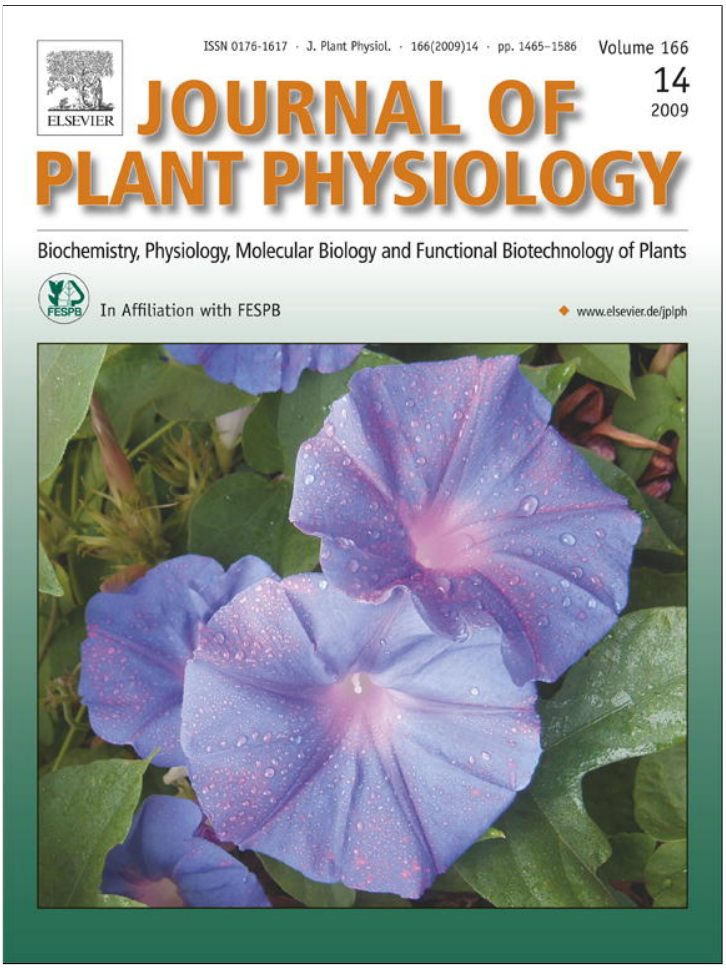

Journal of Plant Physiology **166** (2009) 1479–1487

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JOURNAL OF PLANT PHYSIOLOGY
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Growth and functional responses of different cultivars of *Lotus corniculatus* to aluminum and low pH stress

Ján Pavlovkin[a,*], Peter Pal'ove-Balang[a,b], Lukáš Kolarovič[a], Veronika Zelinová[a]

[a]*Institute of Botany, Slovak Academy of Sciences, Department of Plant Physiology, Dúbravská cesta 14, SK-84523 Bratislava, Slovakia*
[b]*Institute of Biological and Ecological Science, Department of Botany, P.J. Safarik University, Mánesova 23, SK-04154 Košice, Slovakia*

Received 3 December 2008; received in revised form 9 March 2009; accepted 9 March 2009

KEYWORDS
Aluminum stress;
Membrane potential;
Respiration;
Root growth;
Low pH

Summary

Aluminum toxicity is an important stress factor in acid soils. Growth, respiration and permeability properties of root cells were studied in five cultivars of *Lotus corniculatus* subjected to aluminum (Al) or low pH stress. The cultivars showed significant differences in root elongation under stress conditions, which correlated with changes in membrane potential (E_M) of root cortical cells. A pH drop from 5.5 to 4.0 resulted in significant membrane depolarization and root growth inhibition. The strongest inhibition was observed in cv. São Gabriel (33.6%) and least in cv. UFRGS (25.8%). Application of an extremely high Al concentration (2 mM) stopped the root growth in cv. INIA Draco, while inhibition in cv. UFRGS reached only 75%.

The E_M values of cortical cells of *Lotus* roots varied between −115 and −144 mV. Treatment with 250 μM of $AlCl_3$ (pH 4) resulted in rapid membrane depolarization. The extent of the membrane depolarization ranged between 51 mV (cv. UFGRS) and 16 mV (cv. INIA Draco). The membrane depolarization was followed by a loss of K^+ from Al-treated roots (2 mM Al) and resulted in a decrease of the diffusion potential (E_D). The total amount of K^+ in Al-treated roots dropped from 31.4 to 16.8 μmol g^{-1} FW in sensitive cv. INIA Draco, or from 26.1 to 22.7 μmol g^{-1} FW in tolerant cv. UFGRS. The rate of root respiration under control conditions as well as under Al treatment was higher in cv. INIA Draco than in cv. UFRGS. Al-induced inhibition of root respiration was 21–34% of the control.

