

# Root exudation of oxalic acid in *Lotus corniculatus* in response to aluminum toxicity

Armando Martins dos Santos<sup>1†</sup>, Grazielle Pereira Ramos Pedrazza<sup>2</sup> , José Angelo Silveira Zuanazzi<sup>2</sup> , Miguel Dall'Agnol<sup>1</sup> , Roberto Luis Weiler<sup>1\*</sup> , André Pich Brunes<sup>1</sup> , Júlio Antonioli<sup>1</sup> , Diógenes Cecchin Silveira<sup>1</sup> 

<sup>1</sup> Universidade Federal do Rio Grande do Sul, Departamento de Plantas Forrageiras e Agrometeorologia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, RS, Brasil.

<sup>2</sup> Universidade Federal do Rio Grande do Sul, Faculdade de Farmácia, Programa de Pós-Graduação em Ciências Farmacêuticas, Porto Alegre, RS, Brasil.

<sup>†</sup> *In memoriam.*

\*Corresponding author:  
[robertoluisw@yahoo.com.br](mailto:robertoluisw@yahoo.com.br)

Received: June 3, 2021  
Accepted: January 27, 2022

**How to cite:** Santos, A. M.; Pedrazza, G. P. R.; Zuanazzi, J. A. S.; Dall'Agnol, M.; Weiler, R. L.; Brunes, A. P.; Antonioli, J. and Silveira, D. C. 2022. Root exudation of oxalic acid in *Lotus corniculatus* in response to aluminum toxicity. Revista Brasileira de Zootecnia 51:e20210105. <https://doi.org/10.37496/rbz5120210105>

**Copyright:** This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



**ABSTRACT** - The objective of this research was to identify the existence of root exudation of organic acid in *Lotus corniculatus* germplasms subjected to toxic aluminum (Al) levels and investigate the effect of this mechanism on the Al content in the root tissue and in morphological parameters of plant development. Two experiments were performed in nutrient solution to evaluate the Al accumulation and exudation of organic acids, using cultivars INIA Draco and São Gabriel and genotypes UFRGS and UF-T2. The plants were cultivated in Al-free solution, which was applied on the 45th day in half of the pots of each genotype. Root exudation was highly correlated with the reduced accumulation of Al in the root tissue ( $r^2 = 0.75$  at 72 h). Genotype UF-T2, selected for Al tolerance, extruded 80% more oxalic acid in the presence of Al compared with the other germplasms, indicating that this mechanism is involved with Al tolerance in *L. corniculatus*. This experiment showed strong evidence that *L. corniculatus* exhibits Type I tolerance, in which anionic channels are rapidly activated by Al exposure. Exudation of oxalic acid is likely a crucial mechanism that allows the maintenance of *L. corniculatus* growth when exposed to toxic Al conditions, and this characteristic should be used to identify tolerant genotypes in the future.

**Keywords:** birdsfoot trefoil, nutritive solution, oxalic acid, oxidation

## 1. Introduction

Aluminum (Al) toxicity has been identified as the main factor responsible for decreased yields in acidic soils, and has been estimated to reduce plant growth in ~30-40% of arable land in tropical and temperate regions (Meena et al., 2019). In soils with pH<5.0, Al toxicity may be the most limiting factor for plant growth (Singh et al., 2017).

Mechanisms triggered by exposure to Al toxicity involve its interaction with the symplasm, plasm membrane, and root cell wall (Wang et al., 2021). Excess Al in soil solution can interfere with physiological processes, which include nutrient absorption, enzymatic activities, DNA replication, and cell division and inhibition of root function, which decreases the volume of soil explored for water absorption and nutrients, thus decreasing potential production (Bojórquez-Quintal et al., 2017).

During the evolutionary process, plants develop several strategies to reduce the consequences of Al toxicity on growth. These mechanisms can be divided into Al exclusion mechanisms that prevent Al

entering root cells and Al tolerance mechanisms in which Al enters root cells and is detoxified and sequestered into vacuoles or other organs in roots and/or shoots (Zhang et al., 2019). To prevent  $\text{Al}^{3+}$  ions from entering root tip cells, many plants secrete organic acids from the root tip in response to Al stress. Citrate, oxalate, and malate are examples of anions that can form stable complexes with  $\text{Al}^{3+}$ , thereby protecting plant roots (Yang et al., 2019).

