J. Plant Nutr. Soil Sci. 2004, 167, 93–98 **DOI:** 10.1002/jpln.200320309

Factors contributing to the development of aluminum resistance in the Madeiran maize germplasm

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Accepted 28 November 2003

Summary – Zusammenfassung

Forty genotypes representing maize genetic diversity from the Island of Madeira were screened for resistance to aluminum (Al) in nutrient solution. Seeds of maize were obtained from local farmers from field plots spread around the island in a range of altitudes from 99 to 1000 m above sea level. The content of ionic aluminum and the pH of soils cultivated with maize were determined. The mean pH value of all examined soils was 4.9, while the mean ionic Al content was 0.76 cmol kg^{-1}. Seventy-two hours (h) exposure to 100 and 200 μM Al followed by a 48 h recovery period of 4-day old seedlings revealed significant differences in Al resistance in the Madeiran maize germplasm. Root survival and regrowth exceeding 80 % of the seedlings were observed in 22 and 8 of the tested genotypes screened at 100 and 200 μM Al in nutrient solution. High Al resistance among Madeiran maize genotypes appeared not to be associated with the lower pH of soil, and did not correlate with the amount of soil Al^{3+} or the altitude at the site of collection. The high level of Al resistance of the maize cultivars indicates an initial genetic trait enhanced by open pollination of maize.

Zur Entstehung von Aluminiumresistenz von Maissorten aus Madeira beitragende Faktoren

Vierzig repräsentative Maisherkünfte von der Insel Madeira wurden hinsichtlich ihrer Resistenz gegenüber Aluminium (Al) in Nährlösung untersucht. Das verwendete Maissaatgut wurde von einheimischen Landwirten zur Verfügung gestellt. Die Felder repräsentieren verschiedene Standorte der Insel, die sich hinsichtlich Höhenlage (99–1000 Meter über dem Meeresspiegel), pH-Wert und Al^{3+}-Gehalt der Böden unterschieden. Der mittlere pH-Wert aller untersuchten Standorte war 4,9. Der mittlere Al-Ionen Gehalt der untersuchten Böden betrug 0,76 cmol kg^{-1}. Die "eriochrome cyanine"-Technik wurde angewandt, um die Auswirkung der Al-Ionen auf das Längenwachstum der Wurzeln zu bestimmen. Vier Tage alte Keimlinge wurden in Nährlösung für 72 Stunden (h) 100 bzw. 200 μM Al ausgesetzt, gefolgt von einer 48-stündigen Regenerationsphase. Die Herkünfte wiesen große Unterschiede in der Al-Resistenz auf. Bei 22 bzw. 8 von 40 Herkünften überlebten mehr als 80 % der Keimlinge den Al-Stress und zeigten erneutes Wurzelwachstum nach Behandlung mit 100 bzw. 200 μM Al. Die Al-Resistenz der Maisherkünfte stand nicht mit dem pH-Wert des Bodens in Verbindung und war auch nicht mit der Menge von Al^{3+} oder der Höhenlage korreliert. Die Al-Resistenz der Maisherkünfte beruht wahrscheinlich auf einer ursprünglichen genetischen Eigenschaft und wurde vermutlich durch die offene Pollenübertragung verbreitet.

Key words: acid soils / aluminum / germplasm / Madeira / maize / resistance PNSS P03/09P

1 Introduction

Aluminum (Al) toxicity is considered as the main growth- and yield-limiting factor on soils with pH below 5.0 (*Davies*, 1994; *Foy*, 1992). Soil acidity is a serious agricultural problem throughout many parts of the world, affecting as much as 40 % of the world's arable land (*Haug*, 1984). Under acidic conditions, monomeric Al ions are released to the soil solution from soil minerals and polycationic, non-toxic Al complexes that exist at neutral pH. The ionic strength of acid soil solutions is lower than 5000 μM while the concentration of Al is lower than 5×10^{-3} cmol (*Pintro* et al., 1996). Once in soil solution, soluble Al ions can be taken up by the roots and in consequence affect plant growth. The first visual symptom of Al toxicity is a reduction of root growth (*Foy*, 1992).

