poor in nutritive elements, which are calcium (0.77cmolc/kg), magnesium (0.33 cmolc/kg), phosphorus (56 mg /kg) and potassium (0.37 cmolc/kg) (Table 1). The cation exchange capacity of this soil was low (3.52 cmolc/kg). The percentage of saturation by aluminum was 56.80. The acidity of this soil was therefore due to aluminum toxicity. However, soil without aluminum toxicity (amended soil with high organic content) had a pH (water) of 5.30, contained quantities of nutritious elements, which are calcium (11.19 cmolc/kg), magnesium (4.87 cmolc/kg) and phosphorus (1264 mg/kg). The amended soil with high organic content had a high cation exchange capacity (17.39 cmolc/kg). Moreover, the soil without aluminum toxicity did not contain any aluminum element, with a percentage of saturation in aluminum equal to zero.

There was a significant variation in phenolic compounds from maize grown on aluminum toxic soil ($F= 12.59$, $DF=1$, $P= 0.001$) those grown in non-toxic soil ($F= 8.49$, $DF=9$, $P= 0.0001$).

This result suggested that, maize varieties evaluated had different level of phenolic compounds production on aluminum toxic soil. This analysis also revealed a highly significant difference between varieties evaluated for the production of phenolic compounds and that the quantity of phenolic compounds produced in the presence of aluminum was statistically different from the quantity produced on amended soil.

[18] had classified the maize varieties used in this study using the yield, the interval between anthers and the silk and length of seminal roots. Eight varieties were classified following this method into three groups: tolerant, fairly tolerant and sensitive to aluminum toxicity. In the present study, these 8 varieties were used with the addition of 5 unclassified varieties. The least significance difference (LSD) of phenolic compounds quantified from the roots of maize varieties grown on aluminum toxic soil was compared in the 4 groups (tolerant, fairly tolerant, sensitive to aluminum toxicity and unclassified) (Table 2). The results confirmed the classification made by[18] except that of Exp_1 24 which was classified sensitive by[18], but classified tolerant in the present study. In addition, all the unclassified varieties were classified as tolerant.

The amount of phenolic compounds produced on aluminum toxic soil was higher (10.44 x 10^3µg) than those produced on amended soil with high organic content (6.60 x 10^3µg) ($P< 0.0005$). All classes of maize produced similar amount of phenolic compounds on amended soil with high organic content (Table 3) and as such these soil is not recommended for screening of maize varieties for aluminum toxicity.

The characteristics of 13 maize varieties used in this study and their classification based on yield and morphological parameters[6] and the least significant difference (LSD) of phenolic compounds quantified on aluminum toxic soil (Present study) are shown in Table 4

Table 1. Chemical analysis of soils samples used for the determination of phenolic compounds in different maize varieties that are tolerant or sensitive to aluminum toxicity

Soil types	Soil characteristics								
	pH (H_2O)	Al (------------------------	Ca	Mg	K cmolckg^{-1}	Na	CECE ----------------------------)	Ala (%)	P (mgkg^{-1})
Al toxic	3.40	1.99	0.77	0.33	0.37	0.06	3.52	56.8	5.60
Amended	5.30	0.00	11.19	4.87	1.19	0.13	17.39	0.00	12.64

[a]: Percentage saturation of aluminum

Table 2. Comparison of different classes of maize varieties that are sensitive, fairly tolerant, tolerant or unclassified to aluminum toxicity using the least significant difference (LSD) of their phenolic compounds quantified from maize grown on aluminum toxic soil for 3 weeks

(I) Class (J) Class	Difference of means (I) – (j)	Standard error	Signification	Confidence Interval at 95% Lower limit	Confidence Interval at 95% Upper limit
Tolerant Unclassified	-661.11	1308.09	0.62	-3487.07	2164.85
Fairly tolerant	-4194.44*	1688.74	0.02	-7842.75	-546.13
Sensitive	5472.23*	1688.74	0.006	1823.91	912053
Unclassified Tolerant	661.11	1308.09	0.622	-2164.85	3487.07
Fairly tolerant	-3533.33*	1631.48	0.050	-7057.93	-8.73
Sensitive	6133.33*	1631.48	0.002	2608.73	9657.93
Fairly tolerant Tolerant	4194.44*	1688.74	0.027	546.13	7842.74
Unclassified	3533.33*	1631.48	0.050	8.73	7057.93
Sensitive	9666.33*	1949.99	0.000	5453.96	13879.36
Sensitive Tolerant	-5472.22*	1688.74	0.006	-9120.53	-1823.91
Unclassified	-6133.33*	1631.48	0.002	-9657.93	-2608.73
Fairly tolerant	-9666.67*	1949.99	0.000	-13879.37	-5453.96

Based on means observed
*Mean difference is significant at 0.05 level.

Table 3. Comparison of different classes of maize varieties that are sensitive, fairly tolerant, tolerant or unclassified to aluminum toxicity using the least significant difference (at 0.05% level) of their phenolic compounds quantified from maize grown on amended soil with high organic content for 3 weeks

(I) Class (J) Class	Difference of means (I) – (j)	Standard error	Signification	Confidence Interval at 95% Lower limit	Confidence Interval at 95% Upper limit
Tolerant Unclassified	966.67	1894.22	0.615	-2961.71	4895.05
Fairly tolerant	1388.89	2445.43	0.576	-3682.63	6460.40
Sensitive	4055.5	2445.43	0.111	-1015.96	91.27.06
Unclassified Tolerant	-966.67	1895.22	0.615	-4895.05	2961.71
Fairly tolerant	422.22	2362.51	0. 860	-4477.33	5321.77
Sensitive	3088.89	2362.51	0.205	-1810.66	7988.43
Fairly tolerant Tolerant	-1388.89	2445.43	0.576	-6460.40	3682.63
Unclassified	-422.22	2362.51	0.860	-5321.77	4477.33
Sensitive	2666.66	2823.74	0.355	-3189.41	8522.74
Sensitive Tolerant	-40555.55	2445.43	0.111	-9127.07	1015.96
Unclassified	-3088.89	2362.51	0.205	-7988.44	181066
Fairly tolerant	-2666.66	2823.74	0.355	-8522.74	3189.42

