Suelo y Planta. Págs. 25-32.

ALUMINUM TOLERANCE ASSESSMENT IN BUSH BEAN CULTIVARS BY ROOT GROWTH ANALYSIS AND HEMATOXYLIN STAINING

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RESUMEN

DETERMINACION DE LA TOLERANCIA AL ALUMINIO DE CULTIVARES DE JUDIA MEDIANTE ANALISIS DEL CRECIMIENTO RADICULAR Y TINCION CON HEMATOXILINA

Se ha evaluado la tolerancia al aluminio de seis cultivares comerciales de judía de mata baja (*Phaseolus vulgaris* L. cv. "Hilds Maxi", "Superba", "Selección F-15", "Contender", "Eagle" y "Strike") en cultivo hidropónico, mediante la tinción de raíces con hematoxilina, la medida del crecimiento radicular después de exposición corta (48 h) y larga (16 d) al Al y el análisis de curvas de crecimiento radicular. Los cultivares "Hilds Maxi" y "Superba" resultaron ser tolerantes al Al, mientras que "Eagle" y "Strike" eran sensibles al Al. "Contender" y "F-15" mostraron un comportamiento intermedio. La evaluación de la tolerancia después de exposición corta al Al permitía distinguir entre el cultivar más tolerante ("Hilds Maxi") y el más sensible ("Strike"), pero dió resultados equívocos para "Superba" y "Eagle". Para conocer aquellos genotipos sensibles a los efectos indirectos, tardíos del Al es necesario realizar la selección para tolerancia después de exposición larga al Al.

Palabras clave: Aluminio, tinción hematoxilina, *Phaseolus vulgaris*, crecimiento radicular, tolerancia.

SUMMARY

The Al tolerance of six comercially available bush bean cultivars (*Phaseolus vulgaris* L. cv. "Hilds Maxi", "Superba", "Selección F-15", "Contender", "Eagle" and "Strike") has been evaluated in hydroponic culture by means of hematoxylin staining of roots, maximum root length growth after short (48 h) and long-term (16 d) exposure to Al, and root growth curve analysis. "Hilds Maxi" and "Superba" were found to be Al-tolerant, while "Eagle" and "Strike" were Al-sensitive. "Contender" and "F-15" showed an intermediate behavior. Evaluation of Al-tolerance after short-term exposure to Al allowed to distinguish between the most tolerant ("Hilds Maxi") and the most sensitive ("Strike") cultivar, but rendered misleading results for "Superba" and "Eagle". Screening for Al-tolerance after long-term exposure is required to recognize genotypes which are sensitive to the later occurring indirect Al effects.

Key Words: Aluminum, hematoxylin staining, *Phaseolus vulgaris*, root growth, tolerance.

INTRODUCTION

Aluminum toxicity is one of the most important factors limiting crop growth on acid soils (Foy, 1984). Occurrence of high Al activity in soil solution is a widespread agricultural problem, probably affecting about 40% of the world's arable soils (Taylor, 1988 a). The development of Al-tolerant genotypes of major crop plants is an urgent necessity for improving crop productivity in developing countries.

Aluminum-tolerant genotypes have been found for different crops, e.a. wheat, maize, rice, sorghum, soja and snapbean (Devine, 1982; Foy, 1988). Quick screening tests based on root growth after 48 h exposure to Al (Hill *et al.*, 1989) or on Al-accumulation after a few hours exposure (hematoxylin staining) (Polle *et al.*, 1978 a; 1978 b) have been developed for different species.

In the present study we analysed the reponse to Al-toxicity of six comercially available bush bean cultivars by means of hematoxylin staining, root length growth after both short and long-term exposure to Al, and root growth curve analysis, in order to establish the possible differences in Al-tolerance and to select an adequate screening procedure.

MATERIALS AND METHODS

Plan material and growth conditions

Seeds of different bush bean *(Phaseolus vulgaris*, CARL VON LINNE, 1753) cultivars, "Hilds Maxi" (BSV GmbH), "Strike" (CAS, SA), "Eagle" (Asgrow CAS, SA), "Superba" (Batlle, SA), "Contender" (Rocalba, SA) and "Selección F-15" (Fitó) were germinated on perlite with distilled water for eight days. Uniform seedlings were transplanted to continuously aerated half-strength Hoagland's (Hoagland and Arnon, 1950) nutrient solution (plastic beakers, 5 l capacity; 8 plants per beaker). Control plants only received the basic nutrient solution, while the rest of plants received solution supplement with 10 μg Al mL^{-1} as $AlCl_3$-$6H_2O$. All solutions were adjusted initially to pH 4.8 and not adjusted thereafter, for not concealing possible root-induced changes of solution pH values, which might be related to differences between the Al-tolerance of the cultivars.

