DIFERENTIAL RESPONSE OF FOUR MAIZE *(ZEA MAYS* L.) VARIETIES TO ALUMINUM TOXICITY

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RESUMEN

RESPUESTAS DIFERENCIALES A LA TOXICIDAD DE ALUMINIO EN CUATRO VARIEDADES DE *ZEA MAYS* L.

En el presente trabajo hemos estudiado la respuesta de cuatro variedades de *Zea mays* (Adour-250, Honeycomb, HS 1230 y IRAT 200) a la toxicidad por Al, mediante la técnica de tinción de raíces con hematoxilina, el análisis del crecimiento y la determinación de los niveles internos de Al en raíces y partes aéreas. Se observaron diferencias significativas en el crecimiento y la acumulación de Al en la parte aérea entre los genotipos. No se pudo establecer una relación clara entre la acumulación de Al en los tejidos y el crecimiento. De acuerdo con nuestros primeros resultados, Adour-250 es el genotipo más resistente y Honeycomb, IRAT-200 y HS-1230 son los genotipos más sensibles.

Palabras clave: Aluminio. Tinción con hematoxilina. Tolerancia. Toxicidad. Variedades *Zea mays* L.

SUMMARY

In the present work we studied the response to aluminum toxicity in four *Zea mays* genotypes (Adour-250, Honeycomb, HS-1230 and IRAT-200) using hematoxylin staining of roots, determination of growth and analysis of Al concentrations in roots and shoots. Significant differences between genotypes in growth and shoot Al concentrations were observed. There was no clear relation between plant Al concentration and growth. According to our preliminary results, Adour was the most Al-resistant genotype, while Honeycomb, IRAT-200 and HS-1230 were sensitive.

Key words: Aluminum. Hematoxylin staining. Toxicity. Tolerance. *Zea mays* L. varieties.

INTRODUCTION

Besides salinity, Al-toxicity may be considered one of the most widespread mineral toxicity problems affecting crop productivity. The en-

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vironmental chemistry of Al is very complex (Massot *et al.*, 1991 a). Aluminum toxicity is intimately related to soil acidity and principally occurs in tropical regions where abundant rains have washed out alkaline and alkaline-earth cations (Ca, Mg, K and Na) from the root zone of soils (Clark, 1982). These cations of the soil solution and the cation exchange sites are substituted by H^+ or Al ions. Acid mineral soils characteristically have a high Al activity. Aluminum is a small, highly charged ion and has a octahedral shell of six water molecules (Al $(H_2O)_6^{3+}$). This Al-aquo complex is stable in acid environments, where Al itself acts as a pH buffer. When H^+ activity decreases (increase of pH) polimerization occurs and finally Al $(OH)_3$ may precipitate (Driscoll and Schecher, 1988).

High Al activity causes in plants a complex syndrome, which has been studied at different levels (Taylor, 1988). Membrane damage has been proposed as a primary toxicity mechanism, but interaction with nucleic acids and inhibition of cell division as well as mineral nutrition disorders also seem to play an important role.

Significant differences in Al-tolerance between plant species have been reported. Gramineae from the tropics may tolerate the saturation of up to 80 % of exchange capacity by Al. Maize shows moderate tolerance resisting up to 30 % Al saturation (Fageria *et al.*, 1988). Nevertheless important varietal differences within a species have been reported.

In general, corn is poorly adapted to extremely acid soils (Duke, 1982), but recent breeding programms have delivered Al-tolerant genotypes.

In the present work we analysed the Al tolerance of four maize varieties in solution culture, using early indicators of the Al-stress response.

MATERIALS AND METHODS

Plant material and growth condition

Studies were performed with four *Zea mays* L. varieties: *Adour-250* from Semillas Fitó, S. A., Barcelona, adapted to cold humid climate and grown in North Spain (Galicia); *IRAT-200* and *HS-1230* supplied by the seed service of IRAT-CIRAD, Montpellier. IRAT-200 (IRAT-GERVEX catalogue number, 930; synonymes: Ferké 7928, IDSA 29) is original from Ivory Coast and grown in Madagascar; *Honeycomb*, a sweet corn variety from Batlle, S. A., Lérida.

