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Tolerance to Aluminum Toxicity by Tropical Leguminous Plants Used as Cover Crops

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ABSTRACT

The objectives of this work were to compare Al tolerance among 17 species of tropical leguminous plants and to evaluate the most appropriate plant characteristic and Al concentration in nutrient solution for tolerance assessment. In addition, three soybean cultivars were included for comparison purposes. There was a great difference among the 17 plant materials tested, when compared by relative root elongation and critical Al activity to reduce 50% of root net elongation. Based on these parameters and on the comparison of two tropical maize genotypes differing in Al tolerance, the following classification was established: highly tolerant, for Mucuna nivea, M. deeringiana, M. aterrima Vigna unguiculata cv. BR 17 and Lablab purpureus cv. Rongai; tolerant for Cajanus cajan cv. IAPAR 43, Canavalia brasiliensis, Calopogonium mucunoides, Cajanus cajan cv. Fava larga, and Crotalaria paulina; moderately tolerant for Crotalaria ochroleuca, Canavalia ensiformis, Crotalaria spectabilis, and C. mucronata; and sensitive for Neonotonia wightii, Crotalaria breviflora and C. juncea cv.IAC-KR1. The three soybeans cultivars were classified as moderately tolerant (Biloxi) and tolerant (IAC 13 and IAC 9).

Key words: Nutrient solution culture, root growth, dry matter yields, Al speciation

INTRODUCTION

Green manuring is a traditional technique used for conservation and recovery of soils in crop rotation, which is based on the cultivation of high biomass producing cover crops and incorporation of the undecomposed green plant tissue into the soil (Karlen et al., 1994). Among the benefits of green manuring are the improvements of acid soil chemistry (Meda et al., 2001), physical properties (Perin et al., 2002) and biological properties of soils (Debarba and Amado, 1997), which reflects directly on yield increase of agricultural crops (Wutke et al., 1998; Amado et al., 1999; Arf et al., 1999).

Leguminous cover crops are the species mainly employed in green manuring, due to their capacity of nitrogen fixation, which tend to decrease the need for N-based fertilizers in crops (Spagnollo et al., 2002). These plants, however, may have other benefits, such as control of nematodes in soil, protein bank and forage for ruminants (Kerridge, 1978), weed control (Favero et al., 2001), and grain production for human nutrition (Barcelos et al., 1999). However, some studies showed that these plants have a differential capacity to overcome nutritional stresses in soil. Ernani et al., (2001) demonstrated that some tropical leguminous plants had singular responses to lime additions in acid soils, what may be related to acid soil tolerance in those species.

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Soil acidity is a limiting factor to crop growth and it is usually associated to high levels of aluminum (Olmos and Camargo, 1976). The effect of aluminum is initially in root-growth inhibition, resulting in lesser exploration of bulk soil and lesser uptake of water and nutrients. Particularly for leguminous plants, nitrogen fixing may be severely affected by Al toxicity in subsoil (Silva and Sodek, 1997). These consequences, taken together, may decrease carbon sequestration and biomass formation, which is the key for nutrient recycling in soil (Alcântara et al., 2000), physical protection of soil against high temperatures (Oliveira et al., 2002) and erosion (Nascimento and Lombardi Neto, 1999), weed control (Severino and Christoffoleti, 2001), nitrogen incorporation (Debarba and Amado, 1997) and addition of organic carbon to soil (Testa et al., 1992).

Recently there has been great interest in tropical leguminous plants, because of the increased adoption of green manuring in Brazilian *cerrados* (savannah) (Amado et al., 1999) and fruit-producing regions, for example in citrus orchards (Silva et al., 2002b) as a result of the necessity of a stable and sustainable production. Despite of the importance of tropical leguminous cover crops in Brazil, there is no information about Al toxicity reaction by the main species employed for this purpose.

The objectives of this work were to compare Al tolerance among 17 tropical leguminous plants and to evaluate the most appropriate parameter and Al concentration (activity) in nutrient solution for tolerance assessment.