In pastures, low capital investment in the systems makes the application of lime and fertilizers difficult and expensive. In these cases, the introduction of Al-tolerant species could improve the production of forage biomass and profit while minimizing financial costs. The total annual yields of pastures that contain a greater proportion of legumes are generally higher than grass monocultures (Gultekin et al., 2021). *Lotus* species are recognized for their tolerance to acid soils and low natural soil fertility (Escaray et al., 2012), and in South America, legumes are currently being more widely adopted as an alternative to improve the quality and quantity of pasture in regions with acid soils (Kapp-Bitter et al., 2021). The objective of this research was to identify the existence of root exudation of organic acid in *L. corniculatus* germplasms subjected to toxic Al levels and investigate the effect of this mechanism on the Al content in the root tissue and in morphological parameters of plant development.

## 2. Material and Methods

Two experiments were carried out in Porto Alegre, Rio Grande do Sul, Brazil (30°04'13.1" S and 51°08'26.3" W). In the first experiment, treatments included two commercially available *Lotus corniculatus* cultivars (INIA Draco and São Gabriel) and a population of *L. corniculatus* (UFRGS). *Medicago sativa* was included as an Al-sensitive control. Seeds were germinated on moistened paper towels, and 15 days after the germination, plants were transplanted into 0.3-L polystyrene containers with 200 mL of nutrient solution with the following composition (in mmol/L): 0.7  $\text{NH}_4\text{NO}_3$ ; 0.7  $\text{K}_2\text{SO}_4$ ; 0.1 KCl; 2.0  $\text{Ca}(\text{NO}_3)_2$ ; 0.5  $\text{MgSO}_4$ ; 0.1  $\text{KH}_2\text{PO}_4$ ; and in  $\mu\text{mol/L}$ : 1.0  $\text{H}_3\text{BO}_3$ ; 0.5  $\text{MnSO}_4$ ; 1.0  $\text{ZnSO}_4$ ; 0.2  $\text{CuSO}_4$ ; 0.1  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ; and 0.2  $\text{FeCl}_3$ .

There were six replicates of each genotype, with seven plants sown in each pot. Seedlings were carefully transplanted and fixed into the cap of the container. Each pot was continuously aerated by an "aquarium pump". Plants were grown for 45 days, and nutrient solution was added every three days. pH was adjusted daily to 4.2, taking care not to damage the roots. When necessary, distilled water was added to containers between each nutrient solution application to replace the water lost due to evapotranspiration. Plants were artificially illuminated with lamps (type HQI 150W and 4.500 lux) for a 16-h photoperiod. Photosynthetically active radiation was measured with a LI-6300 photometer at  $\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

From the 45th day, 200  $\mu\text{M}$  of Al were added to the nutrient solution applied to three replicate pots of each genotype. No Al was added to the other three pots. Over the next five days, nutrient solution was not added, but all other conditions and management remained the same as prior to the addition of Al. At 24, 72, and 120 h after Al addition, nutrient solution was sampled for the analysis of organic acid exudation. At each sampling event, the plants were carefully removed, and nutrient solutions were homogenized. Two milliliters of nutrient solution were collected from each pot, quickly cooled to 4 °C, and immediately analyzed by high-pressure liquid chromatography (HPLC Waters 2690, Milford, MA, USA).

Oxalic, citric, and malic acids were quantified, but only oxalic acid was exudated in concentrations above the detection limit. Identification of oxalic acid occurred by comparing the retention time of samples to the times of standards. Exudation was evaluated by comparing the areas of root tips of these samples to the area curve. After the third sampling (120 h), plants were harvested. Dry matter yield of the shoots (SDM) and roots (RDM), root volume (RV), root length (RL), and plant height (H) were recorded.

The experiment was repeated to confirm results. In addition to previously evaluated genotypes, in Experiment 2, we also evaluated the Al response of genotype UF-T2 (the  $F_2$  generation was a germplasm selected for tolerance to Al). In the second experiment, only the exudation of oxalic acid was

evaluated, using the same periods and conditions described in Experiment 1. After the evaluation, pots were harvested and separated into shoots and roots. Samples were dried in forced-ventilation oven at 65 °C until constant weight. The Al content in the root tissue samples was determined through nitric acid digestion followed by optical emission spectrophotometer reading (He et al., 2015).