The plants were grown in a growth chamber, illuminated by cool white fluorescent light, supplemented with incandescent lamps, under the following conditions: photon fluence rate at plant height approximately 144 μM photon m^{-2} s^{-1}; photoperiod, 16 h light, 8 h darkness; day/night temperature 26/23° C; day/night relative humidity 60/80%.

Root growth analysis

The length of the longest root per plant was measured non-destructively with a ruler on three plants per cultivar and treatment. Determinations were made on the same plants at days 0, 1, 2, 3, 4, 7, 8, 9, 10, 11, 14, 15 and 16 after the start of the treatment. The significance of differences between control and Al-treated plants was determined by ANOVA. The primary root growth data were fitted to sigmoids and the root extension rate (RER) was calculated for the half time of maximum length (Hunt, 1982).

Hematoxylin staining test

To visualize Al-accumulation in roots, the hematoxylin staining test according to Polle *et al.* (1978 b) was used. Eight day old bush bean seedlings were transplanted to nutrient solution containig 10 μg Al ml^{-1} After 16 h exposure to Al, roots were submerged in distilled water for 60 min and then transfered to the staining solution (2 g hematoxylin and 0.2 g $NaIO_3$ dissolved in 1000 ml distilled water). After 15 min staining, the roots were maintained for further 60 min in distilled water. Then roots were immediately photographed and scored for intensity of staining.

RESULTS

After 16 d growing in solution, both control and Al-treated plants from all cultivars had increased the pH of their nutrient solutions from the initial value of 4.8 to 5.5 ± 0.5. No correlation between increase of solution pH and Al-tolerance could be established.

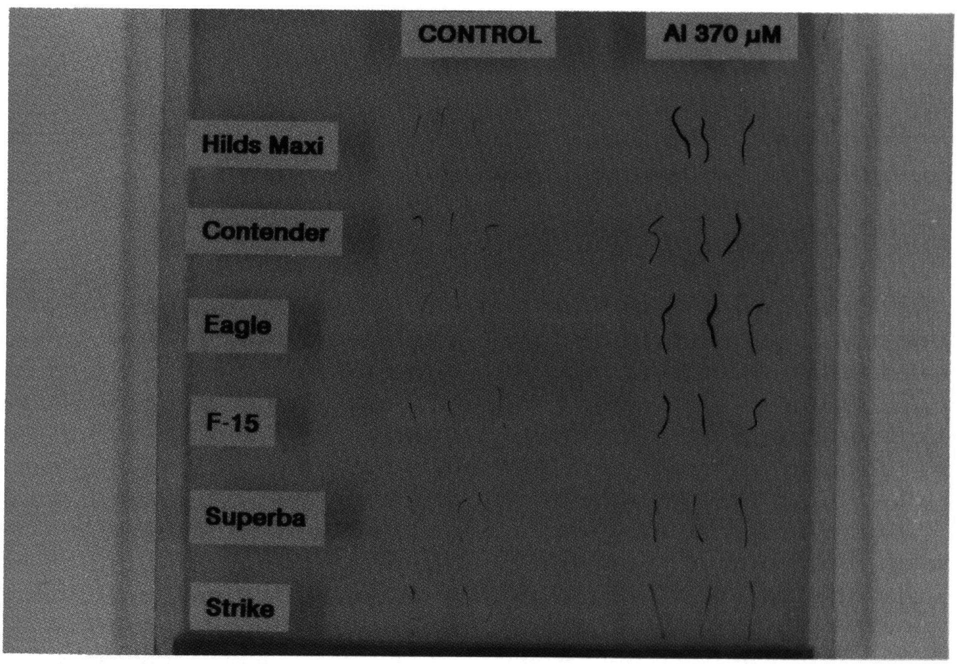

FIG. 1.–*Roots from different bush bean cultivars stained with henatoxylin to visualize Al accumulation. Left side: roots from control plants; right side: roots from plants exposed to 10 µg Al ml^{-1} for 16 h.*