MATERIAL AND METHODS

The experiment was carried out under greenhouse conditions, in 2002, at the Center of Soils and Environmental Resources, Instituto Agronômico (IAC), Campinas, Brazil. The experimental design consisted of complete randomized blocks, in split-plots with three replications. The treatments in the main plots were the Al concentrations (0, 111, 222, 333 and 444 µmol/L of $AlK(SO_4)_2.12H_2O$) and in the subplots, 17 leguminous cover crop species and cultivars, 3 soybean cultivars and 2 tropical maize cultivars contrasting as for Al tolerance. The air temperature and air relative humidity were registered during the entire experimental period. The means and standard deviation for the calculated means for the highest and lowest temperatures were 36.8 ± 2.8°C and 19.5 ± 1.3°C, respectively.. For the air relative humidity the averages were 89.3 ± 2.9% and 38.5 ± 6.0%, respectively.

The species and cultivars used in this study were: *Cajanus cajan*, cvs. Fava larga and IAPAR 43, *Calopogonium mucunoides*, *Canavalia brasiliensis*, *Canavalia ensiformis*, *Crotalaria breviflora*, *Crotalaria juncea*, cv. IAC-KR1, *Crotalaria ochroleuca*, *Crotalaria mucronata*, *Crotalaria paulina*, *Crotalaria spectabilis*, *Lablab purpureus* cv. Rongai, *Mucuna nivea*, *Mucuna deeringiana*, *Mucuna aterrima*, *Neonotonia wightii*, *Vigna unguiculata* cv. BR 17 and soybean (*Glycine max*) cultivars (IAC 13, IAC 9 and Biloxi). Besides the green manure species and soybean cultivars, maize (*Zea mays*) cultivars Taiuba tolerant and Taiuba sensitive to Al were included as references (Furlani et al., 2000).

Seeds were surface-treated with thiabendazole and germinated in moistened papers (type CEL-065) with tap water containing 500 µmol/L of Ca. Preliminary germination test done for each of the plant especies studied, led us to use seedlings of different age (three- to seven-days old) in order to have plants with an average 9.6 cm root length that were transplanted to recipients with nutrient solution. The experimental unit consisted of a plastic vessel with 13 L of nutrient solution with a silver-painted acrylic lid (to avoid light in roots) holding seven seedlings of each specie/cultivar, inserted in holes and supported by sponge beads, in a total of 154 plants/vessel.

The nutrient solution used for plant growth had the following chemical composition (in µmol/L): $N\text{-}NO_3$ 3750, $N\text{-}NH_4$ 300, P 30, K 1280, Ca 1250, Mg 500, $S\text{-}SO_4$ 500, $B\text{-}B(OH)_4$ 11.5, Cu $1.93\ 10^{-2}$, Fe-EDDHA 225, Mn 2.25, $Mo\text{-}MoO_4$ $1.30\ 10^{-1}$ and Zn $0.48\ 10^{-1}$, based on Pavan and Bingham (1982). The nutrient solution was constantly aerated and the 13 L volume was maintained by daily additions of deionized water. Solutions were completely replaced after 3 and 6 days. pH and electrical conductivity (EC) was monitored during the entire experiment and chemically analyzed (macro and micronutrients) before and after solution's refreshment. This allowed the estimation of {Al} activity in solution based on stability constants described by Nordstrom and May (1989) and computation by GEOCHEM-PC 2.0 (Parker et al., 1987).

Before transplanting, root length was measured and after 9 days in nutrient solution plants were harvested and root length was again registered, for determination of root net length (RNL). Shoots and roots were separated, placed in paper bags and taken to a forced-dry oven at 70°C until constant mass, for determination of dry matter yield. The effects of Al on shoot and root dry matter yields were evaluated by means of linear and quadratic polynomial regression.

Al tolerance was quantified by estimation of the mean relative net elongation (RNEm) by the following expression: RNE=RNL in the presence of Al/RNL in control. For each Al level a RNE value was obtained. The estimation of RNEm was based on the means of RNE in the five Al levels. Data was submitted to analysis of variance and Tukey´s test at 5% was used to compare the significant means (Zonta et al., 1987).

Critical Al concentration $[Al]_{50}$ and Al activity $\{Al\}_{50}$ to reduce 50% of RNL were calculated for each plant material. RNL was regressed against Al concentrations and activities using the TableCurve 2D 5.0 software package (Systat, 2002). Polynomial algorithms were the best-fit functions (r^2 = 1,00) and hence permitted the calculation of $[Al]_{50}$ and $\{Al\}_{50}$.