*Corresponding author.
E-mail address: jan.pavlovkin@savba.sk (J. Pavlovkin).

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doi:10.1016/j.jplph.2009.03.005

Introduction

Forage legumes play an important role in the productivity of cultivated pasture due to their capacity for nitrogen fixation and growth in nutrient-poor conditions. The *Lotus* species in particular have a good potential in cultivated pasture, related to their ability to grow in slightly acidic soils and moderate tolerance to aluminum, manganese and sodium stress (Blumenthal and McGraw, 1999; Wheeler and Dodd, 1995). The main *Lotus* species with high forage value cultivated in South America are *Lotus glaber*, *Lotus subbiflorus*, *Lotus corniculatus* and *Lotus uliginosus*. Among these, *L. corniculatus* is undoubtedly the species considered to have the greatest agricultural importance and the widest distribution (Díaz et al., 2005).

As with many other species of agronomic value, productivity of *Lotus* pastures is limited by a number of environmental constrains. Among these, soil acidity is an important factor limiting crop production (Foy, 1988; Haug, 1984). Acidic soils constitute nearly 30% of the arable land in both tropical and temperate belts, and it has been estimated that over 50% of the world's potentially arable lands are acidic (von Uexküll and Mutert, 1995). Oxisols and ultisols represent the majority of the acid soils in the tropical region and alfisols and podsols are common in the cold and temperate zones. Due to the high content of Fe and Al oxides in oxisols, a large fraction of phosphate is fixed in insoluble (and thus unavailable) form to plants, leading to phosphate deficiency, but the main constraint of these soils is the phytotoxicity of Al.

Aluminum, which is the most abundant metal in the Earth's crust, exists in various forms depending on the pH of the soil solution and other physical and chemical parameters of soils. At soil pH higher than 5.5, most Al is present in relatively insoluble aluminosilicates, aluminophosphates and hydroxyoxides and does not exert any harmful effect on plants. However, as the soil becomes more acidic, Al is solubilized and toxic Al species (especially Al^{3+} form) are released into solution (Matsumoto, 2000). Phytotoxicity of Al is characterized by rapid inhibition of root elongation (Sivaguru and Horst, 1998) and subsequent decrease of nutrient uptake (Baligar et al., 2001; Cakmak and Horst, 1991; Cabraia et al., 1989; Mariano and Keltjens, 2005; Mistrík et al., 2000; Pal'ove-Balang and Mistrík, 2007), modification of structure and function of plasma membranes (Ikegawa et al., 2000), interference with a number of metabolic pathways, etc. (Mossor-Pietraszewska, 2001). While the apoplasmic and symplasmic target sites of Al in plant cells are under debate, several studies have focused on the plasma membrane as having a key function. By using electrophysiological measurements, it is possible to define motive forces for ions at the plasma membrane and the activity of membrane transporters involved in perturbation of plant nutrition and metabolism in stress conditions. Because the PM-H^+-ATPase plays an important role in generation of an electrical potential difference (E_M) across the plasma membrane and the generation of an electrochemical gradient of H^+ that is used to drive the transport of other substances in a process called secondary active transport, its functional characterization during Al-induced stress may help to better understand their possible role in Al resistance among different *Lotus* cultivars. Within minutes, Al depolarizes the membrane, affects the activity of channels and other transporters (Illéš et al., 2006; Matsumoto et al., 2001; Miyasaka et al., 1989; Olivetti et al., 1995; Papernik and Kochian, 1997; Pavlovkin and Mistrík, 1999; Sivaguru et al., 2003) and subsequently affects ion homeostasis and cell viability (Sasaki et al., 1997). In spite of several attempts to understand the impact of Al on membrane functions, no conclusive evidence has been obtained to date.

In the present work, analysis of the electrophysiological parameters of root cortical cells was performed on two different *L. corniculatus* cultivars with contrasting resistance to Al stress, selected from five cultivars obtained from Uruguay and Brazil. The impact of Al and low pH on the membrane potential differences, permeability of root cells and root respiration were compared with root growth parameters to examine possible correlations between these processes and resistance of *Lotus* cultivars to Al stress.

Material and methods

Plant material and growing conditions

Seeds of five Latin-American cultivars of *Lotus corniculatus*, INIA Draco, Estanzuela Ganador, San Gabriel, São Gabriel and UFRGS were obtained from Dr. Monica Rebuffo (INIA La Estanzuela, Colonia, Uruguay). Plants were grown on vermiculite under controlled conditions (20 °C, 50% relative humidity, 16 h photoperiod and approximately 120 $\mu mol\, m^{-2}\, s^{-2}$ illumination) and subirrigated with Hornum nutrient solution (Handberg and Stougaard, 1992). After 25 d, the plants were carefully removed from the vermiculite, washed

with distilled water and transferred to 1 L containers filled with nutrient solution containing 1 mM $CaCl_2$, 0.5 mM KNO_3, 0.5 mM NH_4NO_3 and 0.5 mM KCl (control). For Al-treated plants, nutrient solution was supplemented with 0.25 mM, 0.5 mM or 2 mM $AlCl_3$. The pH was maintained at pH 4.0 or 5.5 ($\pm$0.2) throughout the experiment's duration, which varied from 1 to 4 d (depending on the type of experiment).