During evolution, plants developed numerous mechanisms that allow survival in acid soils rich in available ionic Al (*Kochian*, 1995; *Slaski*, 1992). As a result of selection pressure, inter- and intra-species differences in response to Al are widely observed in the plant kingdom. In maize, an Al concentration of 50 μM appears to be highly toxic to the majority of genotypes (*Salazar* et al., 1997; *Collet* et al., 2000). Great differences in resistance to Al have been reported among genotypes originating from Brazil and South America where the intense selection pressure of acid soils with high Al supply resulted in the development of resistant maize cultivars (*Pintro* et al., 1996; *Salazar* et al., 1997; *Collet* et al., 2000). These cultivars often serve as donors of genes controlling Al resistance in breeding programs throughout the world. However, the pool of suitable gene sources is limited, and identification of new Al-resistant genotypes could contribute to the diversification of selection efforts.

The Portuguese Island of Madeira seems to be a good choice for the search for new forms of Al-resistant maize because of

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1436-8730/04/0102-93

soil characteristics and documented presence of highly Al-resistant wheat cultivars (*Dos Santos* et al., 2001; *Pinheiro de Carvalho* et al., 2003). The mean pH and Al^{3+} content of Madeiran wheat-cultivated soils were 4.8 and 0.86 cmol kg^{-1}, respectively (*Pinheiro de Carvalho* et al., 2003). Several major volcanic soil types can be found on the island, including vertisols, cambiosols, phaeozems, leptosols, and andosols. Andosols represent 60 % of the total land area (*Madeira* et al., 1994). Andosols are classified as acid or very acid soils with pH below 5.5 and with total Al ranging from 11 to 34 g per kg of soil (*Madeira* et al., 1994). The soil and climatic conditions of the island are strongly affected by the elevation, and they have determined the evolution of local cultivars (*Madeira* et al., 1994; *Pinheiro de Carvalho* et al., 2003). Since introduction, several centuries ago, the crops including maize were subjected to a long-time adaptation and speciation processes under the selection pressure of local edaphic conditions that resulted in the appearance of today's diversity of ecotypes (*Pinheiro de Carvalho* et al., 2003). The aim of the present study was to evaluate the contribution of several environmental factors to the enhancement of Al resistance among Madeiran maize cultivars and to identify the most Al resistant genotypes that can serve as donors of genes controlling Al resistance in breeding programs.

2 Material and methods

2.1 Plant and soil sampling

Forty Madeiran maize producers from different geographical locations and their fields were identified. Samples of maize seeds and the top 20 cm of soil were collected at the end of the growing season (Tab. 1). The exact location and elevation of the plots were recorded using a GPS Magellan 300. Maize plots were localized at altitudes ranging from 99 to 1000 m above sea level, and thus reflected a variety of pedologic conditions found on the island. Seed samples were stored at –20 °C in the ISOPlexis, Germplasm Bank at the University of Madeira. Maize cultivar ATP-Y obtained from Dr. W. J. Horst was used as a standard for aluminum resistance (*Horst* et al., 1997).

2.2 Soil ionic aluminum and pH measurements

Soil samples from plots corresponding to ISOP (maize) accessions were analyzed for pH and Al content. Soil was dried at 105 °C for 24 hours. 10 g of soil were extracted with 25 ml of 0.01 M KCl for 1 h (*Forster*, 1995), and the pH was measured using a WTW 320 pH meter. Ionic Al was extracted from 5 g of soil using sodium acetate (Morgan reagent), and the Al^{3+} content was measured according to the modified hydroxylamine acid method (*Ross* et al., 1985; *Ross* and *Wang*, 1993).

Table 1: Location of maize plots on the Island of Madeira and corresponding ISOP Germplasm Bank numbers. Soil pH values and content of soil ionic Al are also given.