RESULTS

Aluminum activity in nutrient solution

The estimated aluminum activity (µmol/L), in the solutions with Al varied, on average from 23.8 to 14.9, 42.3 to 26.2, 54.6 to 41.7 and 70.5 to 56.7 before and after the replacement of the nutrient solutions with the following total Al levels: 111, 222, 333 and 444 µmol/L, respectively. The free specie Al^{3+} was predominant in solution (~50%), in relation to other monomeric Al species.

Symptoms of Al toxicity

The presence of Al in nutrient solution caused a delay in the vegetative growth of the plants, with less emission of leaves and decreased development of shoots, directly proportional to Al level in solution. Al toxicity symptoms in roots were evident after 2 days from transplanting, such as lateral root inhibition, darkening and shrinking, which were greater with higher concentration of Al in solution.

The maize cultivar Taiuba sensitive confirmed its reaction to Al, presenting a high sensitivity to 111 µmol/L of Al. Purple color and internerval leaf chlorosis was observed in shoot of Al-stressed plants. On the other hand, the cultivar Taiuba tolerant did not present any symptoms of Al toxicity in shoots in this concentration. Above this concentration, P deficiency and chlorosis was also observed. However, this cultivar confirmed its tolerant reaction to Al toxicity.

There was a great difference among the plant materials as to their reaction to Al. For instance, in *C. juncea*, the 111 µmol/L of Al concentration caused a root-tip curling and apparently interrupted its growth. Conversely, the roots of *Mucuna* plants in dose of 111 µmol/L of Al had the same appearance as control plants, demonstrating a higher capacity of this group of plants to tolerate this concentration of Al.

Dry matter yields

A great variation among plant materials was also obtained for shoot and root dry matter yields, but significant differences were only due to species/cultivar effect, not Al. Considering the mean dry matter production for all the species, there was a significant ($P<0.05$) linear and quadratic effect, indirectly proportional to Al levels. However, low dry matter-producing plants did not present any significant response to Al doses, such as *C. cajan, C. juncea, C. paulina, C. mucunoides, C. spectabilis, N. wighiti., C. breviflora, C. mucronata* and *C. ochroleuca*. These plants presented a low growth rate and, therefore, it was not possible to observe a difference in dry matter yield in the different Al levels after 9 days in nutrient solution. Even those that presented a significant response, like *Mucuna* species, it was observed reduction at Al doses higher than 222 µmol/L and increase in dry matter production for lower doses of Al (data not shown).

Root net length (RNL) and mean relative root net elongation (RNEm)

Root net length, which represented the effective root growth during the experimental period, varied significantly among the plant materials tested. Roots, on average grew (cm) 26.6, 12.3, 6.8, 4.9 and 3.7 in 0, 111, 222, 333 and 444 µmol/L of total Al in nutrient solution, with significant quadratic effects ($P<0.05$). Thus, there was a clear effect of Al toxicity, proportionally related to Al doses. In fig. 1 is shown the values of RNEm for each plant material (refer to material and methods), which can be considered as a quantification of Al tolerance.

Table 1 demonstrated the classification of plant materials as for their reaction to Al toxicity and the critical values of Al concentration and activity in nutrient solution. This classification was established based on data presented in Fig. 1 and the critical Al thresholds. The materials considered tolerant had the same or superior behavior as the tolerant maize cultivar, whereas the same criterion was used for those plant materials with a similar reaction to the sensitive maize cultivar.