Root growth was expressed as root length, measured after 4 d of Al treatment and expressed as root length increment per day (difference of the final root length to the initial root length divided by the number of days).

The same experimental conditions were applied for plants used for membrane potential and respiration measurements.

Measurements of the membrane potential

Measurements of plasma membrane potential (E_M) were carried out at 22 °C on outer cortical cells of 25 mm-long apical root segments of *Lotus* by standard microelectrode techniques described previously (Pavlovkin et al., 1993). After rinsing the roots with 0.5 mM $CaSO_4$, root segments were mounted onto a Plexiglas holder with a soft rubber ring and mounted in a vertical 5 mL plexiglass cuvette, which was perfused with a standard solution containing 0.1 mM KCl, 1 mM $Ca(NO_3)_2$ and $AlCl_3$ at a flow rate of 10 mL min^{-1}. The microelectrode was inserted into the outer cortex cells 2–5 mm from the root tip. Insertion of the microelectrode was observed under a microscope.

Fusicoccin (FC), as a PM-H^+-ATPase stimulator, was used (in 0.1% ethanol at a final concentration of 15 μM) to monitor the functionality of the membrane H^+ pump (Marrè, 1979).

To establish anoxic conditions, the perfusion solution was saturated with N_2 gas by flushing. The flow of the perfusion solution through the measuring chamber at 10 mL min^{-1} was sufficient to establish and to maintain anoxia (Pavlovkin et al., 1986).

Respiration analyses

Two centimeter apical root segments were used for measurement of total respiration rates (V_T; nmol O_2 g^{-1} DW s^{-1}). Respiration was measured polarographically using oxygen, Clark-type electrode (YSI 5300, Yellow Springs Instrument, USA) at 25 °C. The root segments were cut into 5 mm pieces and were sealed in a water-jacketed vessel containing 3 mL of fully aerated 10 mM Na-phosphate buffer (pH 6.8). In order to minimize the problems of non-linear O_2 depletion traces and to eliminate potential wound respiration, handling of roots was kept at a minimum and the uptake of O_2 was measured immediately after excision from the intact root. Linear traces that indicated no wound-dependent increase in the respiration rate were used for the calculations.

Potassium determination

For potassium determination, plants were cultivated for 4 d in a solution without Al (control) or supplemented with 2 mM $AlCl_3$ (Al-treated). On the 5th day, 2.5 cm-long apical segments of the roots were cut off, washed, frozen at −18 °C and, after 4 h, crude extracts were prepared by the addition of deionized water to the frozen tissue. The potassium content in the extract was determined with an ion-selective electrode and was related to the root fresh weight.

Results

Analysis of growth parameters of all the studied cultivars of *L. corniculatus* (INIA Draco, Est. Ganador, San Gabriel, São Gabriel and UFRGS) revealed relatively high sensitivity to low pH of the cultivation media. A drop of the pH from 5.5 to 4.0 resulted in significant root growth inhibition by 25.8–33.6% (Figure 1). On the other hand, relatively low root growth inhibition by 10.8–23.9% was caused by exposure of *Lotus* plants to 0.2 and 0.5 mM Al for 4 d (Figure 2).

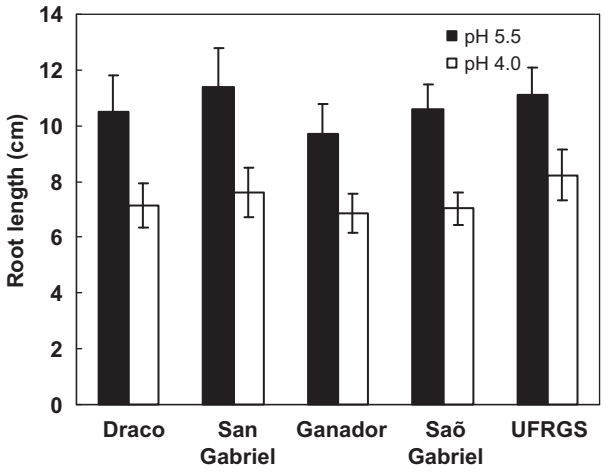

Figure 1. Root length of different *Lotus* cultivars grown for 1 week in growth solution with different pH values (5.5 or 4.0). Mean values $\pm$ SD ($n = 30$).