Tabelle 1: Standorte der Maisanbauflächen auf der Insel Madeira und ihre entsprechenden ISOP-Genbank-Nummern. Die pH-Werte und Al-Ionenkonzentration des Bodens sind ebenfalls angegeben.

ISOP, #	Plot location	Altitude, m	pH	Al^{3+}, cmol kg^{-1}
61	Santa Cruz	236	5.07	0.45
62	Santana	240	5.46	0.40
63	Ponta do Sol	589	4.93	1.03
64	Calheta	538	5.08	1.14
65	Santana	663	5.76	0.94
66	Câmara de Lobos	448	4.51	0.34
67	Ribeira Brava	950	4.74	0.66
68	Santana	579	5.27	1.00
69	Santana	328	4.40	0.90
70	Santana	420	5.31	0.94
71	Santana	661	5.49	1.18
75	Machico	686	4.51	1.03
125	Calheta	715	5.12	1.10
128	Ribeira Brava	1000	4.81	0.99
129	S. Vicente	198	4.29	1.19
130	Ribeira Brava	1000	4.93	1.03
131	Ribeira Brava	500	4.38	0.70
132	Ribeira Brava	500	4.64	0.70
133	S. Vicente	99	4.67	0.61
135	S. Vicente	450	5.13	0.43
137	Santa Cruz	200	4.52	0.64
138	Machico	600	4.64	0.89
140	Porto Moniz	400	4.07	0.85
141	Calheta	513	4.87	0.96
142	Calheta	423	6.15	0.02
146	Santana	310	5.43	0.75
147	Ponta do Sol	780	5.86	0.28
148	Calheta	344	4.37	0.76
149	Ponta do Sol	600	4.70	0.94
150	Ribeira Brava	500	5.37	0.11
151	Câmara de Lobos	600	5.02	0.21
152	Câmara de Lobos	400	4.85	1.00
153	Ponta do Sol	600	4.38	1.23
154	Câmara de Lobos	523	4.51	0.41
155	Santana	591	4.09	0.86
156	Santana	300	4.55	1.04
157	Santana	392	5.08	1.02
158	Machico	212	4.74	0.48
159	Ponta do Sol	343	5.71	0.25
160	Porto Moniz	430	5.61	0.84

All the genotypes mentioned in this paper, as ISOP, are available from the ISOPlexis Germplasm Bank at the University of Madeira, Funchal.

2.3 Screening for aluminum resistance

Two hundred seeds were surface sterilized in 5 % sodium hypochlorite and germinated for 3 days at 25 °C. Sprouted seeds were placed on a raft floating on a surface of aerated full nutrient solution (*Pinheiro de Carvalho* et al., 2003) and grown for 3 days in a growth chamber at 23 °C. For Al exposure, seedlings were transferred for 72 h to fresh nutrient solution containing (in µM) 2900 NO_3^-, 300 NH_4^+, 1000 Ca^{2+}, and 300 Mg^{2+}, with 100 or 200 µM Al supplemented in form of $AlCl_3 \cdot 6H_2O$. Aluminum solution was replaced daily to minimize pH fluctuation and Al depletion. Aluminum activities in nutrient solution were calculated using the program GEOCHEM-PC version 2.0. After Al treatment, the seedlings were transferred to an Al free nutrient solution for 2 days to determine the ability of roots to recover from Al stress. In all treatments, the pH of nutrient solutions was measured every 12 h and adjusted to 4.3 with 0.1 N HCl, if necessary. At the end of the experiment, roots were stained for 10 minutes with a 0.1

solution (w/v) of eriochrome cyanine R_{250} (Sigma), which facilitated the visualization of root survival and regrowth (*Slaski,* 1995). After staining, the roots were extensively rinsed under tap water to remove the excess dye. Root tips of plants that were able to continue growth after exposure to Al remained white, while roots with irreversibly damaged apical meristems were dark purple, indicating the absence of regrowth even after the transfer to an Al free medium.