Seeds were germinated for seven days at 25 °C on filter paper with distilled water. Plants were grown in a growth chamber under the following conditions: photoperiod 12 h light, 12 h darkness, day/night temperature 26 °C/20 °C, day/night relative humidity 75 %/85 %.

Two types of experiments were performed. Experiment I, for visualizing short-term (17 h) Al-uptake by hematoxylin staining of roots and experiment II, for studying the influence of high Al availability on growth and Al-uptake on a longer-term (21 days) basis.

Experiment I:

Seedlings were grown for 24 h in continuously aerated 10 % Hoagland and Arnon (1950) nutrient solution (pH 4). Then, seedlings were transferred for a further 17 h to 10 % Hoagland nutrient solution (pH 4) containing 0 (control), 0.3 mM, 0.6 mM or 1.2 mM Al as $AlCl_3$. After washing the roots with distilled water for 45 minutes, the roots were stained with hematoxyliyn according to Polle *et al.*, 1978, as previously described (Massot *et al.*, 1991 b). After washing, roots were immediately photographed and scored for intensity of staining. A scale from 0 to 6 was applied according to the following criterion: 0, roots without any stain; 1, very light violet colouration; 2 light violet colour in tips and above elongation zone, elongation zone white; 3, light violet colour in elongation zone; 4, more intense violet colour with some lightly stained patches; 5, uniform violet staining; 6, dark violet staining of the whole root. Due to a relatively high variability in the staining of individual roots at a given Al concentration, hematoxylin staining has to be done using a range of different Al concentrations, and the mean staining intensity has to be taken into account for evaluation of tolerance (Polle *et al.*, 1978). Therefore, the given intensity index are the average of the mean values of colour score of three roots per treatment and variety.

Experiment II:

Seedlings were transferred to continuously aerated 10 % Hoagland and Arnon (1950) nutrient solution (pH 4) containing 0 (control) or 1.2 mM Al as $AlCl_3$. After 21 days, plants were harvested and the following parameters were determined on roots and shoots of three plants per treatment and variety: fresh weight, dry weight (material dried at 80 °C for 48 h) and Al concentration. For Al content analysis, the oven dried material was dry ashed at 550 °C for 12 h. Ash was taken up with an acid mixture ($HNO_3 : HCl : H_2O = 1:1:2$). Aluminum concentration in the digest was determined by colorimetry according to Jayman and Sivassubraniam (1974).

Statistics:

Differences between varieties and treatments were determined by two-way layout ANOVA.

RESULTS

Figures 1 and 2 show photographs of the hematoxylin-stained roots from the different maize varieties exposed to different Al-treatments. Increase of intensity of staining with increasing Al-concentration in solution was evident. Colour intensity index for Adour-250, Honeycomb, IRAT-200 and HS-1230 were 2.5, 3.2, 4.2 and 3.8 respectively.

Tables 1 and 2 show the root and shoot growth data. Under control conditions, varietal differences in root and shoot length (Table 1), root fresh weight (data not shown) and root and shoot dry weight (Table 2)