Significant differences were found between the cultivars, which could be divided into two groups: cultivars INIA Draco and San Gabriel showed higher sensitivity to Al, whereas Est. Ganador, São Gabriel and UFRGS were more tolerant. Application of an extremely high Al concentration (2 mM) practically stopped root growth in cv. INIA Draco (95% inhibition), while inhibition in cv. UFRGS reached only 75% of the growth without Al.

In order to detect immediate responses of the root cells to aluminum and pH of the media, the electrical plasma membrane potential (E_M) was recorded before and during aluminum application, as well as after the removal of aluminum from the root media. The E_M values of the outer cortex cells of *Lotus* roots varied between −115 and −144 mV depending on the cultivar and pH of cultivation media (Tables 1 and 2). The initial E_M values of the outer cortex cells were considerably more negative in vacuolized cells localized on the root base than in cells near the root tip. Thus, the apparent energy-dependent component (E_P) of E_M in root tip cells was only half (−26 to −32 mV) that of root base cortex cells (−60 to −70 mV). Al at a concentration of 2 mM induced the greatest depolarization of the outer cortex cells localized at a distance of 2–5 mm from the root tip. With increasing distance from the root tip, rate and magnitude of the depolarization declined. All further experiments were performed in outer cortex cells localized in the region between 2 and 5 mm from the root tip. Measurements of E_M performed at different pH of the external root medium confirmed that the magnitude of E_M is strongly dependent on the pH of the root media. pH values close to neutral (pH 6.5, results not shown) or medium acidic (at 5.5) caused membrane hyperpolarization, while acidification to pH 4 caused immediate depolarization (Table 1). The E_M of cv. INIA Draco decreased to −110 mV (17.9%) and in cv. San Gabriel to −112 mV (11.1%) while in cvs. Est. Ganador, São Gabriel and UFRGS the depolarization was negligible and did not exceed 5% of the control (pH 5.5) values.

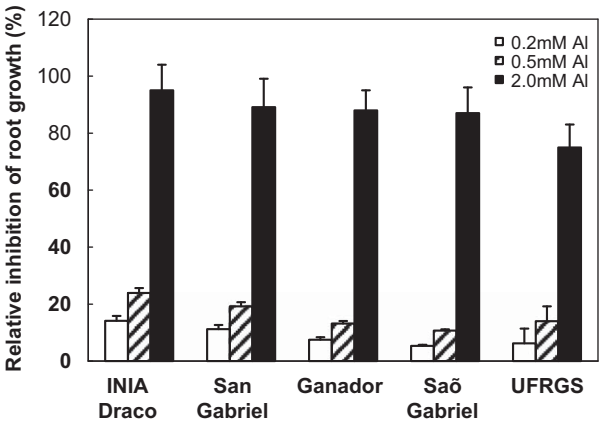

Figure 2. Relative root growth inhibition of *Lotus* cultivars grown for 4 d at different (0.2, 0.5 or 2.0 mM) Al concentrations.

Table 1. The resting potential (E_M) of root cortical cells of different *Lotus* cultivars (short time effect) exposed to a growth medium of pH 5.5 (control) and after the change of pH of the solution to pH 4.0, or pH 4.0 supplemented with 250 μM $AlCl_3$ (±SD, $n = 25$).

Cultivar	pH 5.5 E_M (mV)	pH 4.0 E_M (mV)	pH 4.0+250 μM Al E_M (mV)
INIA Draco	−134±10	−110±4	−95±5
Est. Ganador	−128±5	−122±6	−87±4
San Gabriel	−126±7	−112±5	−97±6
São Gabriel	−126±7	−121±6	−81±5
UFRGS	−132±9	−126±5	−75±5

Table 2. Resting potential (E_M) and diffusion potential (E_D) after a 4-d treatment with pH 5.5, pH 4.0 and pH 4.0+2 mM $AlCl_3$ (±SD, $n = 25$).

Cultivar	pH 5.5		pH 4.0		pH 4.0+2 mM Al	
	E_M (mV)	E_D (mV)	E_M (mV)	E_D (mV)	E_M (mV)	E_D (mV)
INIA Draco	−135±11	−72±4	−121±6	−68±8	−86±6	−31± 6
Est. Ganador	−126±8	−73±6	−117±7	−69±6	−100±8	−50± ±5
San Gabriel	−128±8	−73±4	−113±5	−67±.7	−102±6	−40±7
São Gabriel	−126±5	−73±5	−122±7	−70±4	−106±6	−51±5
UFRGS	−134±8	−72±3	−131±5	−72±4	−110±6	−54±4