2.4 Data analysis

All experiments were run in duplicates for each experimental treatment. The experimental standard deviation of measurements was lower than 15 %. Pearson correlations between soil pH, ionic Al content, altitude, and Al resistance at 100 and 200 μM have been determined. A correlation was considered significant at P values below 0.05. All statistical analyses were performed using Excel and SPSS 10.0 for Windows (*Kinnear* and *Gray*, 1999).

3 Results

Maize seeds and soil samples were collected from plots located at altitudes ranging from 99 to 1000 m above sea level. These plots were characterized by specific pedologic conditions. 15 out of 40 soils belonged to the phaeozems, 15 soils were andosols, 8 cambiosols, 1 vertisol, and 1 was identified as leptosol. The pH of the soil samples ranged from 4.07 to 6.15, at the Porto Moniz and Ponta do Pargo locations, respectively, with 23 plots of pH below 5.0. The mean pH value of all examined plots was 4.9. The content of ionic Al in soil sampled from tested plots was between 0.02 and 1.23 cmol Al^{3+} per kg of soil, both from the Ponta do Sol locations. The mean Al^{3+} content was 0.76 cmol kg^{-1}. The aluminum content of the soil was negatively correlated with the soil pH ($R_{Pearson} = -0.351$, $P < 0.05$), and was not correlated with the altitude (Fig. 1). It was also not significantly correlated with the soil type (e.a. Andosols, Phaeozems, and Cambiosols) ($R_{Pearson} = -0.287$, $P = 0.072$).

Maize cultivars collected from 40 farmer plots were screened for Al resistance using the eriochrome cyanine test. Aluminum resistance of the genotypes was expressed as a capability of roots to growth after 72 h exposure to 100 or 200 μM of Al. Speciation analysis using GEOCHEM-PC predicted that the free activity of Al^{3+} was 1.59×10^{-5} M and 1.58×10^{-5} M for the 100 and 200 μM treatment solution, respectively. The two solutions differ dramatically in the amounts of precipitated hydroxyl form of Al: 6.47×10^{-5} mol l^{-1} and 1.65×10^{-4} mol l^{-1} for 100 and 200 μM solution, respectively. The Madeiran maize germplasm appeared to be highly resistant to Al at a concentration of 100 μM since 55 % of the tested genotypes did not show symptoms of roots injury (root regrowth over 80 %). At 200 μM Al in the medium, half of the tested genotypes was classified as very sensitive where less than 20 % of seedlings within a genotype were able to continue growth after exposure to Al. Even using the more stringent stress conditions of 200 μM Al, we were able to identify 8 highly Al-resistant maize genotypes (Fig. 2).

Although we found that the Madeiran maize germplasm was generally resistant to Al at concentration of 100 μM while sensitive at 200 μM Al (Fig. 2), a detailed analysis of Al resistance revealed a variety of complex and intermediate responses to this metal. These include genotypes very sensitive to Al (ISOP 130 and 153), moderately sensitive (ISOP 065 and 147), and very resistant (ISOP 71, 138, 141, 152, 157) (Tab. 2). Some genotypes, however, appeared to be resistant to 100 μM Al, while they were sensitive to this metal at higher concentration (ISOP 129 or 149).

Enhanced Al resistance expressed as the ability of over 50 % of roots to regrow after exposure to 100 or 200 μM Al was not significantly correlated with both altitude and soil pH. Moreover, there was no significant correlation between soil Al^{3+}

