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Differential response of three bean (*Phaseolus vulgaris*) cultivars to aluminium

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SUMMARY

The response of three bean (*Phaseolus vulgaris*) cultivars to 10 mg l^{-1} Al in nutrient solution was studied using tolerance indicies based on root elongation, root dry weight, shoot dry weight and leaf expansion growth. Plant Al and Ca concentrations were determined. According to the tolerance index based on root elongation, the cultivars Hilds Maxi and Selección F-15 were Al tolerant, while Eagle was Al sensitive. Aluminium supply significantly affected shoot growth in Selección F-15 and this cultivar exhibited unusually high Al concentrations in shoots. Eagle, the most Al-sensitive cultivar, was able to restrict Al uptake and transport. In the presence of Al, calcium uptake was significantly decreased in all cultivars. Scoring for tolerance using root elongation as a single criterion may be misleading in genotypes which accumulate high amounts of Al in shoots.

Key-words: aluminium, bean, *Phaseolus vulgaris*, root tolerance, shoot tolerance.

INTRODUCTION

High Al availability due to soil acidity is the most widespread metal toxicity problem in agriculture (Haug, 1984). Crops grown on tropical and subtropical soils are mainly affected by this phenomenon (Clark, 1982). The development of Al-tolerant genotypes of major crop plants is an urgent necessity for improving food production in the developing countries.

Several authors have found variegate differences for Al tolerance in bean plants (Foy *et al.* 1972; Malavolta *et al.* 1981; Massot *et al.* 1991). The mechanisms of Al tolerance in bean plants have not been established. Snapbeans differing in Al tolerance exhibited different Al concentrations in roots, but not in shoots (Foy *et al.* 1972). No correlations between plant-induced changes in pH and Al tolerance could be established in *Phaseolus vulgaris* (Foy *et al.* 1972).

Aluminium toxicity is known to induce mineral nutrient disorders in plants (Foy *et al.* 1978; Massot *et al.* 1990). In bean cultivars, tolerance to excess Al has been associated with the ability to resist Al-induced Ca-deficiency (Foy *et al.* 1978). Nevertheless, Al-induced alterations of nutrient balance in shoots do not seem to be caused by direct harm from Al in upper plant parts but by prior injury to roots (Foy 1984). According to this, the root

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tolerance index is considered the most sensitive indicator for Al tolerance (Rengel & Robinson 1989).

In the present study we analysed the influence of aluminium on the Al and Ca concentrations of three bean cultivars. The ability to use root and shoot tolerance indices to indicate Al tolerance of plants was evaluated.

MATERIALS AND METHODS

Seeds from different bean (*Phaseolus vulgaris* L.) cultivars (Hilds Maxi, Selección F-15, and Eagle) were germinated on perlite, moistened with distilled water, for 8 days. Uniform seedlings were transplanted to plastic beakers (5 l capacity, five plants per beaker) containing continuously aerated 10% Hoagland's nutrient medium. Controls received the basic nutrient medium only, while the rest of the plants received a medium containing 10 mg l^{-1} Al as $AlCl_3 6 H_2O$. The pH was initially adjusted to 4·8 and was not adjusted thereafter in order not to conceal possible root-induced changes in pH values that might be related to differences between Al tolerances of the cultivars. At day 8 after transplantation plants were given fresh medium and were grown for another 8 days. The experiment was performed in a growth chamber illuminated by cool white fluorescent light, supplemented with incandescent light under the following conditions: photon fluence rate at plant height approximately 144 $\mu E\ m^{-2}\ s^{-1}$, photoperiod 16-h light, 8-h darkness, day/night temperature 26/23°C, day/night relative humidity 60/80%. At the end of the experiment (16 days after transplantation), solution pH had risen to $5{\cdot}3 \pm 0{\cdot}2$ in all the cultivars exposed to Al. No significant differences were detected between cultivars in the ability to increase the pH of the solution. After harvest, growth was estimated by measuring the longest root length, shoot length, and the dry weight of roots, stems, primary leaves, 1st trifoliolate leaves and following trifoliolate leaves, and the total leaf area (planimeter, LiCor model 3000). Root and shoot tolerance indices based on root and shoot length (RI_l and SI_l) and on root and shoot dry weight (RI_w and SI_w) were calculated according to Rengel & Robinson (1989).

Prior to mineral analysis, roots were washed with 0·01 N HCl followed by distilled water. Oven dried (70°C) material from roots, stems, primary and 1st trifoliolate leaves was dry ashed (450°C) and the ash was taken up with an acid mixture (HNO_3: HCl: H_2O = 1:1:2). Aluminium concentration was analysed by ICP-ES (Yvon JY38-VHR). Calcium concentrations were determined by AAS (Perkin Elmer 703). Per cent inhibition of Ca uptake (PI) was calculated according to Rengel & Robinson (1989), following the equation:

$$PI = ([U_0 - U_1]/U_0) \cdot 100$$

where U_0 and U_1 are the amounts per plant of a nutrient present in the control and the Al-treated plant respectively.