Figure 1 shows roots from the six bush bean cultivars stained with hematoxylin. Roots from control plants (without Al supply) were hardly coloured (left side), while those exposed to Al for 16 h showed the typical violet colouration due to Al-accumulation (right side). In Al-treated roots from all cultivars the elongation zone was less stained, indicating lower Al-accumulation in these cells. Roots from "Hilds Maxi" and "Contender" appeared almost completely stained, while those from "Superba" and "Strike" exhibited large unstained zones. "Eagle" and "F-15" showed an intermediate behavior.

TABLE 1

Root length (cm) and relative root length (% of control) of bush bean cultivars treated with 10 μg Al ml^{-1} for 48 h or 16 d. Given values are means ± standart deviation.

Exposure time	48 Hours			16 Days		
Cultivar	Control	Aluminum	%	Control	Aluminum	%
F-15	10.7 ± 0.4	11.5 ± 0.1	107.5	43.0 ± 0.6	41.8 ± 0.8	97.2
Hilds Maxi . . .	8.3 ± 0.3	9.0 ± 0.9	108.4	39.5 ± 0.5	45.2 ± 1.1	114.4
Superba.	10.3 ± 0.3	8.4 ± 0.2*	81.6	38.1 ± 0.8	37.3 ± 1.5	97.9
Eagle.	10.3 ± 0.6	9.6 ± 0.1	93.2	42.9 ± 0.2	29.8 ± 0.1*	69.5
Contender . . .	12.4 ± 0.5	9.5 ± 0.5*	76.6	46.0 ± 1.0	37.1 ± 0.1*	80.6
Strike	11.7 ± 1.7	7.5 ± 0.0*	64.1	40.8 ± 2.0	23.0 ± 1.3*	56.4

* Denotes significantly different from control.

Table 1 shows the values of maximum root length of control and Al-treated plants. After 48 h exposure to Al, significant decreases of the maximum root length were found for "Superba", "Contender" and "Strike". Long-term exposure to Al (16 d) significantly affected the maximum root length of "Contender", "Eagle" and "Strike".

To evaluate the Al-effects on root extension growth over the whole experimental period, and for calculating the root extension growth rates, the primary root growth data obtained at different time samples were fitted to sigmoids. These growth curves are shown in Fig. 2, and the calculated root growth rates (RER) are given en Table 2. Table 2 also shows the half time of maximum length ($t_{1/2}$) and the standardized root extension rates (SRER) calculated as $RER_{Al}/RER_{control}$. The Al-treatment did not significantly affect the root growth rates of "Hilds Maxi" and "Superba". The cultivars "Eagle" and "Strike" showed an about 40% decrease of RER, while "F-15" and "Contender" Exhi-