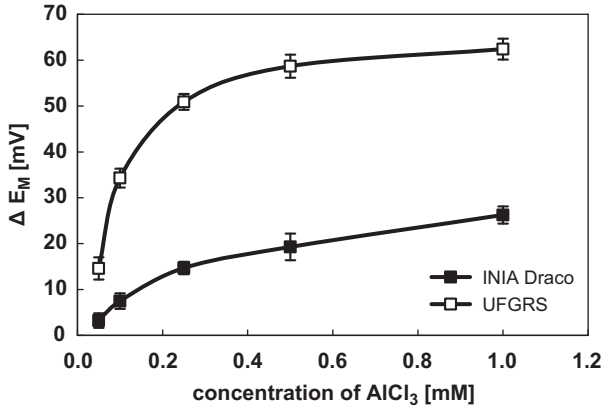

Figure 3. Concentration dependence of Al-induced membrane depolarization in root outer cortical cells (2–5 mm from the end of the root tip) of *Lotus* cultivars INIA Draco and UFGRS. Mean values ± SD ($n = 3$–12).

Replacement of the root medium with the pH 4.0 medium with identical pH but supplemented with 250 μM of $AlCl_3$ resulted in rapid and significant membrane depolarization. The magnitude of depolarization in cv. UFGRS was 51 mV in cv. São Gabriel 40 mV, and in cv. Est. Ganador 35 mV. Membrane depolarization in root cortical cells of cv. INIA Draco and cv. San Gabriel was much lower and did not exceed 16 mV. The E_M of root cells of three cultivars over the next 20–24 h partially (cv. Ganador, cv. Est. Gabriel) or completely (cv. UFGRS) recovered.

In short-term experiments, all Al concentrations tested induced a dose-dependent membrane depolarization. The depolarization was significantly greater in the root cells of cv. UFGRS, especially at lower Al concentrations (up to 500 μM), when the depolarization was much stronger than in cv. Draco (Figure 3).

The main objective in short-term electrophysiological experiments was to characterize the sensitivity of five *Lotus* cultivars to Al stress and dynamics of Al-induced changes of E_M during exposure to 250 μM $AlCl_3$. Al-induced membrane depolarization occurred within 2 min after Al application in all cultivars. However, the magnitude of the E_M decrease was greater in cultivars UFGRS, São Gabriel and Est. Ganador than in San Gabriel and INIA Draco. Complete membrane repolarization was achieved by removing aluminum from the perfusion solution with all cultivars (Figure 4). The E_M of root cells treated with 250 μM Al repolarized to control values within 24 h.

FC, the PM-H^+-ATPase activator, applied to the perfusion solution (15 μM) caused a rapid increase of E_M in control plants that was very similar in cv. UFRGS and cv. Draco (Figure 5), regardless of quantitatively different Al-induced hyperpolarization between these two cultivars. On the other hand, after a 4-d Al treatment, a difference between these two cultivars was observed. Al caused only a small decrease of range and velocity of the membrane hyperpolarization in cultivar UFRGS, whereas in Draco much stronger inhibition was apparent (Figure 5).

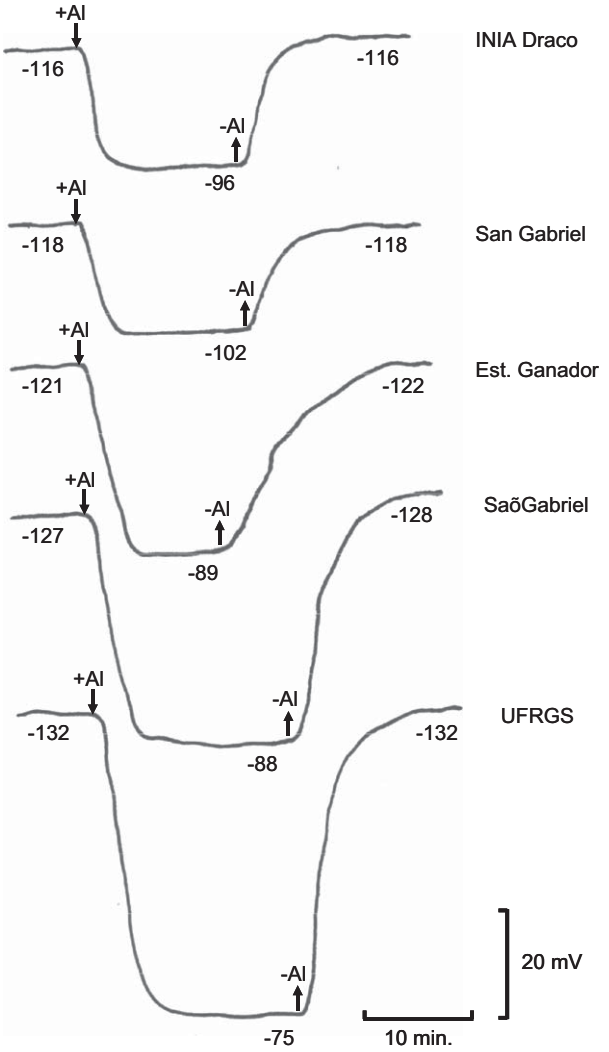

Figure 4. Representative E_M traces of root cortex cells (2–5 mm behind root tip) of five different *Lotus* cultivars exposed to 2 mM Al (pH 4.0). Numbers at the E_M trace indicate recorded mV.