The results given are the averages of at least three determinations per organ and per treatment. The significance of differences between treatments on cultivars was determined by two-way layout analysis of variance, followed by Duncan's multiple range test.

RESULTS

Aluminium supply differently affected the growth of the bean cultivars (Table 1). Root elongation and root dry weight were significantly decreased in Eagle, but not in Selección F-15. Hilds Maxi showed a significant Al-induced increase of both root characteristics.

Table 1. Main root and shoot length (cm), total leaf area (cm^2) and root and shoot dry weight (g) of bean cultivars grown in control or 10 mg l^{-1} Al containing nutrient solution

Cultivar	Hilds Maxi		Selección F-15		Eagle	
Treatment	Control	Aluminium	Control	Aluminium	Control	Aluminium
Root length	39·5^{a}*	45·2^{b}	43·0^{c}	41·8^{c}	42·9^{c}	29·8^{d}
Shoot length	13·4^{a}	13·5^{a}	11·0^{b}	11·5^{b}	11·7^{b}	10·6^{c}
Leaf area	216·4^{a}	206·5^{a}	208·1^{a}	145·6^{b}	208·6^{a}	154·0^{b}
Root dry wt	0·15^{a}	0·35^{b}	0·12^{a}	0·11^{a}	0·29^{b}	0·24^{c}
Shoot dry wt	0·89^{a}	1·05^{b}	0·63^{c}	0·38^{d}	1·03^{b}	0·74^{ac}

*Values within a line followed by the same letter are not significantly different ($P > 0·05$).

Table 2. Aluminium tolerance index based on root length (RI_l), shoot length (SI_l), leaf area (LI), and root (RI_w) and shoot (SI_w) dry weight

Cultivar	Hilds Maxi	Selección F-15	Eagle
RI_l	1·14	0·97	0·69
SI_l	1·01	1·04	0·91
LI	0·95	0·70	0·74
RI_w	2·33	0·92	0·83
SI_w	1·18	0·60	0·72

Except in the cultivar Eagle, elongation of shoots was unaffected by Al. Total shoot dry weight and leaf expansion, the latter measured as total leaf area, were significantly reduced in Eagle and Selección F-15. A significant increase of shoot dry weight was observed in Hilds Maxi.

Table 2 shows the tolerance index based on the root, shoot or leaf growth data presented in Table 1. According to all indices, Hilds Maxi was Al tolerant and Eagle Al sensitive. The behaviour of Selección F-15 was unclear. Tolerance indices based on root and shoot elongation or root dry weight indicate Al tolerance, but the indicies based on leaf expansion growth or shoot dry weight were lower than in the Al-sensitive Eagle.

Control plants exhibited relatively high Al concentrations (Table 3), due to germination on perlite. Aluminium supply significantly increased the Al concentrations in roots and upper plant parts of all cultivars, except Eagle. In this cultivar, the increase of Al concentration in primary and first trifoliolate leaves was not significant. The bean cultivars exposed to Al did not show significant differences between their root Al-concentrations, while significant differences were found in upper plant parts (Table 3). Generally, Eagle exhibited significantly lower Al concentrations than Hilds Maxi. Plants from selección F-15 showed extraordinarily high Al concentrations in leaves and stems. In this cultivar, Al concentrations in tops were higher than in roots. For both control and Al-exposed

Table 3. Aluminium concentration (mg kg^{-1} dry wt) and Al-amount in shoot/Al-amount in root ratio of bean cultivars growing in control or 10 mg l^{-1} Al-containing nutrient solution. (PL = primary leaves; TL = 1st trifoliolate leaves)

Cultivar	Hilds Maxi		Selección F-15		Eagle	
Treatment	Control	Aluminium	Control	Aluminium	Control	Aluminium
Root	583·7^{a}*	750·7^{b}	282·9^{c}	778·0^{b}	303·0^{c}	747·9^{b}
Stem	130·2^{a}	306·6^{b}	269·5^{b}	1445·3^{c}	80·4^{d}	122·9^{a}
PL	213·2^{a}	517·5^{b}	194·0^{a}	1981·4^{c}	107·0^{d}	254·6^{d}
TL	107·3^{a}	234·6^{b}	211·2^{b}	1182·2^{c}	91·1^{a}	155·0^{ab}
Shoot/root	1·1^{a}	0·9^{a}	3·0^{b}	6·1^{c}	0·8^{a}	0·6^{d}

*Values within a line followed by the same letter are not significantly different ($P > 0{\cdot}05$).

