



**PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO**  
**DOUTORADO EM AGRONOMIA**

**CAMILA HATSU PEREIRA YOSHIDA**

**AN APPROACH ON ALUMINUM TOLERANCE MECHANISMS AND THE  
MITIGATING ROLE OF SILICON IN POPCORN**



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*“Aqueles que passam por nós,  
não vão sós,  
não nos deixam sós.  
Deixam um pouco de si,  
levam um pouco de nós.”  
(Antoine de Saint-Exupéry)*



# UMA ABORDAGEM SOBRE OS MECANISMOS DE TOLERÂNCIA AO ALUMÍNIO E PAPEL MITIGADOR DO SILÍCIO EM MILHO PIPOCA

## RESUMO

O cultivo de milho pipoca (*Zea mays* L. var. everta) é amplamente destinado ao consumo humano, mas sua produtividade pode ser afetada em solos ácidos, onde a toxicidade do alumínio (Al) é um problema. No primeiro experimento, investigamos os efeitos do Al em duas linhagens de milho pipoca: 11-133, tolerante ao Al, e 11-60, sensível ao Al. Observamos que o Al não afetou o crescimento da parte aérea, mas causou amarelecimento na lâmina foliar mais velha da linhagem sensível. A linhagem 11-133 apresentou mecanismos de quelação de Al, resultando em menor alteração na estrutura radicular e menor presença de espécies reativas de oxigênio (EROs) e malondialdeído (MDA), o que foi atribuído a um sistema enzimático mais eficiente, principalmente devido ao metabolismo da glutatona (GSH). No segundo experimento, investigamos o papel mitigador do silício (Si) frente ao Al em plantas de milho pipoca. Observamos que na presença de Al houve redução da taxa de crescimento das raízes, mas a adição de Si mitigou esse efeito negativo, resultando na redução da concentração de Al nas pontas de raízes e em menor impacto morfoanatômico. O Si também modulou o metabolismo das plantas expostas ao Al, aumentando as concentrações de frutose, sacarose, aminoácidos totais e nitrato, enquanto as concentrações de proteínas totais diminuíram nas plantas tratadas com Si. Esses resultados indicam o potencial do Si como uma estratégia para melhorar o desenvolvimento de culturas em solos ácidos e mitigar os efeitos negativos da toxicidade do Al em plantas de milho pipoca.

**Palavras-chave:** Toxicidade por alumínio; morfologia da raiz; sistema antioxidante, ácidos orgânicos, metabolismo primário.

## AN APPROACH ON ALUMINUM TOLERANCE MECHANISMS AND THE MITIGATING ROLE OF SILICON IN POPCORN CORN

### ABSTRACT

The cultivation of popcorn maize (*Zea mays* L. var. *everta*) is widely intended for human consumption, but its productivity can be affected in acidic soils, where aluminum (Al) toxicity is a problem. In this study, we investigated the effects of Al on two popcorn maize lineages: 11-133, Al-tolerant, and 11-60, Al-sensitive. We observed that Al did not affect the shoot growth, but it caused yellowing in the older leaf blade of the sensitive lineage. The tolerant lineage showed Al chelation mechanisms, resulting in minor alterations in the root structure and lower presence of reactive oxygen species (ROS) and malondialdehyde (MDA), which is attributed to a more efficient enzymatic system, mainly due to the glutathione (GSH) metabolism. In the second experiment, we investigated the mitigating role of silicon (Si) against Al in popcorn plants. We observed that in the presence of Al there was a reduction in the root growth rate, but the addition of Si mitigated this negative effect, resulting in a reduction in the Al concentration in at root tips and a lower morphoanatomical impact. Si also modulated the metabolism of plants exposed to Al, increasing fructose, sucrose, total amino acids, and nitrate concentrations, while total protein concentrations decreased in Si-treated plants. These results indicate the potential of Si as a strategy to improve the development of crops in acidic soils and mitigate the negative effects of Al toxicity on popcorn plants.