Table 4. Characteristics of maize varieties (a) and their classification (b) based on the least significant difference (LSD) of phenolic compounds quantified on aluminum toxic and amended soils

(a)

Varieties	Origin	Adaptation	Colour
ATP S4 25W	IRAD	Low altitude	White
Cam Inb gp1 17	IRAD	Low altitude	Yellow
88094	IRAD	Mid altitude	White
Entrada 3	IRAD	Low altitude	White
91105	IRAD	Mid altitude	White
87036	IRAD	Mid altitude	White
CLA 18	CIMMYT	Low altitude	Yellow
CML 254	CIMMYT	Low altitude	White
CML 247	CIMMYT	Low altitude	White
Entrada 29	CIMMYT	Low altitude	White
M131	IRAD	Mid altitude	White
Exp1 24	IRAD	Low altitude	White
Tuxpeño sequia	CIMMYT	Low altitude	White

(b)

Variety	LSD of Phenolic compound		aPrevious Clas-sification	Present classifi-cation
	bAluminum toxic soil	bAmended soil		
ATP S4 25W	23222.22 ± 785.67	11888.88 ± 471.40	Tolerant	Tolerant
Cam Inb gp1 17	6999.99 ± 2985.56	5777.77 ± 2199.88	Tolerant	Tolerant
88094	5888.88 ± 157.13	6222.22 ± 1257.07	Tolerant	Tolerant
Entrada 3	4888.88 ± 0.00	5888.88 ± 785.67	Tolerant	Tolerant
91105	11999.99 ± 5342.58	1111.11 ± 0.00	Unclassified	Tolerant
87036	8111.11 ± 1728.48	4555.55 ± 3299.83	Unclassified	Tolerant
CLA 18	7777.77 ± 3771.23	7111.11 ±0.00	Unclassified	Tolerant
CML 254	4333.33 ± 157.13	7999.99 ± 942.81	Unclassified	Tolerant
CML 247	22333.33 ± 1728.48	13555.55 ± 314.27	Unclassified	Tolerant
Entrada 29	21111.11 ± 2514.16	11111.11 ± 942.81	Fairly tolerant	Fairly tolerant
M131	7777.77 ± 5028.31	1777.77 ± 942.80	Fairly tolerant	Fairly tolerant
Exp1 24	9222.22 ± 2985.56	1222.22 ± 471.40	Sensitive	Tolerant
Tuxpeño sequia	333.33 ± 157.13	6333.33 ± 1099.94	Sensitive	Sensitive

[a]: Classification by[17]
[b]: Least significant difference of phenolic compounds

4. Discussion

The quantity of phenolic compounds produced in the presence of aluminum was almost double of that produced on soil without aluminum. This result showed that, in the presence of exchangeable aluminum (Al^{3+}), maize varieties produce more phenolic compounds as a mechanism of defense against aluminum toxicity[22]. The conception of antioxidant action of phenolic compounds is not novel[21]. The induction of phenolic compound biosynthesis was observed in maize in response to aluminum[15]. Antioxidant action of phenolic compounds is due to their high tendency to chelate aluminium[23].

It can be observed that all maize varieties produced phenolic compounds either on aluminum toxic soil or on soil without aluminum. Maize varieties known as tolerant to aluminum toxicity tend to produce more phenolic compounds when they are planted on aluminum toxic soil[17].

Therefore, extra quantities of phenolic compounds produced in the presence of aluminum show that *ATP* S_4 *25W* and *91105* exuded more than 10 x 10^3µg of phenolic compounds by the effect of aluminum and are revealed as very tolerant to aluminum toxicity. Other maize varieties classified as tolerant using the quantity of phenolic compounds exuded are: *M131*, *Entrada 29*, *87036, Cam Inb* gp_1*17*, *CLA 18*, *CML 247* and Exp_1*24.*

The variety Exp_1*24* that is native of IRAD Cameroon is known as susceptible to aluminum toxicity by its performance on acidic soil. However, this variety was classified as tolerant to aluminum toxicity using quantity of phenolic compounds it produced. This variety may have a different mechanism to tolerate aluminum toxicity such as detoxification of Al by aminoacid metabolism partways[24]

Maize varieties, which are tolerant to aluminum toxicity may possess some physiological mechanisms that permit them produce more phenols when grown on aluminum toxic soil. A sensitive variety, *Tuxpeño sequia* may not have the physiological mechanisms against the excess of aluminum and is therefore unable to exude phenolic compounds to detoxify its roots from aluminum. Consequently, the growth is inhibited by aluminum, which penetrates into its roots and interferes with its development[12][25]

5. Conclusions

Using phenolic compounds exuded the classification of maize varieties as tolerant, fairly tolerant or sensitive made in a previous study was confirmed by this study. Furthermore, unclassified varieties of maize were classified as tolerant using the extraction and quantification of phenolic compounds. As such, the quantity of phenolic compounds produce by maize varieties cultivated in pots with exchangeable aluminum can be effectively used as an early criterion to screen maize varieties for their tolerance to aluminum toxic soils.

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