Table 4. Calcium concentration (mg g^{-1} dry wt) and Ca amount per shoot/Ca amount per root ratio of bean cultivars grown in control or 10 mg l^{-1} Al-containing nutrient solution. PI = percent inhibition of uptake; PL = primary leaves, TL = 1st trifoliolate leaves

Cultivar	Hilds Maxi		Selección F-15		Eagle	
Treatments	Control	Aluminium	Control	Aluminium	Control	Aluminium
Root	0·47^{a}*	0·08^{b}	0·24^{c}	0·11^{bd}	0·12^{d}	0·11^{bd}
Stem	40·83^{a}	6·49^{b}	7·38^{b}	5·14^{b}	8·37^{b}	12·75^{c}
PL	81·86^{a}	8·90^{b}	71·04^{c}	67·38^{c}	56·02^{cd}	44·37^{d}
TL	88·32^{a}	39·80^{b}	48·92^{b}	29·98^{c}	84·83^{a}	53·40^{b}
Shoot/root	620·9^{a}	424·1^{b}	572·6^{c}	797·1^{d}	972·1^{e}	823·1^{d}
PI		72·9		44·0		36·0

*Values within a line followed by the same letter are not significantly different ($P > 0{\cdot}05$).

plants, the Al amount in shoot/Al amount in root ratio (Table 3) was substantially higher in Selección F-15 than in Hilds Maxi and Eagle.

Aluminium supply significantly affected the Ca concentrations in all cultivars (Table 4). The per cent inhibition of Ca uptake was substantially higher in Hilds Maxi than in the other cultivars. Hilds Maxi exhibited the lowest shoot/root ratio of Ca content. In all cultivar leaves, Ca concentrations stayed within the range that is considered to be sufficient for normal growth (Bergmann, 1988).

DISCUSSION

Our results on root and shoot tolerance indicies of the bean cultivars Hilds Maxi and Eagle confirm previous studies (Massott *et al.* 1991), that scored these cultivars as Al tolerant and Al sensitive, respectively. In the cultivar Selección F-15, the root tolerance index was different from the shoot tolerance index.

Some Al-tolerant snapbean cultivars have been included in the category of plants in which Al concentrations in shoots are not consistently different from those of sensitive plants, but the roots of tolerant plants often contain less Al than those of sensitive plants (Foy 1984). This did not appear to be the case for the bean cultivars studied here. Significant differences between Al concentrations were only found in shoots. Eagle, the most Al sensitive cultivar, exhibited the lowest Al concentrations.

The use of root tolerance index was inappropriate for classifying the cultivar Selección F-15. Plants from this cultivar showed no significant Al-toxicity effects on root growth, but performance of upper plant parts was severely inhibited. In soybeans grown on Al-saturated acid soil, an increase of shoot dry weight by liming has been observed, while root dry weight was unaffected. The inhibition of shoot growth of legumes grown in acid soil may be related to inhibition of nodulation (Sartain & Kamprath 1975). But nitrogen was not a limiting factor in our experiment with nitrate-containing nutrient solution. Recent studies by Tan *et al.* (1991) on a Al-sensitive genotype of sorghum grown on acid soil have shown that, after liming, no Al-induced root growth inhibition occurred, but shoot growth was inhibited by Mg-deficiency. In our study, solution pH increased during the experiment to a final value of $5{\cdot}3 \pm 0{\cdot}2$. At this pH, Al tends to precipitate from the solution as $Al(OH)_3$ (Driscoll & Schecher, 1988). This can explain the absence of toxic effects in roots of the cultivar Selección F-15, in which shoot growth reduction may have been a consequence of Al-induced nutrient deficiency. Nevertheless, the high concentrations of Al found in shoots of the cultivar Selección F-15 indicate that Al was available to the plants and direct toxic effects of Al on shoot growth cannot be ruled out. The high shoot/root Al ratio indicates that plants from Selección F-15 have a low capacity to restrict translocation of Al from roots to shoots. In Eagle, the cultivar which most efficiently limited Al translocation from roots to shoots, inhibition of shoot growth was probably a consequence of impaired root performance.

Inhibition of Ca uptake by Al has been observed in different legume species (Foy *et al.* 1969, 1972; Horst 1985; Alva & Edwards 1990). Our finding that the Ca concentration was more affected by Al in the Al-tolerant cultivar, Hilds Maxi, than in Eagle and Selección F-15 does not agree with results from others (Foy *et al.* 1972), who found higher Ca concentrations in Al-tolerant than in Al-sensitive snapbeans. Nevertheless, at least in certain legumes, Ca-deficiency is not a primary effect of Al toxicity (Horst *et al.* 1983). The interference of Al with calmodulin is considered a key reaction in the Al-toxicity syndrome (Haug 1984). However, it has been found that the stoichiometric binding of Al ions to calmodulin takes place irrespective of the presence or absence of saturating calcium concentrations (Haugh & Weis, 1986). This observation suggests that there may be no direct correlation between the tissue Ca concentrations, the toxic effect of Al on calmodulin, and Al tolerance. Other protection mechanisms, such as Al chelation by organic acids, may play a role (Haug & Weis, 1986).

We conclude from our results, that Al tolerance in Hilds Maxi was neither caused by an Al-exclusion mechanism nor by higher Ca uptake. Our results on Eagle confirm that restriction of transport of Al from root to shoot may not be an efficient tolerance mechanism, because of Al injury in roots. Nevertheless, the occurrence of Al injury in upper plant parts prior to root damage cannot be ruled out, especially, when high Al concentrations accumulate in shoots, because of low capacity for restricting Al transport. Under such circumstances, e.g. cultivar Selección F-15 in our study, the tolerance index based on root elongation may not be a useful indicator of Al sensitivity, and shoot growth has to be considered.

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