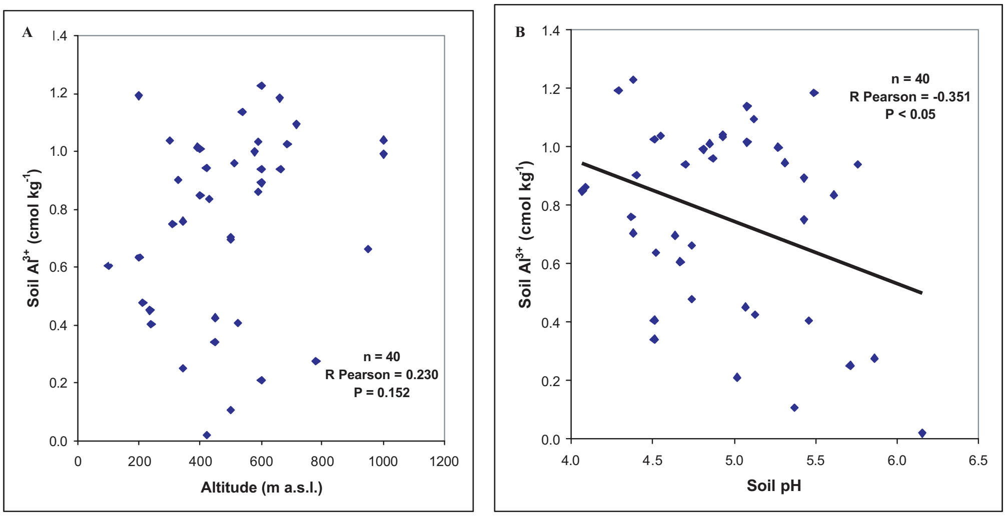

Figure 1: The correlation between altitude (A) or soil pH (B) and the ionic soil aluminum content of maize plots on the Island of Madeira.
Abbildung 1: Korrelation zwischen Höhe (A) oder pH-Wert des Bodens (B) und dem Al^{3+}-Gehalt im Boden der ausgewählten Maisstandorte auf Madeira.

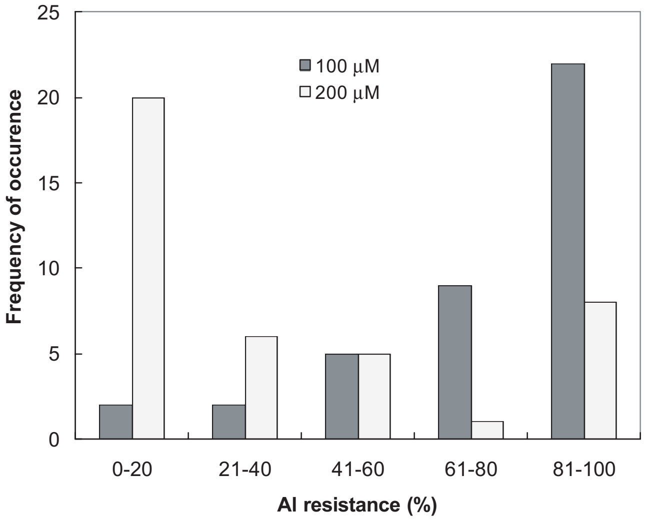

Figure 2: Frequency distribution of Al resistance of maize cultivars collected from 40 farmer plots on the Island of Madeira. A cultivar was considered Al-resistant if more than 50 % of the roots were able to continue growth after exposure to 100 or 200 μM Al for 72 h.
Abbildung 2: Häufigkeitsverteilung der Al-Resistenz von Maisherkünften von 40 Maisstandorten auf Madeira. Eine Herkunft wurde als Al-resistent angesehen, wenn nach 72 Stunden in 100 oder 200 μM Al mehr als 50 % der Wurzeln noch in der Lage waren zu wachsen.

Table 2: Differential responses of the selected Madeiran maize genotypes to Al stress imposed in nutrient solution in comparison to the Al resistant cultivar ATP-Y (*Horst* et al., 1997). Resistance is assessed as regrowth ability in Al-free nutrient solution after Al-treatment.
Tabelle 2: Unterschiedliche Reaktionen ausgewählter Maissorten Madeiras gegenüber Al-Stress in Nährlösung im Vergleich mit der Al-resistenten Standardsorte ATP-Y (*Horst* et al., 1997).