The diffusion potential (E_D) was determined in order to distinguish between passive and active, i.e. energy-dependent components of the E_M by application of anoxia (perfusion with N_2-saturated standard solution). Under control conditions (pH 5.5) in root cells of all cultivars, anoxia caused a rapid membrane depolarization to −72 mV, the value considered to be the level of the diffusion potential (E_D). At more acidic pH (pH 4.0), the E_D value was lower; however, the differences among

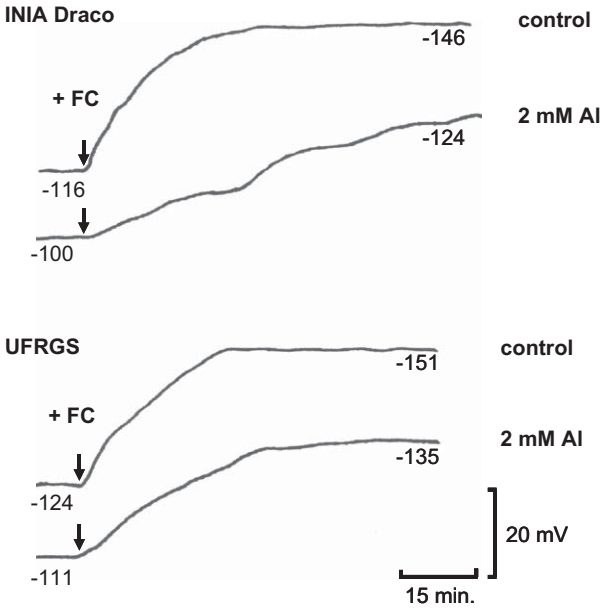

Figure 5. Effect of fusicoccin (15 μM) on the membrane potential in outer cortex cells (2–5 mm from the end of the root cap) of *Lotus* roots (pH 4) with the previous 4-d exposure to 2 mM Al. Numbers at the E_M trace indicate recorded mV.

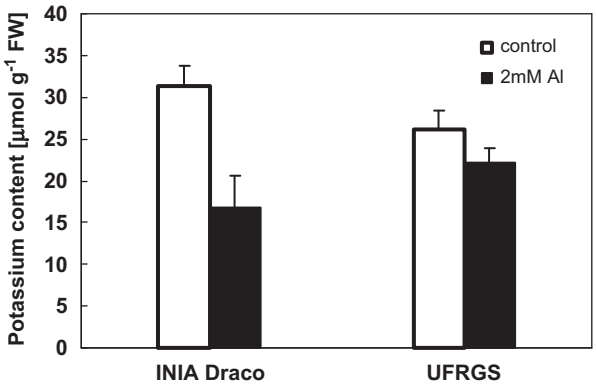

Figure 6. Potassium content in roots of cv. INIA Draco and cv. UFRGS after a 4-d treatment with 2 mM $AlCl_3$.

cultivars or among the pH values of root media were not significant.

Anoxic conditions in Al-treated roots induced significant E_D decrease, becoming greater with time of Al exposure. Extremely low values of E_D were recorded after 4-d treatment with 2 mM $AlCl_3$ and differences among cultivars were observed. Cultivars INIA Draco and San Gabriel were more sensitive to Al than the cultivars Est. Ganador, São Gabriel, and UFRGS (Table 2).

The decrease in E_M and E_D was followed by a loss of K^+ ions from Al-treated (2 mM) roots (Figure 6). Lowered values of E_D induced by Al treatment likely reflect the leakage of K^+ ions from the root cells. The total amount of K^+ in the control roots was higher in cv. INIA Draco than in cv. UFGRS, whereas in Al-treated roots (4 d) the K^+ content in the sensitive cv. INIA Draco dropped from 31.4 to 16.8 μmol g^{-1} FW (46.5% decrease), but in the more tolerant cultivar UFGRS only from 26.1 to 22.7 μmol g^{-1} FW (29.2% decrease).

The rapid membrane depolarization, beginning just minutes after Al applications but significant effect of Al on potassium efflux from *Lotus* root segments, was found only after 48 h of Al treatment.