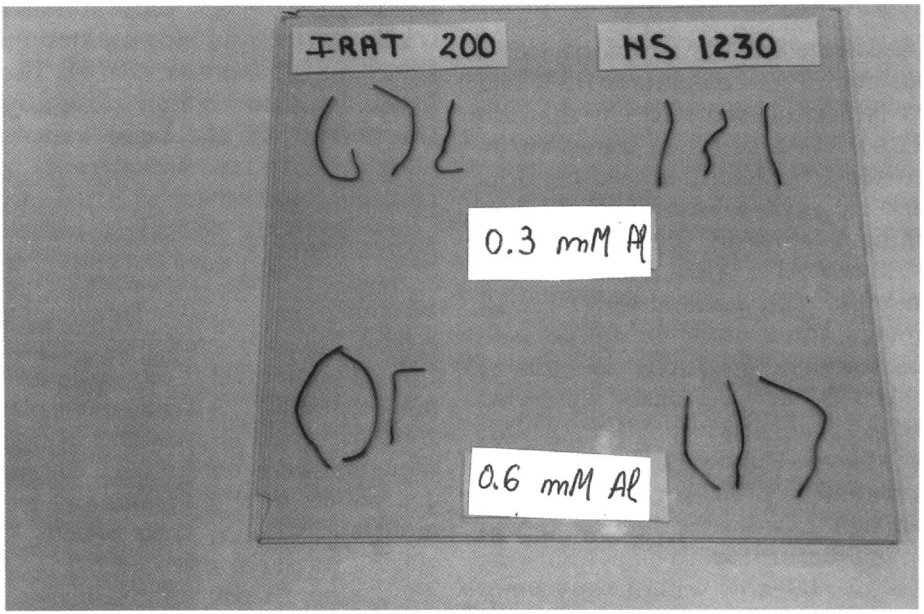

FIG. 1.—*Roots from the* Zea mays *L. varieties HS-1230 and IRAT 200 exposed to different Al concentrations and stained with hematoxylin.*

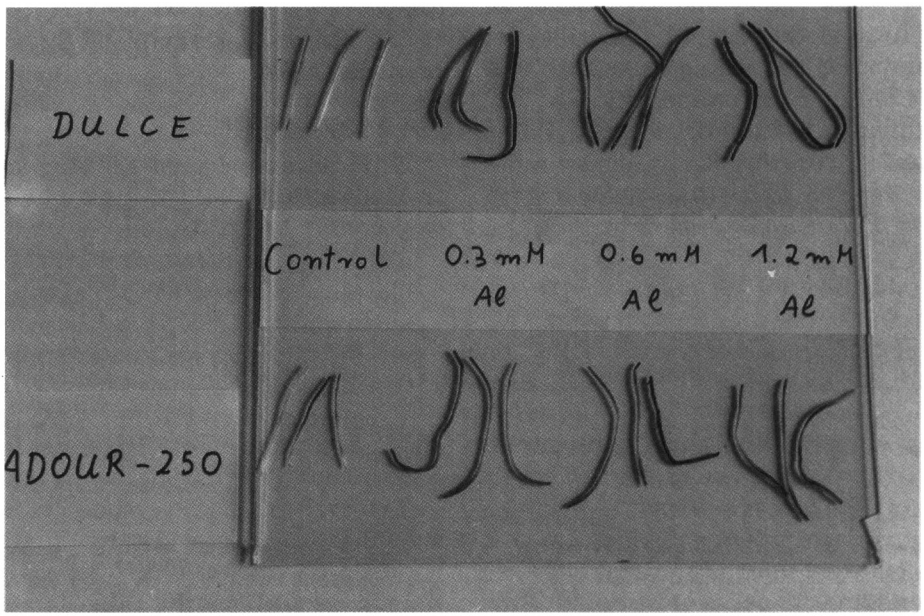

FIG. 2.—*Roots from the* Zea mays *L. varieties Adour-250 and Honeycomb ("dulce") exposed to different Al concentrations and stained with hematoxylin.*

TABLE 1

Length of the longest root (cm) and shoot length (cm) of four maize varieties grown in control or 1.2 mM Al containing nutrient solution.

	ROOT LENGTH			SHOOT LENGTH		
	Control	1.2 mM AL	TI	Control	1.2 mM Al	TI
Adour	59.2	27.8	47.0	74.2	38.9	52.4
Honeycomb	42.2	21.2	50.2	76.5	26.6	34.8
HS-1230	42.2	17.3	41.0	63.5	21.0	33.1
IRAT	49.8	18.5	37.1	66.7	19.4	29.1

ANOVA Source of variation	F	Prob.
Varieties	4.5	0.02
Al treatment	89.3	5.1×10^{-8}
Interaction	0.8	0.51

ANOVA Source var.	F	Prob.
Varieties	4.6	0.02
Al treatment	187.1	3.0×10^{-10}
Interaction	0.1	0.42

were observed. The supply of 1.2 mM Al significantly decreased growth of all varieties. The Al-induced decrease of length of the longest root (Table 1) was quite similar for all varieties and tolerance index based on this parameter were not significantly different. The same was true

TABLE 2

Dry weight (g) of roots and shoots of four maize varieties grown in control or Al-containing nutrient solution.