Genotype	Aluminum resistance (%)	
	100 μM Al	200 μM Al
ATP-Y	100 ± 0	100 ± 0
ISOP 71	100 ± 0	92 ± 6
ISOP 141	100 ± 0	90 ± 5
ISOP 157	99 ± 1	85 ± 8
ISOP 152	97 ± 4	63 ± 10
ISOP 138	91 ± 10	75 ± 23
ISOP 149	88 ± 8	23 ± 11
ISOP 129	86 ± 20	11 ± 3
ISOP 147	83 ± 2	49 ± 6
ISOP 65	72 ± 19	49 ± 17
ISOP 153	36 ± 11	4 ± 1
ISOP 130	11 ± 8	0 ± 0

content and Al resistance of maize cultivars at both 100 and 200 μM Al supply. However, 200 μM Al supply revealed that the most Al-resistant cultivars were obtained from locations of high soil Al^{3+} content (Fig. 3).

4 Discussion

Using the eriochrome cyanine screening technique (*Aniol*, 1990; *Slaski*, 1992), we identified a great number of Al-resistant cultivars among the Madeiran maize germplasm. To obtain the separation between cultivars in response to Al in nutrient solution culture, we had to subject maize seedlings to very stringent stress conditions of 72 h exposure at 100 and 200 μM Al. Shorter times of exposure (i.e. 24 h), commonly used in the screening of maize populations (*Martins* et al., 1999; *Collet* et al., 2000), were not effective since the majority of Madeiran genotypes exhibited high levels of Al resistance in the presence of 100 μM of this metal. For maize, 100 μM Al typically results in irreversible root damage and such concentration exceeds about twice what is considered to be the lower level of toxicity of this metal (*Pintro* et al., 1996). Interestingly, the effect of total Al concentration on root regrowth (100 or 200 μM) was not in agreement with the predicted concentration of the free Al^{3+} in bulk solution (about 15 μM for both concentrations). It is possible that the pool of the toxic, monomeric form of Al might be replenished from the precipitated hydroxyl forms at different rates at 100 and 200 μM as root growth and, associated with that, changes in pH of Al treatment solution occurred, resulting in the observed pronounced differences in the degree of root damage.

We assumed that the enhancement of Al resistance among Madeiran maize cultivars could be driven by selection pressure of the environmental factors unique to the islands as it was recently documented for wheat (*Pinheiro de Carvalho* et al., 2003). Indeed, the pH and Al^{3+} contents of maize plots showed that Madeiran soils were mainly acidic with high levels of free ionic Al. As expected, a negative correlation between increasing soil pH and Al^{3+} content was observed. However, differences in Al resistance among the Madeiran germplasm were not correlated with the altitude, soil pH or even ionic Al content. We anticipated that strongly acidic conditions observed in Madeiran soils should enhance Al solubility and the increase of the exchangeable Al pool or hydroxo-Al mononuclear complexes (pH < 6.0) in mineral soils (*Bertsch* and *Bloom*, 1996). On Madeira, total Al ranged from 4.1 to 12.5 cmol (kg soil)$^{-1}$ (*Madeira* et al., 1994), while in our experiments Al^{3+} content in soils from 40 maize plots varied between 0.02 and 1.23 cmol Al^{3+} (kg soil)$^{-1}$. Lower ionic Al content as observed in maize plots compared to wheat plots (*Pinheiro de Carvalho* et al., 2003) could result partially from the traditional agricultural practice. This technique implies abundant application of organic humus before planting maize (Pinheiro de Carvalho, personal observation). The Al^{3+} content in the maize plots was similar or even higher than in tropical soils (*Edmeades* et al., 1985; *Collet* et al., 2000). It seems, however, that Al present in soil does not contribute to the actual Al resistance in maize genotypes since the enhanced resistance or sensitivity was observed both in soils with higher or lower Al^{3+} contents. These observations indicate an independent plant adaptation to low pH and to Al toxicity (*Kidd* and *Proctor*, 2001). The absence of a correlation between analyzed environmental factors and resistance for the majority of maize genotypes may suggest that the Al content in the tested soils had no direct effect on the enhancement of Al resistance of the cultivars on the island. Thus, we may presume that a high number of Al-resistant maize genotypes identified during the course of this study was the result of a selection pressure exerted by cultivation of maize on soils with low pH and high Al^{3+} content outside Madeira. A high biodiversity of the Madeiran maize could be perhaps a