Both experiments were completely random designs with three replicates per treatment, wherein each pot constituted one replicate. Each experiment was analyzed separately with SAS software (Statistical Analysis System, version 8.0). Morphological parameters were analyzed in a factorial germplasm × Al. Oxalic acid exudation was analyzed in a factorial of treatments (germplasms with and without Al) × time after exposure to Al. Correlation analysis was performed between Al accumulation in the root tissue and root exudation of oxalic acid at the three observation times evaluated. The magnitudes of correlation coefficients were classified according to Silveira et al. (2021).

Analysis of variance (ANOVA) was conducted. Treatment means were separated by Tukey's test, at a 5% probability, when F test was significant. We used the following mathematical model:

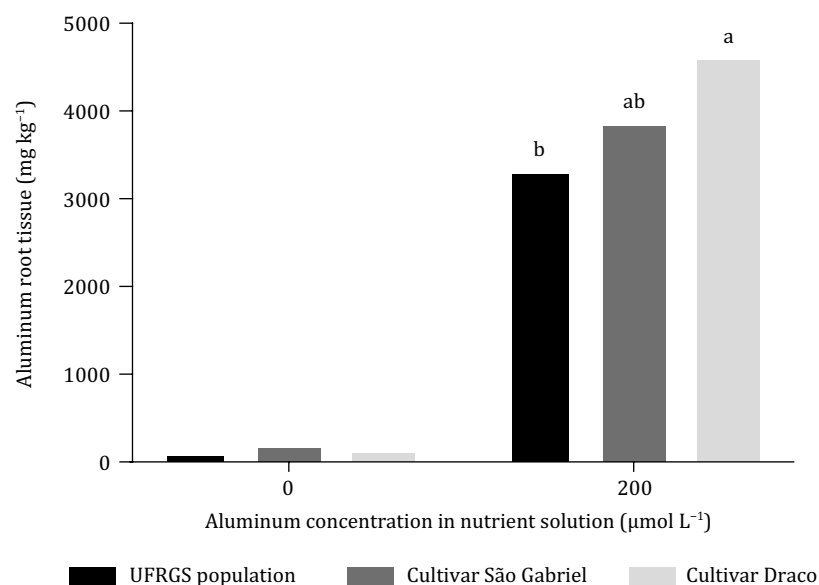
$$Y_{ij} = \mu + G_i + e_{ij}$$

in which  $Y_{ij}$  is the value of treatment of the  $j$ -th pot, evaluated in the  $i$ -th genotype;  $\mu$  is the grand mean;  $G_i$  is the fixed effect of the  $i$ -th treatment; and  $e_{ij}$  is the experimental error associated with the  $Y_{ij}$  observation.

### 3. Results

There was a significant effect of Al ( $P < 0.01$ ) and a difference among the germplasms evaluated ( $P < 0.05$ ) for Experiment II. The Al content in roots was  $< 150 \text{ mg kg}^{-1}$  of tissue (Figure 1). However, after 120 h of Al exposure, the Al content increased in all genotypes. The highest concentration of Al was found in cv. INIA Draco ( $4533 \text{ mg kg}^{-1}$  tissue), which was 37% more than in the UFRGS genotype ( $3303 \text{ mg kg}^{-1}$  tissue), which had the least amount of Al uptake among the Al-exposed plants. The cv. São Gabriel had an intermediate Al content ( $3800 \text{ mg kg}^{-1}$  tissue).

Oxalic acid exudation patterns showed a significant difference among evaluated germplasms ( $P < 0.01$ ) for the presence or absence of Al ( $P < 0.01$ ) and for the Al exposure period ( $P < 0.01$ ). Overall, the



Lowercase letters differentiate genotypes in each Al concentration by Tukey's test (5%). (Experiment II).

**Figure 1** - Aluminum (Al) content in root tissue of three *Lotus corniculatus* genotypes cultivated in nutrient solution, in the presence and absence of 200  $\mu\text{M}$  of Al measured 120 h after exposure.

exposure to Al increased the root exudation in all germplasms evaluated in both experiments. The two commercial cultivars and the population of *L. corniculatus* evaluated released more oxalic acid than alfalfa at all three sampling times after exposure (Table 1). In the second experiment, the Al-tolerant UF-T2 showed oxalic acid exudation superior to all other treatments (Table 1).

As expected, there was no difference among the germplasms for oxalic acid exudation for treatments not exposed to Al with minimal levels identified in the solution (exudation <4 mmol g<sup>-1</sup> of RDM) in both experiments, including the Al-tolerant genotype (UF-T2).