**Keywords:** Aluminum toxicity; root morphology; antioxidant system, primary metabolism, organic acid, primary metabolism.

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## References

- Adrees, M., Ali, S., Rizwan, M., Zia-Ur-Rehman, M., Ibrahim, M., Abbas, F., Farida, M., Qayyum, M. F., & Irshad, M. K. (2015). Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. *Ecotoxicology and Environmental Safety*, 119, 186-197. <https://doi.org/10.1016/j.ecoenv.2015.05.011>
- Cañizares, L. D. C. C., Silva Timm, N., Ramos, A. H., Neutzling, H. P., Ferreira, C. D., & Oliveira, M. (2020). Effects of moisture content and expansion method on the technological and sensory properties of white popcorn. *International Journal of Gastronomy and Food Science*, 100282. <https://doi.org/10.1016/j.ijgfs.2020.100282>
- Chandra, J., & Keshavkant, S. (2021). Mechanisms underlying the phytotoxicity and genotoxicity of aluminum and their alleviation strategies: A review. *Chemosphere*, 278, 130384. <https://doi.org/10.1016/j.chemosphere.2021.130384>
- Chandra, J., Parkhey, S., Varghese, D., Varghese, B., & Keshavkant, S. (2020). Aluminium Rhizotoxicity in *Cicer arietinum*. *Russian Journal of Plant Physiology*, 67(5), 945-952. 2020. <https://doi.org/10.1134/S1021443720050027>
- Dong, Z., Li, Y., Xiao, X., Chen, Y., & Shen, X. (2018). Silicon effect on growth, nutrient uptake, and yield of peanut (*Arachis hypogaea* L.) under aluminum stress. *Journal of Plant Nutrition*, 41(15), 2001-2008. <https://doi.org/10.1080/01904167.2018.1485163>
- Du, B., Nian, H., Zhang, Z., & Yang, C. (2010). Effects of aluminum on superoxide dismutase and peroxidase activities, and lipid peroxidation in the roots and calluses of soybeans differing in aluminum tolerance. *Acta Physiologiae Plantarum*, 32(5), 883-890. <https://doi.org/10.1007/s11738-010-0476-z>
- Garzón, T., Gunsé, B., Moreno, A. R., Tomos, A. D., Barceló, J., & Poschenrieder, C. (2011). Aluminium-induced alteration of ion homeostasis in root tip vacuoles of two maize varieties differing in Al tolerance. *Plant Science*, 180(5), 709-715. <https://doi.org/10.1016/j.plantsci.2011.01.022>
- Giannakoula, A., Moustakas, M., Mylona, P., Papadakis, I., & Yupsanis, T. (2008). Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. *Journal of plant physiology*, 165(4), 385-396. <https://doi.org/10.1016/j.jplph.2007.01.014>
- Giannakoula, A., Moustakas, M., Syros, T., & Yupsanis, T. (2010). Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. *Environmental and Experimental Botany*, 67(3), 487-494. <https://doi.org/10.1016/j.envexpbot.2009.07.010>
- Gupta, N., Gaurav, S. S., & Kumar, A. (2013). Molecular basis of aluminium toxicity in plants: A review. *American Journal of Plant Sciences*, 4, 21-37. <https://doi.org/10.4236/ajps.2013.412A3004>
- Hasanuzzaman, M., Bhuyan, M. B., Raza, A., Hawrylak-Nowak, B., Matraszek-Gawron, R., Al Mahmud, J., Nahar, K., & Fujita, M. (2020). Selenium in plants: Boon or bane?.

*Environmental and Experimental Botany*, 178, 104170.

<https://doi.org/10.1016/j.envexpbot.2020.104170>

Jiang, D., Wu, H., Cai, H., & Chen, G. (2022). Silicon confers aluminium tolerance in rice via cell wall modification in the root transition zone. *Plant, Cell & Environment*,

<https://doi.org/10.1111/pce.14307>

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M. S., Saleem, M. H., Adil, M., Heidari, P., & Chen, J. T. (2019). An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation.