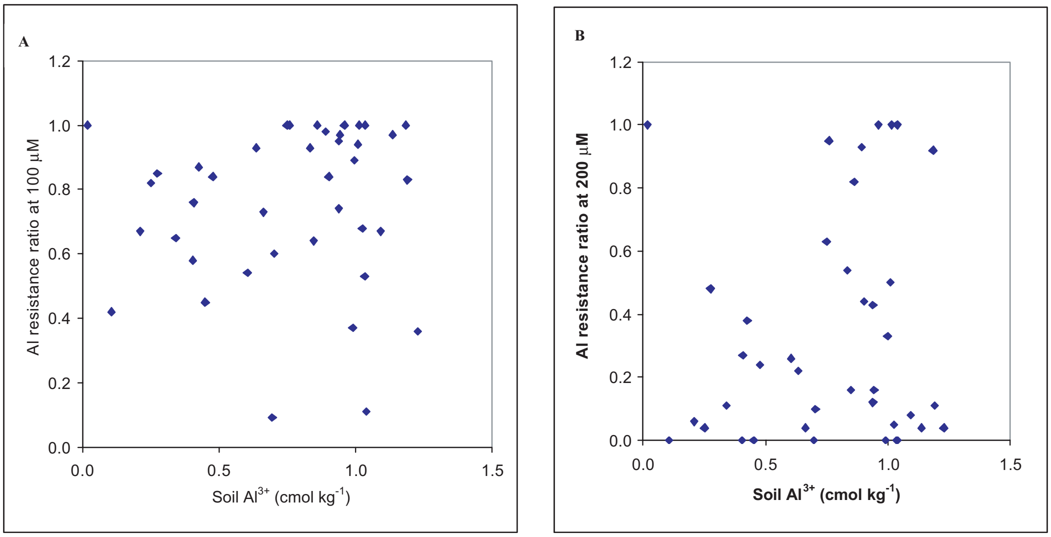

Figure 3: The correlation between ionic soil Al content on the Island of Madeira and the aluminum resistance of maize cultivars grown in nutrient solution at 100 (A) or 200 (B) µM Al supply. The aluminum resistance was determined using the eriochrome cyanine staining method (*Aniol*, 1990; *Slaski*, 1995).

Abbildung 3: Korrelation zwischen dem Al^{3+}-Gehalt des Bodens von Maisanbauflächen auf Madeira und der Al-Resistenz der Maisherkünfte in Nährlösung mit 100 (A) oder 200 µM Al-Angebot (B). Die Al-Resistenz wurde mit der "eriochrome cyanine staining"-Methode bestimmt (*Aniol*, 1990; *Slaski*, 1995).

result of the introduction of maize cultivars brought in by the Portuguese as early as the 18th century from various phytogeographical locations including Azores, Canary Islands, Europe, and more recently from the African continent (*Silva* and *Meneses*, 1984; *Ribeiro*, 2001). Cultivars adapted to the highly Al-toxic soils were retained by the farmers for cultivation and were able to survive till the present time. Additionally, a high number of Al-resistant maize genotypes could result from the open pollination of the crop, which promotes the sharing of gene pools between different cultivars and is independent of the influence of pedologic conditions.

Acknowledgments

Portuguese Foundation for the Science and Technology (FCT, Fundação para a Ciência e Tecnologia) has sponsored this work through the Centre for Macaronesian Studies (CEM) and the project POCTI/ 35003/ AGR/ 1999. Acknowledgments are due to Mr. *R. Correia* for the technical assistance in conducting the fieldwork, to Dr. *A. Aniol* for valuable advice during the preparation of this manuscript and to Dr. *W. J. Horst* for providing seeds of the Al-resistant cultivar ATP-Y.

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