Addition of Al affected the root exudation of genotypes differently in both experiments. Following the same pattern of *L. corniculatus* genotypes evaluated for Al accumulation in root tissue, the UFRGS population had high average oxalic acid levels of 15 mmol g<sup>-1</sup> of RDM (Experiment 1; Table 1) and 11 mmol g<sup>-1</sup> of RDM (Experiment 2; Table 1). São Gabriel had average oxalic acid levels of 12 and 7.5 mmol g<sup>-1</sup> of RDM, while INIA Draco had the lowest levels of oxalic acid, 10.5 and 6.0 mmol g<sup>-1</sup> of RDM for Experiments 1 and 2, respectively. Although alfalfa increased oxalic acid exudation in the presence of Al, the mean oxalic acid level measured in the solution was < 7.5 mmol g<sup>-1</sup> of RDM (species evaluated in Experiment 1 only). In the second experiment, the Al-tolerant UF-T2 genotype had exudation superior to all other germplasms (Table 1). The exudation was twice that of UFRGS and São Gabriel genotypes and three times more than that extruded by INIA Draco. This greater exudation may be related to a higher frequency and magnitude action of the anionic channel of the plasmatic membrane.

Root exposure to Al during the first two evaluation periods (24 and 72 h after Al exposure) in Experiment 1 and in the first evaluation (24 h) of Experiment 2 showed higher levels of oxalic acid compared with the final evaluation (120 h after Al exposure). At the last evaluation, a decrease of root exudation occurred in all germplasms, with birdsfoot trefoil germplasm containing an average of 6.87 mmol g<sup>-1</sup>

**Table 1** - Oxalic acid exudation rates of *Lotus corniculatus* germplasms after exposure to 200 µM Al for 24, 72 and 120 h

| Germplasm with and without Al exposure | Experiment I                     |           |           |
|--|----------------------------------|-----------|-----------|
|  | Time after exposure to 200 µM Al |           |           |
|  | 24 h                             | 72 h      | 120 h     |
| UFRGS zero                             | 4.496dAB                         | 7.647dA   | 1.765cB   |
| UFRGS Al                               | 17.059aA                         | 20.168aA  | 7.941aB   |
| São Gabriel zero                       | 3.319dA                          | 3.571eA   | 0.714cB   |
| São Gabriel Al                         | 13.319bA                         | 15.294bA  | 6.597aB   |
| INIA Draco zero                        | 4.580dA                          | 3.950eA   | 0.924cB   |
| INIA Draco Al                          | 12.563bA                         | 12.773bcA | 7.773aB   |
| Alfalfa zero                           | 1.807dA                          | 1.429eA   | 0.336cA   |
| Alfalfa Al                             | 8.445cA                          | 11.639cA  | 4.034bB   |
| Germplasm with and without Al exposure | Experiment II                    |           |           |
|  | Time after exposure to 200 µM Al |           |           |
|  | 24 h                             | 72 h      | 120 h     |
| UFRGS zero                             | 0.9746dA                         | 0.8475dA  | 0.0042dA  |
| UFRGS Al                               | 16.5678bA                        | 7.7119bB  | 8.7712bB  |
| São Gabriel zero                       | 0.9322dA                         | 0.3390dA  | 0.0042dA  |
| São Gabriel Al                         | 13.0932bcA                       | 6.4407bcB | 5.1695cB  |
| INIA Draco zero                        | 1.5254dA                         | 0.1695dA  | 0.0042dA  |
| INIA Draco Al                          | 9.5339cA                         | 4.7458cB  | 4.9576cB  |
| Alfalfa zero                           | 0.3814dA                         | 0.0042dA  | 0.0042dA  |
| Alfalfa Al                             | 27.7542aA                        | 12.2881aB | 15.9746aB |

Lowercase letters indicate significant differences between treatments (germplasms with and without aluminum) within each time period, and uppercase letters compare treatments among times (24, 72, and 120 h), both by Tukey's test ( $P \leq 0.05$ ).

RDM. This was superior to alfalfa (4.09 mmol g<sup>-1</sup> RDM) in the Al-exposed treatment. In the second experiment, the UF-T2 genotype had 16.01 mmol g<sup>-1</sup> RDM. As expected, in the absence of Al stress, all genotypes had similar exudation patterns (P>0.05).