The rate of root respiration under control conditions was higher in cv. INIA Draco than in cv. UFRGS and this trend remained similar also during Al treatment. Independent of the duration of Al treatment (1 h or 4 d) Al inhibited root respiration by 21–34% (Figure 7). When the cultivars were compared, the Al-tolerant roots of UFRGS showed a significantly lower rate of O_2 consumption than those of the Al-sensitive cv. INIA Draco. This was found not only under Al stress, but also under control conditions.

Discussion

Because Al can interact with a number of extra- and intracellular structures, many different mechanisms of Al toxicity have been hypothesized. These mechanisms include disruption of the plasma membrane and plasma membrane transport processes that can result in plant nutritional and metabolic disorders. Results based on the measurement of the electrical membrane potential of root cells show a different extent of Al-induced depolarization. In the present experiments, the Al-induced membrane depolarization was concentration-dependent and immediately reversible. The rapidity and the reversibility of the Al-induced depolarization indicate that Al may influence the structure and permeability of plant cell membranes (Olivetti et al., 1995). Results of our electrophysiological measurements in the outer cortical cells are in agreement with the expected mode of response that should reflect the different cultivar status. In short-term experiments, aluminum caused a rapid depolarization of the plasma membrane in cells of both the more tolerant cultivar UFGRS and the sensitive INIA Draco. The depolarization was, however, much more extensive in the more tolerant cultivar UFGRS. Similar results were demonstrated on snapbean (*Phaseolus vulgaris*) exposed to Al treatment where Al-tolerant Dade showed significantly higher

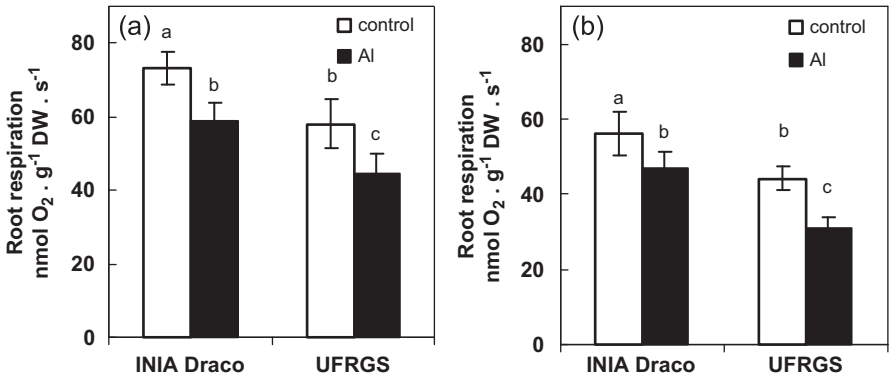

Figure 7. Root respiration of *Lotus* cultivars expressed on a dry-weight (DW) basis. Draco: Al-sensitive; UFRGS: Al-tolerant. Plants were exposed to Al in solution for 1 h (a) and 4 d. (b) Experiments were carried out as described in Material and methods. The columns followed by the same letter do not differ for $P < 0.05$.

membrane depolarization than Al-sensitive Romano (Olivetti et al., 1995). The authors suggested that this depolarization may act as a signal that is transduced into metabolic responses which enable Dade to eventually acquire tolerance to Al.

Removal of free aluminum from the medium led to complete regeneration of E_M values. Consistent with cultivar-dependent differences in sensing aluminum, the recovery process was slower in cells of the sensitive cultivar Inia Draco compared to the tolerant UFGRS. Rapid membrane depolarization might be explained by massive influx of calcium through ligand-gated calcium channels. This could be a reaction to an increased concentration of glutamate (the ligand) outside the cells, since efflux of glutamate is initiated by aluminum (Sivaguru et al., 2003). Aluminum may either accumulate on the cell surface (Horst et al., 1999) or enter the plant cells (Vázquez, 2002) when the root is exposed to aluminum ions. Illéš et al. (2006) showed that the cells regenerate membrane functions in recovery experiments, which apparently means that aluminum remaining on the cell surface lost its toxic effect. Alternatively, removal of aluminum from the cell surface via its internalization and sequestering within the vacuole could also contribute to the recovery. This scenario is in agreement with the present results. Vacuolar deposits in aluminum-treated maize roots support the tentative conclusion that vacuolar compartmentalization of the internalized aluminum might be the method of its intracellular detoxification (Vázquez, 2002).