*International journal of molecular sciences*, 21(1), 148. <https://doi.org/10.3390/ijms21010148>

Liu, W., Xu, F., Lv, T., Zhou, W., Chen, Y., Jin, C., Lu, L., & Lin, X. (2018). Spatial responses of antioxidative system to aluminum stress in roots of wheat (*Triticum aestivum* L.) plants. *Science of the Total Environment*, 627, 462-469.

<https://doi.org/10.1016/j.scitotenv.2018.01.021>

Nunes-Nesi, A., Brito, D. S., Inostroza-Blancheteau, C., Fernie, A. R., & Araújo, W. L.

(2014). The complex role of mitochondrial metabolism in plant aluminum resistance. *Trends in Plant Science*, 19(6), 399-407. <https://doi.org/10.1016/j.tplants.2013.12.006>

Paraginski, R. T., Souza, N. L., Alves, G. H., Ziegler, V., Oliveira, M., & Elias, M. C. (2016) Sensory and nutritional evaluation of popcorn kernels with yellow, white and red pericarps expanded in different ways. *Journal of Cereal Science*, 69, 383-391.

<https://doi.org/10.1016/j.jcs.2016.05.013>

Pavlovic, J., Kostic, L., Bosnic, P., Kirkby, E. A., & Nikolic, M. (2021). Interactions of silicon with essential and beneficial elements in plants. *Frontiers in Plant Science*, 12, 1224.

<https://doi.org/10.3389/fpls.2021.697592>

Pereira, J. F. & Ryan, P. R. (2019). The role of transposable elements in the evolution of aluminium resistance in plants. *Journal of experimental botany*, 70(1), 41-54.

<https://doi.org/10.1093/jxb/ery357>

Pinto, V. B., Ferreira, P. G., Vidigal, P. M. P., Mendes, T. A. O., Dal-Bianco, M., Magalhaes, J. V., & Viana, J. M. S. (2021). Uncovering the transcriptional response of popcorn (*Zea mays* L. var. everta) under long-term aluminum toxicity. *Scientific reports*, 11(1), 1-12.

<https://doi.org/10.1038/s41598-021-99097-z>

Pirzadah, T. B., Malik, B., Tahir, I., Rehman, R. U., Hakeem, K. R., & Alharby, H. F. (2019). Aluminium stress modulates the osmolytes and enzyme defense system in *Fagopyrum* species. *Plant Physiology and Biochemistry*, 144, 178-186.

<https://doi.org/10.1016/j.plaphy.2019.09.033>

Rahim, F., Almeida, V. C., Viana, J. M. S., Ribeiro, C., Risso, L. A., & Ribeiro, M. P. (2019). Identification of contrasting tropical popcorn inbreds for studying aluminum toxicity tolerance inheritance. *Euphytica*, 215(3), 47. <https://doi.org/10.1007/s10681-019-2372-y>

Ramírez-Benítez, J. E., Chee-González, L., & Hernandez-Sotomayor, S. M.T. (2008).

Aluminium induces changes in organic acids metabolism in *Coffea arabica* suspension cells with differential Al-tolerance. *Journal of inorganic biochemistry*, 102(8), 1631-1637.

<https://doi.org/10.1016/j.jinorgbio.2008.03.002>

Ribeiro, C., Cambraia, J., Peixoto, P. H. P., & Fonseca Júnior, E. M. D. (2012). Antioxidant system response induced by aluminum in two rice cultivars. *Brazilian Journal of Plant Physiology*, 24(2), 107-116. <https://doi.org/10.1590/S1677-04202012000200004>

Silva, S. (2012). Aluminium toxicity targets in plants. *Journal of Botany*, 2012, 1-8. <https://doi.org/10.1155/2012/219462>

Singh, S., Tripathi, D. K., Singh, S., Sharma, S., Ribeiro, N. K., Chauhan, D. K., & 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>