Oxalic acid exudation doubled between the initial (24 h) and final (120 h) observation for all evaluated genotypes. UF-T2 had the highest oxalic acid exudation (27.7 mmol g<sup>-1</sup> RDM) 24 h after exposure. Exudation of UFRGS genotype (18.20 mmol g<sup>-1</sup> RDM) was more than that of INIA Draco and São Gabriel (13.08 mmol g<sup>-1</sup> RDM). Alfalfa exudated < 9.50 mmol g<sup>-1</sup> RDM 24 h after exposure.

After the last evaluation (120 h) for Experiment I, distinct morphological differences between alfalfa and birdsfoot trefoil genotypes were observed (Table 2). In the absence of Al, alfalfa was superior (P<0.01) to birdsfoot trefoil in all evaluated characteristics. Additionally, INIA Draco had lower RDM, RV, RL, and H compared with UFRGS population and São Gabriel, which indicated it was a less vigorous and productive cultivar.

**Table 2 - Morphological characteristics evaluated after the last evaluation of oxalic acid (experiment I)**

| Genotype    | Characteristic |        |           |        |           |        |           |        |           |        |
|-------------|----------------|--------|-----------|--------|-----------|--------|-----------|--------|-----------|--------|
|             | SDM (g)        |        | RDM (g)   |        | RV (mL)   |        | H (cm)    |        | RL (cm)   |        |
|             | Zero           | Al     | Zero      | Al     | Zero      | Al     | Zero      | Al     | Zero      | Al     |
| UFRGS       | 0.92Ab         | 0.95Aa | 0.92Ab    | 0.83Aa | 2.15Ab    | 2.13Aa | 7.78Ab    | 7.22Aa | 8.52Ab    | 8.37Aa |
| São Gabriel | 0.88Ab         | 0.83Aa | 0.86Ab    | 0.76Aa | 1.87Ac    | 1.73Ab | 7.28Ab    | 6.81Aa | 9.11Ab    | 7.32Bb |
| INIA Draco  | 0.91Ab         | 0.58Bb | 0.57Ac    | 0.37Bb | 1.27Ad    | 1.17Ac | 6.47Ac    | 5.89Ab | 5.64Ac    | 5.23Aa |
| Alfafa      | 2.32Aa         | 0.84Ba | 1.50Aa    | 0.44Bb | 2.83Aa    | 1.21Bc | 12.3Aa    | 6.56Ba | 13.3Aa    | 5.86Bc |
| Mean±SD     | 1.03±0.53      |        | 0.78±0.35 |        | 1.80±0.57 |        | 7.54±2.02 |        | 7.92±2.61 |        |
| CV          | 5.93           |        | 5.26      |        | 7.56      |        | 6.56      |        | 5.99      |        |

SDM - shoots dry matter; RDM - roots dry matter; RV - root volume; H - plant height; RL - root length; SD - standard deviation; CV - coefficient of variation.

Uppercase letters indicate significant differences in the same germplasm when subjected to the presence or absence of aluminum in nutrient solution, and lowercase letters indicate significant differences among different germplasms subjected to the presence or absence of aluminum in nutrient solution by Tukey's test (P≤0.05).

## 4. Discussion

Increased concentrations of Al in the root tissue can indicate a greater sensitivity to Al and indicate less efficient mechanisms of detoxification and exclusion (Bojórquez-Quintal et al., 2017). Aluminum held in roots may be connected to cell walls, plasmatic membrane, cytosol, or cellular nucleus, which can inhibit elongation and division, in addition to several other metabolic alterations in cellular function (Moustaka et al., 2016.). Here, the only organic compound exudated in measurable amounts was oxalic acid. In species with an elevated tolerance to Al, such as *Fagopyrum esculentum*, exudation of oxalic acid has been identified as the main factor associated with Al tolerance (Wang et al., 2015). Citric, malic, and oxalic acids are the most commonly identified acids, forming robust bonds and chelating Al (Meng et al., 2017).

Several authors have identified root exudation of organic acids in Al-tolerant cultivars from different species. Palove-Balang et al. (2012) identified Al tolerance in two genotypes of birdsfoot trefoil and concluded that the UFRGS population sustained less damage to cell walls and had a higher exudation rate of citrate and oxalate compared with INIA Draco and was thus considered more tolerant. He et al. (2015) identified the response mechanism for wheat cultivars with a higher Al tolerance through secretion of organic acids from the root apex activating the anion channels. Sun et al. (2020) verified that citrate synthesis and exudation play a key role in Al resistance in alfalfa, in which higher levels of citrate concentration and exudation are associated with Al binding in Al-tolerant cultivars compared with Al-sensitive cultivars.