Several of our results concerning of E_M differ only to a small extent from those presented by Kinraide et al. (1992) in wheat roots and Lindberg et al. (1991) in cells of fibrous roots of sugar beet, who measured an initial E_M value of about −100 mV in perfusion medium containing 1 mM K^+ because of different experimental solutions used. An initial E_M of approximately −114 to −140 mV was found in the outer cortex cells of *Lotus* roots 3–5 mm from the root apex perfused with 0.1 mM K^+. Similarly high E_M values were registered by Olivetti et al. (1995) in snapbean (*P. vulgaris*) roots and by Miyasaka et al. (1989) in wheat roots. However, in contrast to our results, no changes in initial E_M values were registered by these authors in cells from the zones of cell division, cell elongation and mature root cells exposed to Al and different pH. In particular, the energy-dependent component (E_P) of E_M maintained by the H^+-ATPase measured in root cortex cells varied between −48 and −61 mV depending on the cultivar and pH (Table 2). The impact of external pH on the values of E_M and E_D has been demonstrated in various plant species (Kitasato, 1968; Spanswick, 1972; Ullrich-Eberius et al., 1984; Ullrich-Eberius et al., 1989) and the marked decrease of E_M and E_D at low pH was interpreted as the effect of increased H^+ influx across the plasmalemma.

To characterize the immediate effect of Al on the PM-H^+-ATPase of root cells, we performed a set of experiments with FC. The results show that the functional activity of the H^+-ATPase is not directly influenced by Al treatment (Figure 5). This result is confirmed by experiments that revealed that Al does not counteract FC-caused hyperpolarization. The response may indicate independent sites or modes of action for alteration of H^+-ATPase activity by Al and FC. Independent action is suggested by the permanent nature of the FC-caused hyperpolarization, which was never counteracted by Al addition.

Measurements of Al-induced K^+ efflux in the bulk solution revealed great differences between cultivars, but only a negligible effect of Al on K^+ efflux from the root segments (apical and basal part) was observed.

Al is often reported to decrease V_T (Bennet et al., 1985; de Lima and Copeland, 1994; Keltjens, 1988; Mossor-Pietraszewska, 2001). However, no effect, including no increase of O_2 consumption, may also be expected (Cumming et al., 1992). In the present study, the addition of Al to the nutrient medium of *Lotus* plants inhibited O_2 uptake by excised root apices. This inhibition could have been caused by Al affecting the electron transport through the cytochrome pathway or oxidative phosphorylation. Experiments with isolated mitochondria indicated that Al interacts directly with the mitochondrial respiratory pathway (de Lima and Copeland, 1994). On the other hand, respiration fall may also be caused by the decrease of growth rates. This means that the decrease in the rate of root growth lowers the overall requirements for respiratory ATP, and thus the rate of total respiration (Atkin et al., 2000). When cultivars were compared, the Al-tolerant UFRGS had a significantly lower rate of O_2 consumption than the Al-sensitive Draco. This was found not only under stress, but also under the following control conditions. Thus, UFRGS seems to be inherently ''cheaper'' to produce in terms of carbon (C) expended. Considering that a root utilizes a major proportion of photosynthates (Lambers et al., 2002), lower respiration rates in the root system could enable more C to be utilized within shoot growth. Therefore, the selection of cultivars, such as UFRGS, exhibiting lower but more efficient C consumption in the root could be an important criterion for improving forage production of legumes. However, to confirm whether this trait is of agricultural importance, field experiments should be performed. In addition, it seems that the capability of plants to tolerate Al in their root environment may be linked not only with their anticipated ability to ''save'' energy for stress responses and stress survival by reducing V_T (C expenditures) but also with their ability to increase $V_T$when the demand for respiratory energy (ATP) to energize defense processes is elevated. This assumption could explain the results of the study of Cumming et al. (1992), where treatment with Al increased root respiration in an Al-tolerant bean cultivar (Dade) and reduced it in the sensitive one (Romano).

The results presented here demonstrate the effect of Al on E_M of outer cortical *Lotus* root cells. Al decreased the E_M but did not influence the plasma membrane PM-H^+-ATPase. The extent of depolarization was closely related to the sensitivity of individual *Lotus* cultivars to Al. More tolerant cultivars showed considerably stronger membrane depolarization (E_M) than the sensitive ones, supporting the hypothesis about the role of E_M in cell signaling (Kinraide et al., 1992; Kochian, 1995). In spite of numerous experiments on different plant species in relation to Al toxicity, our work presents, for the first time, information about the Al impact on the membrane potential and respiration of root cells of different cultivars of *L. corniculatus* in relation to their sensitivity to Al. Due to the observed correlation between the extent of E_M depolarization and the sensitivity of studied *Lotus* cultivars to Al, this parameter as well as the technique of E_M measurement should be used for rapid screening of resistant cultivars of *Lotus* for application in agriculture.

Acknowledgements

The authors are grateful for financial support to Dr. Monica Rebuffo (INIA La Estanzuela, Colonia, Uruguay) by FP6-2003-INCO-DEV2-517617 from the European Union, for providing *Lotus* seeds.

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