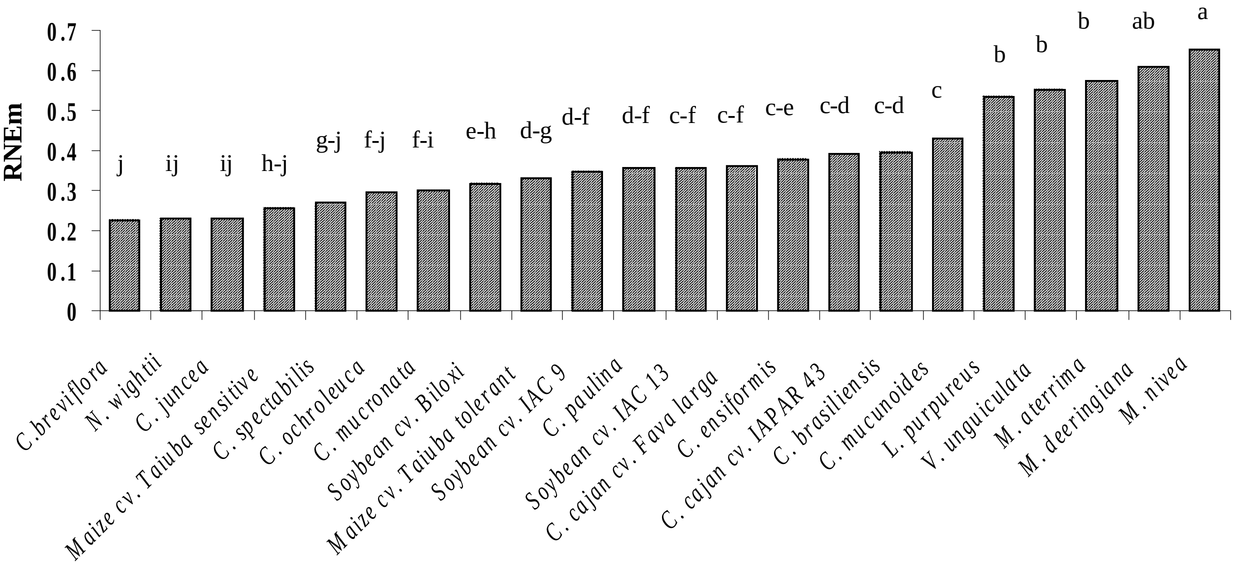

Figure 1 - Mean relative root net elongation (RNEm) of 20 leguminous plants and 2 maize cultivars after 9 days of cultivation in nutrient solution, with five Al levels. Columns with same letters do not differ statistically at Tukey´s test ($P<0.05$).

DISCUSSION

Aluminum toxicity causes significant changes in biochemical and structural pattern of plant cells, reflecting on reduction of cell multiplication (Minocha et al., 1992) and cell growth, altering auxin action in cell-wall loosening and synthesis (Ma et al., 1999). Normally the root is the plant organ most affected by Al toxicity, and more specifically the root tip is considered to be the main site for Al toxicity (Archambault et al., 1997). As a result, root elongation is considered to be the most sensitive parameter in a short period of time, and thus may represent the whole-plant reaction to Al. Noble et al., (1988) demonstrated that in 24 h it was possible to observe damages caused by Al in roots of soybean plants, directly proportional to Al concentrations in nutrient solution, as demonstrated in the present experiment.

Recent studies by Silva et al. (2000) showed by fluorescence images, that within just 30 min, Al entered in cell symplast, accumulating in the nucleus of meristematic cells of soybean roots, causing a decrease in root growth. The observation of Al toxicity symptoms, like root darkening and root-tip shrinking, in this experiment in a short period of time (48 h), could be related to the rapid entrance of Al in root cells. The root morphological alterations, like shrinking and tip curling, observed in roots of some of the plant materials tested could be attributed to alterations in cytoskeleton (Blancaflor et al., 1998; Horst et al., 1999). These authors showed that changes in organization and stability of microtubules and microfilaments in root cells of maize were correlated to Al toxicity, besides the rapid inhibition of root elongation.

In the present experiment, dry matter yields did not permit a discrimination of plant materials for Al tolerance. On the other hand, root elongation was the best parameter to compare Al tolerance among the plant materials. Braccini et al. (2000b) also reported that in the presence of Al, root elongation of coffee genotypes was more affected than root dry matter. Bernal and Clark (1998) observed that differences in dry matter production in sorghum genotypes were not as significant as root elongation, the latter being the best discrimination parameter. The results obtained by Mascarenhas et al. (1984) indicated that root length of seedlings was better than shoot and root dry matter yield to evaluate Al tolerance among soybean genotypes.