Other functional differences between the INIA Draco and UFRGS population may also be associated with their greater tolerance to toxic Al. Palove-Balang et al. (2012) reported differences in depolarization of the plasmatic membrane in different populations of *L. corniculatus* when exposed to toxic levels of Al. The results showed that the most tolerant germplasm (UFRGS population) had more membranes resistant to depolarization compared with the most sensitive cultivar (INIA Draco) (Figure 1). In other studies, Al application promoted a fast connection to the plasmatic membrane, especially in the root apical region, which was identified as the most sensitive region to Al exposure (Scavo et al., 2019). This bond promotes a structural change on the plasmatic membrane, which stimulates an increase of cytosolic  $Ca^{2+}$  for the induction of callose synthesis (Singh et al., 2018) and the formation of free radicals that lead to lipid peroxidation and protein oxidation, subsequently causing rupture and depolarization of the plasmatic membrane (Furlan et al., 2018).

Superior root growth and SDM from the UFRGS population compared with INIA Draco may have been influenced by the greater root exudation of oxalic acid. In large amounts, this organic acid can bind and neutralize Al molecules close to the rhizosphere minimizing the effect of Al on plant tissues.

Two organic acid exudation patterns have been classified based on their secretion pattern (Chen et al., 2013). A Type I response has a higher exudation rate in the early hours of Al exposure, while Type II responses show that the exudation of organic acids increases over time (Table 1). Different mechanisms are involved in these two distinct patterns. Type I responses suggest that Al activates a pre-existing anionic canal in the plasmatic membrane with gene induction being unnecessary (Chen et al., 2013). In contrast, Type II responses indicate that a protein induction may be necessary to trigger a protective response. These proteins may be associated with the metabolism or transport of organic acids, but these mechanisms are not yet fully understood (Huang et al., 2017). There is strong evidence that birdsfoot trefoil exhibits a Type I response, which has previously been demonstrated in experiments with more Al-tolerant genotypes of wheat (*Triticum aestivum*), corn (*Zea mays*), sunflower (*Helianthus annuus*), and soybean (*Glycine max*) (Hayes and Ma, 2003). Studies demonstrated Al exclusion via organic acid exudation in birdsfoot trefoil, inhibiting part of its phytotoxic action on the cell (Kochian et al., 2002).

The presence of Al, even for a short period, reduced SDM and RDM of INIA Draco but, as expected, Al-sensitive alfalfa showed the greatest negative response to Al exposure. With alfalfa, a highly significant reduction in RV, RL, and H was observed in plants treated with Al. In contrast, yield characteristics of UFRGS and São Gabriel were unaffected. In the presence of Al, the UFRGS population and São Gabriel were superior to the INIA Draco and alfalfa for the variables evaluated.

These results demonstrated the protective role of oxalic acid for plant growth under toxic Al conditions. The reduction of Al accumulation was related to higher root exudation of oxalic acid, suggesting that this mechanism inhibits the accumulation of Al in tissue of *L. corniculatus*. Oxalic acid binds to Al in the rhizosphere, which blocks its toxic action in the plant when exposed to acidic conditions by restricting uptake in solute. This experiment showed that higher concentrations of oxalic acid were highly correlated with reduced accumulation of Al in the root tissue ( $r^2 = 0.69, 0.75, \text{ and } 0.60$  for observations at 24, 72, and 120 h, respectively). The UF-T2 genotype exhibited a Type I response that contributed to a higher Al tolerance.

## 5. Conclusions

The genotype selected for aluminum tolerance (UF-T2) showed increased root exudation of oxalic acid, indicating that this mechanism is involved with Al tolerance in *Lotus corniculatus*. This experiment showed strong evidence that birdsfoot trefoil presents the Type I tolerance pattern, in which anionic channels are rapidly activated by Al exposure.

## Conflict of Interest

The authors declare no conflict of interest.