	ROOT DRY WEIGHT			SHOOT DRY WEIGHT		
	Control	1.2 mM Al	TI	Control	1.2 mM Al	TI
Adour	0.20	0.18	90.0	1.03	0.27	26.2
Honeycomb	0.12	0.01	5.6	0.63	0.12	19.0
HS-1230	0.35	0.13	37.1	0.94	0.12	12.8
IRAT-200	0.31	0.08	25.8	0.91	0.09	19.3

ANOVA Source of variation	F	Prob.
Variety	5.0	0.01
Al treatment	24.3	1.4×10^{-4}
Interaction	5.6	7.9×10^{-3}

ANOVA Source var.	F	Prob.
Variety	4.0	0.02
Al treatment	161.5	8.8×10^{-10}
Interaction	1.7	0.20

for shoot length. Shoot dry weight of all varieties was severely affected by Al, and tolerance index based on this parameter were lower than those based on root or shoot length. Highly significant differences between varieties in the response to Al-toxicity were observed when root dry weight was considered (Table 2). In Adour, the root dry weight was not significantly affected by the Al supply, while the other varieties showed a severe decrease.

Figure 3 shows the Al concentrations in roots (A) and shoots (B) of the four maize varieties. Under control conditions, the varieties significantly differed in the Al concentrations of shoots and roots (Fig. 3 a and b, Table 3). Aluminum supply significantly increased the Al concentration within both roots and shoots. Aluminum principally accumulated in roots and significantly lower concentrations were found in shoots. The differences between varieties in root and shoot Al concentrations were highly significant (Table 3). The varieties Adour and Honeycomb accumulated significantly higher Al concentrations in roots than HS-1230 and IRAT. In contrast, the varieties HS-1230 and IRAT showed significantly higher Al concentrations in shoots than Honeycomb and Adour.

TABLE 3

Analysis of variance of results from figure 3.

Al μg/g in roots Source of variation	F	Prob.	Al μg/g in shoots Source var.	F	Prob.
Variety	224.9	5.4×10^{-13}	Variety	70.6	1.8×10^{-9}
Al treatment.	1628.1	8.0×10^{-14}	Al treatment.	104.7	1.9×10^{-8}
Interaction.	219.1	3.5×10^{-3}	Interaction.	5.7	7.2×10^{-3}

DISCUSSION

Our results obtained with the hematoxylin staining methods indicate small varietal differences in Al-uptake after short-term exposure to Al. Adour-250 exhibited the lowest colour intensity index and, according to Polle *et al.* (1978), this would be the most tolerant genotype, followed by Honeycomb, HS-1230 and IRAT-200. This result was only partially confirmed by growth data after longer exposure to Al. Honeycomb and Adour-250 exhibited the highest root length tolerance index, but differences between varieties were not significant. Root dry weight as an indicator for Al tolerance has been used, among others, in annual ryegrass (Rengel and Robinson, 1989). In our study, Al-influence on root dry weight revealed important varietal differences. Tolerance index