Root elongation has been considered the most sensitive characteristic to quantify Al tolerance due to the fact that the elongation zone is the site where Al toxicity is primarily detected (Blancaflor et al., 1998). Particularly, the distal transition zone (DTZ) is the most sensitive site for Al action in root (Kollmeier et al., 2000). These authors showed that application of Al solution exclusively in DTZ inhibited root elongation in a similar pattern to whole-root application.

Table 1 - Reaction to Al toxicity and critical values for Al concentration $[Al]_{50}$ and Al activity $\{Al\}_{50}$ for 50% reduction of root net length of 20 leguminous plants and 2 maize cultivars

Plant material	$[Al]_{50}$	$\{Al\}_{50}$	Reaction to Aluminum
Crotalaria juncea	39	11	sensitive
Maize cv. Taiuba sensitive	39	11	sensitive
Crotalaria breviflora	42	12	sensitive
Neonotonia wightii	43	12	sensitive
Crotalaria mucronata	58	15	moderately tolerant
Crotalaria spectabilis	65	16	moderately tolerant
Maize cv. Taiuba tolerant	68	17	moderately tolerant
Canavalia ensiformis	73	18	moderately tolerant
Crotalaria ochroleuca	75	18	moderately tolerant
Soybean cv. Biloxi	80	19	moderately tolerant
Crotalaria paulina	88	21	tolerant
Soybean cv. IAC 13	92	21	tolerant
Cajanus cajan cv. Fava larga	95	22	tolerant
Calopogonium mucunoides	97	22	tolerant
Soybean cv. IAC 9	100	23	tolerant
Canavalia brasiliensis	104	23	tolerant
Cajanus cajan cv. IAPAR 43	111	25	tolerant
Lablab purpureus cv. Rongai	189	37	highly tolerant
Vigna unguiculata	192	38	highly tolerant
Mucuna aterrima	229	44	highly tolerant
Mucuna deeringiana	262	49	highly tolerant
Mucuna nivea	334	59	highly tolerant

The differential tolerance to Al by the species tested in this experiment could be related to Al-exclusion mechanisms (Silva et al., 2000; Silva et al., 2002a) and/or symplast tolerance (Watanabe et al., 2001). The latter authors demonstrated in *Melastoma malabathricum*, a highly Al-tolerant and Al-accumulator, that oxalic acid was an important link agent to Al in the cell apoplast and symplast, allowing its accumulation, without serious damages in growth.

Exclusion mechanisms are based on the reduction of Al^{3+} activity in root tips, like the exudation of low molecular-weight organic compounds, which may form stable complexes with Al, reducing its toxicity to roots, such as citrate (Miyasaka, et al., 1991), malate (Delhaize et al., 1993), polipetides (Basu et al., 1994) and flavonoids (Kidd et al., 2001), given that more than one type of organic acid may be released by Al-stressed roots (Larsen et al., 1998; Ma et al., 2000). There are strong evidences that Al-stimulation of organic acid excretion may be highly specific for Al, and not other ions, by anionic channel in plasma membrane (Piñeros and Kochian, 2001). Other possible mechanism of Al^{3+} activity reduction could be the H^+ influx to the inside of cell, reducing the Al^{3+} solubility by higher pH. Degenhardt et al. (1998) presented the first evidence of an Al-tolerance mechanism based on pH increase in rizosphere of an *Arabidopsis thaliana* mutant, tolerant to Al toxicity. On the other hand, Braccini et al. (2000a) demonstrated that rizosphere pH-changes of coffee plants were not related to higher capacity of this specie to tolerate excess Al in soil.

Since the mechanisms to Al toxicity so far have elucidated excluded organic compounds in rizosphere, one may consider that the cultivation of plant materials in the same nutrient solution, as in the present experiment, may influence the plant reaction to Al toxicity. Nevertheless, several experiments for genotype selection to Al tolerance in the same nutrient solution have been successfully conducted (Furlani et al., 2000; Giaveno et al., 2001). Galvez and Clark (1991) demonstrated that two sorghum genotypes maintained their relative differences to Al toxicity tolerance independently if they were grown separately or in the same nutrient solution. A plausible explanation for this would be that the rizosphere chemistry changes might not influence on bulk solution chemistry. Whether rizosphere-chemistry changes by organic compounds or pH increase promoted by the plants tested in this work, is a strategy of differential tolerance to Al toxicity, remains a matter to be elucidated in further studies.