## Author Contributions

Conceptualization: A.M. Santos, J.A.S. Zuanazzi and M. Dall'Agnol. Data curation: J. Antonioli and D.C. Silveira. Formal analysis: A.M. Santos, G.P.R. Pedrazza, A.P. Brunes, J. Antonioli and D.C. Silveira. Investigation: A.M. Santos, G.P.R. Pedrazza, J.A.S. Zuanazzi, M. Dall'Agnol, R.L. Weiler and J. Antonioli. Methodology: A.M. Santos, G.P.R. Pedrazza, J.A.S. Zuanazzi, M. Dall'Agnol and A.P. Brunes. Project administration: A.M. Santos, J.A.S. Zuanazzi, M. Dall'Agnol and A.P. Brunes. Supervision: A.M. Santos, G.P.R. Pedrazza and M. Dall'Agnol. Visualization: A.P. Brunes. Writing-original draft: A.M. Santos, G.P.R. Pedrazza, J.A.S. Zuanazzi, M. Dall'Agnol, R.L. Weiler, A.P. Brunes, J. Antonioli and D.C. Silveira. Writing-review & editing: A.M. Santos, G.P.R. Pedrazza, J.A.S. Zuanazzi, M. Dall'Agnol, R.L. Weiler, A.P. Brunes, J. Antonioli and D.C. Silveira.

## References

- Bojórquez-Quintal, E.; Escalante-Magaña, C.; Echevarría-Machado, I. and Martínez-Estévez, M. 2017. Aluminum, a friend or foe of higher plants in acid soils. *Frontiers in Plant Science* 8:1767. <https://doi.org/10.3389/fpls.2017.01767>
- Chen, Q.; Wu, K. H.; Wang, P.; Yi, J.; Li, K. Z.; Yu, Y. X. and Chen, L. M. 2013. Overexpression of *MsALMT1*, from the aluminum-sensitive *Medicago sativa*, enhances malate exudation and aluminum resistance in tobacco. *Plant Molecular Biology Reporter* 31:769-774. <https://doi.org/10.1007/s11105-012-0543-2>
- Escaray, F. J.; Menendez, A. B.; Gárriz, A.; Pieckenstein, F. L.; Estrella, M. J.; Castagno, L. N.; Carrasco, P.; Sanjuán, J. and Ruiz, O. A. 2012. Ecological and agronomic importance of the plant genus *Lotus*. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. *Plant Science* 182:121-133. <https://doi.org/10.1016/j.plantsci.2011.03.016>
- Furlan, F.; Borgo, L.; Rabêlo, F. H. S.; Rossi, M. L.; Martinelli, A. P.; Azevedo, R. A. and Lavres, J. 2018. Aluminum-induced stress differently modifies *Urochloa* genotypes responses on growth and regrowth: root-to-shoot Al-translocation and oxidative stress. *Theoretical and Experimental Plant Physiology* 30:141-152. <https://doi.org/10.1007/s40626-018-0109-2>
- Gultekin, Y.; Filley, S. J.; Smallman, M. A.; Hannaway, D. B. and Ates, S. 2021. Pasture production, persistence of legumes and lamb growth in summer-dry hill pastures. *Grass and Forage Science* 76:159-172. <https://doi.org/10.1111/gfs.12497>
- Hayes, J. E. and Ma, J. F. 2003. Al-induced efflux of organic acid anions is poorly associated with internal organic acid metabolism in triticale roots. *Journal of Experimental Botany* 54:1753-1759. <https://doi.org/10.1093/jxb/erg188>
- He, H.; He, L. and Gu, M. 2015. Signal transduction during aluminum-induced secretion of organic acids in plants. *Biologia Plantarum* 59:601-608.
- Huang, S.-C.; Chu, S.-J.; Guo, Y.-M.; Ji, Y.-J.; Hu, D.-Q.; Cheng, J.; Lu, G.-H.; Yang, R.-W.; Tang, C.-Y.; Qi, J.-L. and Yang, Y.-H. 2017. Novel mechanisms for organic acid-mediated aluminium tolerance in roots and leaves of two contrasting soybean genotypes. *AoB Plants* 9:plx064. <https://doi.org/10.1093/aobpla/plx064>
- Kapp-Bitter, A. N.; Dickhoefer, U.; Kreuzer, M. and Leiber, F. 2021. Mature herbs as supplements to ruminant diets: effects on *in vitro* ruminal fermentation and ammonia production. *Animal Production Science* 61:470-479.
- Kochian, L. V.; Pence, N. S.; Letham, D. L. D.; Pineros, M. A.; Magalhaes, J. V.; Hoekenga, O. A. and Garvin, D. F. 2002. Mechanisms of metal resistance in plants: aluminum and heavy metals. *Plant and Soil* 247:109-119. <https://doi.org/10.1023/A:1021141212073>
- Meena, R. S.; Kumar, S.; Bohra, J. S.; Lal, R.; Yadav, G. S. and Pandey, A. 2019. Response of alley cropping-grown sesame to lime and sulphur on yield and available nutrient status in an acidic soil of Eastern India. *Energy, Ecology and Environment* 4:65-74. <https://doi.org/10.1007/s40974-019-00113-w>
- Meng, Q.; Zhang, Y. and Dong, P. 2017. Use of glucose as reductant to recover Co from spent lithium ions batteries. *Waste Management* 64:214-218. <https://doi.org/10.1016/j.wasman.2017.03.017>
- Moustaka, J.; Ouzounidou, G.; Bayçu, G. and Moustakas, M. 2016. Aluminum resistance in wheat involves maintenance of leaf Ca<sup>2+</sup> and Mg<sup>2+</sup> content, decreased lipid peroxidation and Al accumulation, and low photosystem II excitation pressure. *BioMetals* 29:611-623. <https://doi.org/10.1007/s10534-016-9938-0>
- Palove-Balang, P.; Čiamporová, M.; Zelinová, V.; Pavlovkin, J.; Gurinová, E. and Mistrík, I. 2012. Cellular responses of two Latin-American cultivars of *Lotus corniculatus* to low pH and Al stress. *Central European Journal of Biology* 7:1046-1054. <https://doi.org/10.2478/s11535-012-0098-0>
- Scavo, A.; Abbate, C. and Mauromicale, G. 2019. Plant allelochemicals: Agronomic, nutritional and ecological relevance in the soil system. *Plant and Soil* 442:23-48. <https://doi.org/10.1007/s11104-019-04190-y>