Sousa, A., Abdelgawad, H., Han, A., Teixeira, J., Matos, M., & Fidalgo, F. (2016). Oxidative metabolism of rye (*Secale cereale* L.) after short term exposure to aluminum: Uncovering the glutathione–ascorbate redox network. *Frontiers in plant science*, 7, 685. <https://doi.org/10.3389/fpls.2016.00685>

Sousa, A., Saleh, A. M., Habeeb, T. H., Hassan, Y. M., Zrieq R., Wadaan, M. A., Hozzein, W. N., Selim, S., Matos, M., & Abdelgawad, H. (2019). Silicon dioxide nanoparticles ameliorate the phytotoxic hazards of aluminum in maize grown on acidic soil. *Science of the Total Environment*, 693, 133636. <https://doi.org/10.1016/j.scitotenv.2019.133636>

Tolrà, R., Barceló, J., & Poschenrieder, C. (2009). Constitutive and aluminium-induced patterns of phenolic compounds in two maize varieties differing in aluminium tolerance. *Journal of Inorganic Biochemistry*, 103(11), 1486-1490. <https://doi.org/10.1016/j.jinorgbio.2009.06.013>

Vega, I., Nikolic, M., Pontigo, S., Godoy, K., Mora, M. D. L. L., & Cartes, P. (2019). Silicon improves the production of high antioxidant or structural phenolic compounds in barley cultivars under aluminum stress. *Agronomy*, 9(7), 388. <https://doi.org/10.3390/agronomy9070388>

Verma, D., Lakhanpal, N., & Singh, K. (2019). Genome-wide identification and characterization of abiotic-stress responsive SOD (superoxide dismutase) gene family in *Brassica juncea* and *B. rapa*. *BMC Genomics*, 20(1), 1-18. <https://doi.org/10.1186/s12864-019-5593-5>

Xiao, Z., & Liang, Y. (2022). Silicon prevents aluminum from entering root tip by promoting formation of root border cells in rice. *Plant Physiology and Biochemistry*, 175, 12-22. <https://doi.org/10.1016/j.plaphy.2022.02.003>

Zerrouk, I. Z., Rahmoune, B., Auer, S., Rösler, S., Lin, T., Baluska, F., Dobrev, P. I., Motyka, V., & Ludwig-Müller, J. (2020). Growth and aluminum tolerance of maize roots mediated by auxin-and cytokinin-producing bacillus toyonensis requires polar auxin transport. *Environmental and Experimental Botany*, 104064. <https://doi.org/10.1016/j.envexpbot.2020.104064>

Zheng, S. J. & Yang, J. L. (2005). Target sites of aluminum phytotoxicity. *Biologia Plantarum*, 49(3), 321-331. <https://doi.org/10.1007/s10535-005-0001-1>

Zhou, X., Gu, Z., Xu, H., Chen, L., Tao, G., Yu, Y., & Li, K. (2016). The effects of exogenous ascorbic acid on the mechanism of physiological and biochemical responses to

nitrate uptake in two rice cultivars (*Oryza sativa* L.) under aluminum stress. *Journal of Plant Growth Regulation*, 35(4), 1013-1024. <https://doi.org/10.1007/s00344-016-9599-9>