In acid soils, limestone application may reduce Al activity in solution, and it is a common practice in Brazil. Al-tolerant plants, in general, do not respond significantly to limestone addition in acid soils. Ernani et al. (2001) demonstrated that *C. juncea* was the tropical specie that most responded to limestone application in two acid soils (Latosol and Cambisol, Brazilian soil taxonomy) of Santa Catarina state, Brazil. These authors also documented that liming did not alter dry matter production in *M. deeringiana* and *M. nivea* in Latosol and *C. ensiformis, M. aterrima* and *C. spectabilis* in the Cambisol. Moreover, Hairiah and Van Noordwijk (1986), cited by Hairiah et al. (1990) verified that *Mucuna pruriens* var. *utilis* did not respond to 1 t/ha of limestone added to an acid soil of Nigeria. These results may be confronted to Al reaction of the plant materials tested in the present experiment (see Table 1).

The center of origin of the species tested may have a relation to Al reaction. Calegari (1995) reported that the species herein tested were originated from regions where the soils were predominately acid (von Uexküll and Mutert, 1995), mainly in South America, India and Africa. This could explain, in part, why the leguminous plants tested presented a predominant tolerant reaction to Al, showing the capacity of these plants to overcome selection pressure by excess Al in soil. The fact that some plant materials were considered sensitive to Al, could be due to the presence of neutral to alkaline soils in some sites of the tropics, where possibly these species could have been originated or domesticated (Kerridge, 1978).

Al tolerance is undoubtedly an ecological and agronomical advantage to plants and crops. Besides the Al tolerance observed in the leguminous plants tested in the present experiment, some studies indicated the possibility and necessity of breeding programs of leguminous cover crops considering its performance in acid soils, with high Al and low P contents, in order to explore the genetic variability existent in each specie (Mugwira and Haque, 1993). Research for plants considering this aspect would be useful for low-input agricultural productions and recovery of degraded soils and therefore minimize the impacts in environment.

The present work presented first comparison of Al tolerance in tropical leguminous plants used as cover crops in Brazil. It was possible to demonstrate that nutrient solution is still a practical and efficient tool for plant selection considering nutritional stresses.

CONCLUSIONS

1. Al concentration of 111 µmol/L in nutrient solution (or 23.8 µmol/L of Al activity) was the best to discriminate the plant materials tested;
2. Root elongation, rather than shoot and root dry matter, was the best parameter to compare Al tolerance among the leguminous plants;
3. Five leguminous cover crops were considered highly tolerant, four were tolerant, six were moderately tolerant and three were sensitive to Al toxicity.

RESUMO

Leguminosas herbáceas são plantas de cobertura utilizadas como adubo verde que também atuam na recuperação de solos agrícolas. Devido aos benefícios econômicos e ambientais da adubação verde, tem aumentado consideravelmente a adoção desta técnica no Brasil. Entretanto, não há informações sobre estas espécies quanto à toxicidade de alumínio (Al). O presente estudo teve como objetivo avaliar 17 espécies de leguminosas quanto a tolerância ao alumínio. Também foram incluídas nos testes, três cultivares de soja e duas de milho. Houve uma grande diferença entre as espécies testadas, possibilitando de acordo com a metodologia empregada, a seguinte classificação: muito tolerantes (*Mucuna nivea*, *M. deeringiana*, *M. aterrima Vigna unguiculata* cv. BR 17 e *Lablab purpureus* cv. Rongai); tolerante (*Cajanus cajan* cv. IAPAR 43, *Canavalia brasiliensis*, *Calopogonium mucunoides*, *Cajanus cajan* cv. Fava larga, e *Crotalaria paulina*); moderadamente tolerante (*Crotalaria ochroleuca*, *Canavalia ensiformis*, *Crotalaria spectabilis*, e *C. mucronata*); sensível (*Neonotonia wightii*, *Crotalaria breviflora* e *C. juncea* cv.IAC-KR1). As três cultivares de soja foram classificadas em moderadamente tolerante (Biloxi) e tolerantes (IAC 13 e IAC 9).

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