- Silveira, D. C.; Pelissoni, M.; Buzatto, C. R.; Scheffer-Basso, S. M.; Ebone, L. A.; Machado, J. M. and Lângaro, N. C. 2021. Anatomical traits and structural components of peduncle associated with lodging in *Avena sativa* L. *Agronomy Research* 19:250-264. <https://doi.org/10.15159/AR.21.001>
- Singh, C. K.; Singh, D.; Tomar, R. S. S.; Karwa, S.; Upadhyaya, K. C. and Pal, M. 2018. Molecular mapping of aluminium resistance loci based on root re-growth and Al-induced fluorescent signals (callose accumulation) in lentil (*Lens culinaris* Medikus). *Molecular Biology Reports* 45:2103-2113. <https://doi.org/10.1007/s11033-018-4368-4>
- Singh, S.; Tripathi, D. K.; Singh, S.; Sharma, S.; Dubey, N. K.; Chauhan, D. K. and Vaculík, M. 2017. Toxicity of aluminium on various levels of plant cells and organism: a review. *Environmental and Experimental Botany* 137:177-193. <https://doi.org/10.1016/j.envexpbot.2017.01.005>
- Sun, G.; Zhu, H.; Wen, S.; Liu, L.; Gou, L. and Guo, Z. 2020. Citrate synthesis and exudation confer Al resistance in alfalfa (*Medicago sativa* L.). *Plant and Soil* 449:319-329. <https://doi.org/10.1007/s11104-020-04490-8>
- Wang, H.; Chen, R. F.; Iwashita, T.; Shen, R. F. and Ma, J. F. 2015. Physiological characterization of aluminum tolerance and accumulation in tartary and wild buckwheat. *New Phytologist* 205:273-279. <https://doi.org/10.1111/nph.13011>
- Wang, P.; Dong, Y.; Zhu, L.; Hao, Z.; Hu, L.; Hu, X.; Wang, G.; Cheng, T.; Shi, J. and Chen, J. 2021. The role of  $\gamma$ -aminobutyric acid in aluminum stress tolerance in a woody plant, *Liriodendron chinense*  $\times$  *tulipifera*. *Horticulture Research* 8:80. <https://doi.org/10.1038/s41438-021-00517-y>
- Yang, J. L.; Fan, W. and Zheng, S. J. 2019. Mechanisms and regulation of aluminum-induced secretion of organic acid anions from plant roots. *Journal of Zhejiang University-SCIENCE B* 20:513-527. <https://doi.org/10.1631/jzus.B1900188>
- Zhang, X.; Long, Y.; Huang, J. and Xia, J. 2019. Molecular mechanisms for coping with Al toxicity in plants. *International Journal of Molecular Sciences* 20:1551. <https://doi.org/10.3390/ijms20071551>