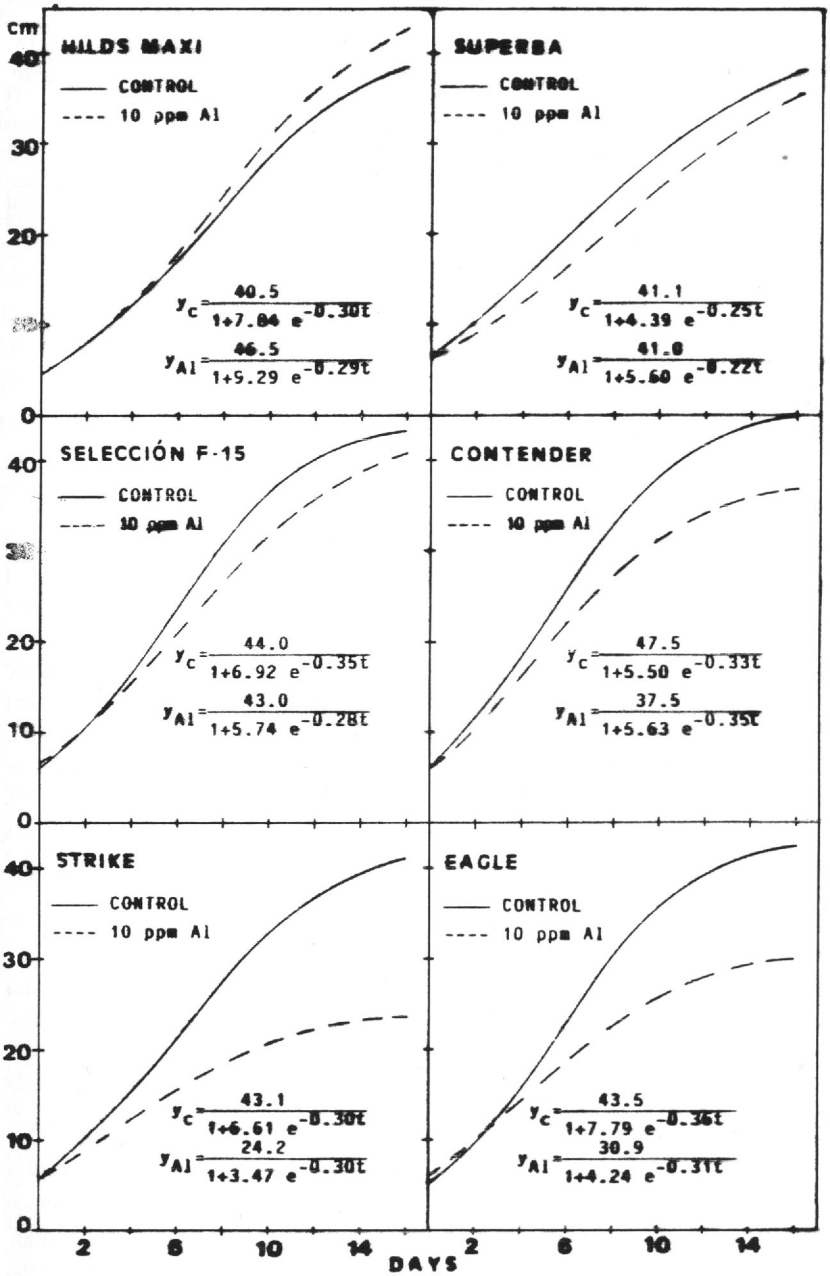

FIG. 2.–*Extension growth curves (cm/days) for roots from different bush bean cultivars grown either in control or Al-supplemented nutrient solution.* Hilds Maxi: *control* $r^2 = 0.9398$; *Al-treated* $r^2 = 0.8721$. Superba: *control* $r^2 = 0.9848$; *Al-treated* $r^2 = 0.9607$. Selección F-15: *control* $r^2 = 0.9448$; *Al-treated* $r^2 = 0.9185$. Contender: *control* $r^2 = 0.9526$; *Al-treated* $r^2 = 0.9654$. Strike: *control* $r^2 = 0.8442$; *Al-treated* $r^2 = 0.9068$. Eagle: *control* $r^2 = 0.9305$; *Al-treated* $r^2 = 0.9458$. *For all curves n = 39.*

TABLE 2

Root extensión growth rates (RER (cm d^{-1}) of roots from control and Al-treated plants at half time of maximum growth ($t_{1/2}$). The standardized root extension growth rate (SRER) was calculated as $RER_{Al}/RER_{control}$).

	Control		Aluminum		
Cultivar	RER	$t_{1/2}$	RER	$t_{1/2}$	SRER
F-15	3.90	5.45	3.04	6.18	0.78
Hilds Maxi	3.08	6.78	3.33	7.79	1.08
Superba.	2.50	5.95	2.25	7.48	0.90
Eagle.	3.92	5.70	2.42	4.60	0.62
Contender	3.89	5.21	3.29	4.93	0.85
Strike	3.23	6.30	1.81	4.14	0.56

bited an intermediate behavior. Aluminum supply increased the half times of maximum growth of "Superba", "Hilds Maxi" and "F-15", and decreased those of "Strike" and "Eagle". The half time of "Contender" was practically unaffected.

Scoring of cultivars for Al-tolerance, based on root staining (Al-accumulation), root growth after 48 h, and 16 d exposure to Al, and root extension growth rates (RER) is given in Table 3. All screening methods identified "Hilds Maxi" and "Strike" as the most Al-tolerant and most Al-sensitive cultivars, respectively. But the results on the relative tolerance of "Contender", "F-15", "Superba" and "Eagle" were different depending on the screening parameter used.