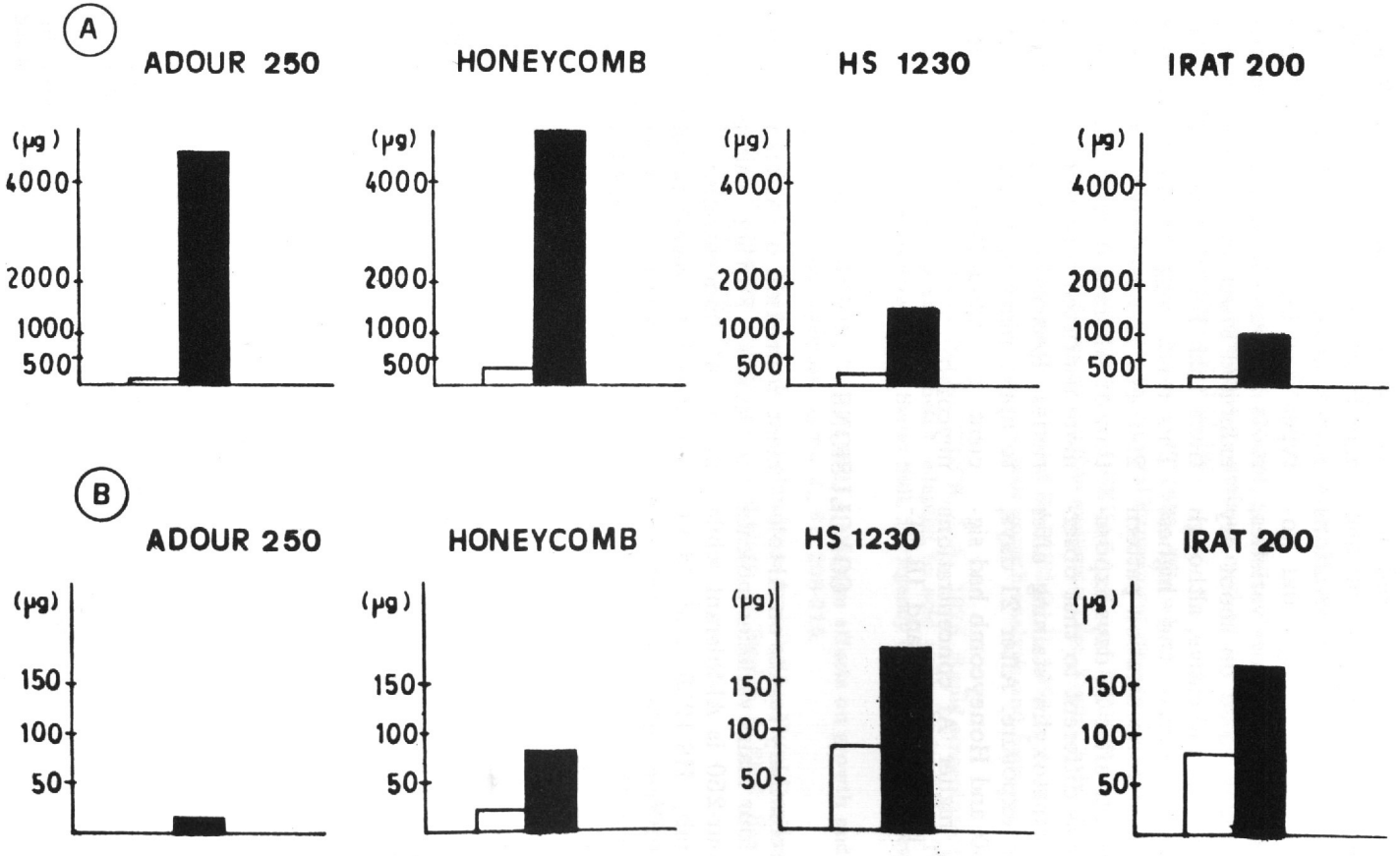

FIG. 3.—*Aluminum concentrations (µg g^{-1}) in roots (A) and shoots (B) of* Zea mays *L. varieties exposed to 1.2 mM Al. White columns, control; black columns, Al treatment.*

based on root dry weight indicated highest tolerance in Adour-250, followed by HS-1230, IRAT-200 and Honeycomb. Similar to observations in annual ryegrass (Rengel and Robinson, 1989), in our maize varieties, tolerance index based on shoot dry weight were less indicative, although Adour-250 exhibited the highest value. The Al concentration pattern found in roots after 21 days exposure to Al was different to that observed by hematoxylin staining after short-term exposure. After 21 days, Adour-250 and Honeycomb had significantly higher Al concentrations in roots than HS-1230 and IRAT-200, while after short-term exposure less Al was accumulated in Adour and Honeycomb, as shown by the colour intensity index. Both genotypes with high Al concentration in roots (Adour-250 and Honeycomb) exhibited lower shoot Al-concentrations than HS-1230 and IRAT-200. This result suggests that in Adour-250 and Honeycomb the translocation of Al from roots to shoots was more restricted than in the other varieties. Restriction of Al transport to upper plant parts was more efficient in Adour-250 than in Honeycomb.

CONCLUSIONS

Our preliminary results on Al-tolerance in four maize varieties indicate that Adour-250 is Al-tolerant while Honeycomb, HS-1230 and IRAT are Al-sensitive. Aluminum-tolerance in Adour-250 seems due to tolerance mechanisms in roots and efficient restriction of Al translocation to shoots. Further studies, using lower Al concentrations must be performed to reveal possible differences in tolerance between Honeycomb, HS-1230 and IRAT.

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