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Lotus corniculatus and toxic Aluminum: an update of the progress obtained by the LOTASSA Project

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Introduction

Environmental stresses are among the most important production limiting factors in the Southern Cone Region, and soil acidity and the presence of toxic aluminum (Al) are very important in this scenario, especially in Brazil. There are several Al tolerance mechanisms described in literature, root exudation of organic biomolecules being the most studied and explored by researchers (Kang and Ishii, 2006). These molecules form stable complexes with Al, reducing its activity (MA, 2000). Plant populations and genotypes, in a same species, may differ deeply in tolerance to several soil fertility problems and in nutrient utilization efficiency. A correct understanding of toxicity caused by Al and related tolerance levels is important for developing appropriate tests for the selection of tolerant germoplasms and for developing genotypes with superior performance in soils that present this characteristic. Compared to other grazing leguminous, several *Lotus* species present a high adaptation potential to soil acidity, making this genus a strong candidate to be used for this condition. This paper aims characterize the *L. corniculatus* genotypes' response to toxic Al.

Material and methods

Three experiments were realized for the characterization of Al tolerance. The first was realized in soil, where six levels of liming were applied: 0, 10, 20, 30, 40 and 80% of the recommended dose by the SMP method (Comissão de Fertilidade, 2004). Five *L. corniculatus* genotypes (*São Gabriel, San Gabriel, Estanzuela, Draco e UFRGS 2004/2*) were tested. Additionally, alfalfa was used as an Al sensitive species. The dry matter production of the aerial part (DMPAP), foliar area (FA), height (H), number of ramifications and number of live trifoliate leaves were evaluated. In a second experiment, two genotypes of *Lotus corniculatus* (Draco and UFRGS) which presented contrasting responses to the Al in the previous experiment, were evaluated in nutrition solution. The solutions used were composed of 200 µmol L^{-1} of Ca ($CaCl_2$) and three levels of Al^{+3} (50, 100 or 200 µmol L^{-1} of Al, $AlCl_3$), using a control solution (0 µmol L^{-1} of Al). The initial and final root lengths and

root growth were evaluated after 20 days of growth. Finally, a third experiment designed to evaluate the exudation of organic acids by three *Lotus corniculatus* genotypes (Draco, São Gabriel e UFRGS) and alfalfa was realized. Plants with 15 days were transplanted to potd containing a complete nutrition solution and maintained in these conditions for 67 days. Between the 30^{th} and 37^{th} and after the 60^{th} day, 200µM of Al was added to half the pots of each genotype, being the other half maintained without aluminum. The nutrition solution samplings for the evaluations of the organic acids exudation were carried out 24, 72 and 120 hours after the last Al addition. Two ml of each pot were collected and analyzed through chromatography (HPLC). The exudation of oxalic, malic and citric acids was evaluated. After the third sampling (120 h), the plants were collected, evaluating the dry matter of the aerial and root parts, root length and plant height.

Results and discussion

Evaluating the six genotypes in each liming level, a severe limitation in the 57,9% treatment of saturation by Al treatment was observed. In the 34,4% treatment, the UFRGS and São Gabriel genotypes were superior to the others, with average DMPAP values of 0,06g, while the alfalfa presented the lowest values (0,0227g). In the 22,2% level, the UFRGS genotype was better, with an DMPAP of 0,1165g, with the alfalfa being the less productive genotype once again. The 14,1% level promoted, in general, the highest productions in all genotypes, including alfalfa, with an average of 0,0832g. Overall, the UFRGS and São Gabriel genotypes were the most efficient in situations of higher soil acidity (34,4% SMP treatment), presenting good DMPAP productions, giving them potential for breeding.

Regarding the data in nutrition solution, in the absence of Al, the three genotypes presented similar root length, while in the presence of Al (50, 100 and 200 µmol L^{-1}) an average of 60% reduction of root length of alfalfa seedlings were observed at the 27^{th} day of growth . However, for the Draco and UFRGS genotypes, no significant reduction was observed in any of the three doses of Al tested at the 27^{th} day. At this time, the Draco and UFRGS genotypes presented similar root length in the 0 and 50 µmol L^{-1} doses. In the 100 µmol L^{-1} dose, the UFRGS genotype presented the longest root length, while the Draco genotype was intermediate and the alfalfa presented the shortest length. For the root growth, evaluated by the difference between the length at the 20^{th} and the 7^{th} day after germination, it was observed that between the 100 and 200 µmol L^{-1} level there was no significant reduction, indicating that the 200 µmol L^{-1} level may have been too high. Amongst the tested genotypes, the UFRGS had a good response, presenting a higher growth compared to the alfalfa, while the Draco genotype presented a growth 32% smaller than the UFRGS genotype and 40% superior to the alfalfa.

The root exudation results demonstrated that of the three organic acids tested, the only one quantified by the chromatography was the oxalic. An average exudation of 14,0 mMolar, 24 and 72 hours after the Al exposure was observed, reducing significantly to 6,6 mMolar (53%) 120 hours after the addition of Al. In all three evaluations, the three Lotus genotypes treated with Al presented exudation three times superior to the alfalfa, indicating that this is a mechanism of tolerance activated by the presence of Al. Of all the Lotus genotypes evaluated, the UFRGS genotype exhibited a greater oxalic acid exudation compared to the

others, 24 and 72 hours in the presence of Al. In the third evaluation (120 hours) the three Lotus genotypes tested did not differ among each other, but were superior to the alfalfa. Regarding the parameters evaluated after the last sampling (120 hours), in the absence of Al an accumulation of DM (aerial and roots) about 200% superior to the alfalfa was observed, which presented an accentuated reduction in production after exposure to Al, even for short periods. This production reduction was not observed in the Lotus genotypes. The protective role played out by the oxalic acid has already been demonstrated in experiments realized with other species and could be related to the tolerance observed in *Lotus corniculatus*.

Conclusions

The 34,4% level of Al saturation was the most efficient for the selection in soil due to the great differentiation presented among the genotypes in this level.

The UFRGS and São Gabriel genotypes presented moderate tolerance to soil acidity, while the DRACO genotype was the most effected by the Al.

The results obtained in nutrition solution confirmed those obtained by using acid soils, indicating that this methodology can be used to characterize Al tolerance in a faster and cheaper manner.

The alfalfa confirmed its low tolerance to Al, reducing greatly its growth in doses greater than 50 µmol L^{-1}.

There was a significant increase in the oxalic acid exudation in *Lotus corniculatus* genotypes cultivated in nutrition solution in the first 72 hours after exposure to toxic Al, specially in the UFRGS genotype. This is the first report of production of organic acid in response to Al in *L. corniculatus*.

References

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