TABLE 3

Scoring of six bush bean cultivars according to intensity of root staining by hematoxylin (hemtox), maximum root length (m. r. l.) after short (48 h) and long-term (16 d) exposure to Al and root extension rate (RER).

Hemtox.	Hilds Maxi > Contender > Eagle > F-15 >> Superba > Strike
m. r. l. (48 h) . . .	Hilds Maxi = F-15 > Eagle > Superba > Contender > Strike
m. r. l. (16 d) . . .	Hilds Maxi > Superba = F-15 > Contender > Eagle > Strike
RER.	Hilds Maxi > Superba > Contender > F-15 > Eagle > Strike

DISCUSSION

Polle *et al.* (1978 b) have found that the classification of Al-tolerance in wheat cultivars based on hematoxylin staining was in good agreement with classification based on root growth. Aluminum-sensitive cultivars, which had shown strong root growth inhibition by Al, generally exhibited intense hematoxylin staining. This does not hold true for our bush bean cultuvars. "Strike" and "Hilds Maxi" which were the less and most stained cultivars, respectively showed strong and no root growth inhibition after 48 h exposure to Al. Several authors have demonstrated that most of the root Al accumulates in the apoplast, and the role of Al-immobilization in root cell walls for Al-tolerance has been discussed in extense by Taylor (1988b). In wheat and several other species, Al-tolerance associated with low root Al-concentrations seems related to low cation exchange capacity of root cell walls, leading to lower al uptake. But this may not hold true for all species as shown by others (for references see Taylor 1988 b) and by our own results on bean cultivars.

The high Al-tolerance associated with high Al-accumulation in roots found in "Hilds Maxi", and the Al-sensitivity of "Strike" which showed low hematoxylin staining, may be explained by a high ("Hilds Maxi") or low ("Strike") capacity to precipitate hydroxy Al- polymers, excluding Al from the plasma membrane. According to Taylor (1988b), the polymerization reaction would proceed more rapidly in Al-tolerant cultivars causing higher Al concentrations in roots from Al-tolerant than Al-sensitive plants. Nevertheless, this hypothesis can explain neither the low hematoxylin staining of "Superba", which showed relative high Al-tolerance after 16 d exposure to Al, nor the intense staining of "Contender", which exhibited intermediate tolerance according to the root growth data. Therefore, hematoxylin staining alone may not be considered an adequate screening method for Al-tolerance in bush beans.

Scoring of cultivars for Al-tolerance based on root extension growth after short-term exposure to Al did not agree with the classification based on root growth after long-term exposure for "Eagle" and "Superba". "Eagle", which was not significantly affected after 48 h exposure to Al, showed intense root growth decrease after 16 d exposure; the opposite was observed for "Superba". Root growth after short-term exposure to Al principally reflects the effects of Al on elongation and division of root cells (Sartain, Kamprath, 1978), but after longer exposure, later occurring Al-effects on mineral nutrition and other metabolic functions may substantially contribute to growth inhibition. Resistance to those indirect Al-effects seem to play an important role in Al-tolerance of crops (Foy, 1988) and can only be evaluated after long-term exposure. The occurrence of those late effects can be clearly visualized by the growth curves and by the decreased half times of maximum growth, observed for "Eagle" and "Strike" in our study.

CONCLUSIONS

We can conclude from our work that comercially available bush bean cultivars show differences in Al-tolerance. Intense hematoxylin staining may be used as a criterion for Al-tolerance, but has to be acompanied by root growth measurements to avoid exclusion of valuable genetic material (e. a. "Superba" in our experiment). Screening tests after long-term exposure have to be performed to exclude material which is sensitive to later occurring Al-effects (e. a. "Eagle" in our study). Taking into account the results obtained by the different screening methods used in this study, the six cultivars may be classified for Al-tolerance as follows: "Hilds Maxi" and "Superba" Al-tolerant; "Eagle" and "Strike" Al-sensitive; "F-15" and "Contender" intermediate behavior.

Further evaluation of Al-tolerance of these bush bean cultivars is in progress, taking into account productivity under field and laboratory conditions.

ACKNOWLEDGEMENTS

We are grateful to DGICYT (project PB 88-0234) for financial support and to CIRIT for a personal grant to N. Massot. We thank Dr. B. Gunsé for technical assistance.

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Recibido: 2-2-90.
Aceptado: 21-6-90.