## References

- Anjum NA, Sharma P, Gill SS, Hasanuzzaman M, Khan EA, Kachhap K, Mohamed AA, Thangavel P, Devi GD, Vasudhevan P, Sofo A, Khan NA, Misra AN, Lukatkin AS, Singh HP, Pereira E, Tuteja N (2016) Catalase and ascorbate peroxidase—representative H<sub>2</sub>O<sub>2</sub>-detoxifying heme enzymes in plants. *Environ Sci Pollut Res* 23: 19002–19029. <https://doi.org/10.1007/s11356-016-7309-6>
- Awasthi JP, Saha B, Regon P, Sahoo S, Chowra U, Pradhan A, Roy A, Panda SK (2017). Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. *PLoS ONE* 12: e0176357. <https://doi.org/10.1371/journal.pone.0176357>
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Brito DS, Neri-Silva R, Ribeiro KVG, Peixoto PHP, Ribeiro C (2020) Effects of aluminum on the external morphology of root tips in rice. *Braz J Bot* 43: 413–418. <https://doi.org/10.1007/s40415-020-00620-9>
- Caniato FF, Guimarães CT, Schaffert RE, Alves VMC, Kochian LV, Borém A, Klein PE, Magalhaes JV (2007) Genetic diversity for aluminum tolerance in sorghum. *Theor Appl Genet* 114: 863–876. <https://doi.org/10.1007/s00122-006-0485-x>
- Chandra J, Keshavkant S (2021) Mechanisms underlying the phytotoxicity and genotoxicity of aluminum and their alleviation strategies: A review. *Chemosphere* 278:130384. <https://doi.org/10.1016/j.chemosphere.2021.130384>
- Chandra J, Parkhey S, Varghese D, Varghese B, Keshavkant S (2020) Aluminum Rhizotoxicity in *Cicer arietinum*. *Russ J Plant Physiol* 67: 945–952. <https://doi.org/10.1134/S1021443720050027>
- Chowra U, Yanase E, Koyama H, Panda SK (2017) Aluminium-induced excessive ROS causes cellular damage and metabolic shifts in black gram *Vigna mungo* (L.) Hepper. *Protoplasma* 254:293–302. <https://doi.org/10.1007/s00709-016-0943-5>
- Čiamporová M (2002) Morphological and structural responses of plant roots to aluminium at organ, tissue, and cellular levels. *Biologia plant* 45:161–171. <https://doi.org/10.1023/A:1015159601881>
- Clark RB (1975) Characterization of phosphatase of intact maize roots. *J Agric Food Chem* 23:458–460. <https://doi.org/10.1021/jf60199a002>
- Foyer CH, Noctor G (2011) Ascorbate and Glutathione: The heart of the redox hub. *Plant Physiol* 155:2–18. <https://doi.org/10.1104/pp.110.167569>
- Furlan F, Borgo L, Rabêlo FHS, Rossi ML, Linhares FS, Martinelli AP, Azevedo RA, Lavres J (2020) Aluminum-induced toxicity in *Urochloa brizantha* genotypes: A first glance into root Al-apoplastic and -symplastic compartmentation, Al-translocation and antioxidant performance. *Chemosphere* 243:125362. <https://doi.org/10.1016/j.chemosphere.2019.125362>
- García-Pinilla S, Gutiérrez-López GF, Hernández-Sánchez H, Cáez-Ramírez G, García-Armenta E, Alamilla-Beltrán L (2020) Quality parameters and morphometric characterization of hot-air popcorn as related to moisture content. *Dry Technol* 39:77–89. <https://doi.org/10.1080/07373937.2019.1695626>
- Garzón T, Gunsé B, Moreno AR, Tomos AD, Barceló J, Poschenrieder C (2011) Aluminium-induced alteration of ion homeostasis in root tip vacuoles of two maize varieties differing in Al tolerance. *Plant Science* 180:709–715. <https://doi.org/10.1016/j.plantsci.2011.01.022>
- Gay C, Gebicki JM (2000) A Critical Evaluation of the Effect of Sorbitol on the Ferric–Xylenol Orange Hydroperoxide Assay. *Anal Biochem* 284:217–220. <https://doi.org/10.1006/abio.2000.4696>
- Giannakoula A, Moustakas M, Syros T, Yupsanis T (2010) Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. *Environ. Exp Bot* 67: 487–494. <https://doi.org/10.1016/j.envexpbot.2009.07.010>
- Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. occurrence in higher plants. *Plant Physiol* 59:309–314. <https://doi.org/10.1104/pp.59.2.309>

- Guo P, Qi YP, Cai YT, Yang TY, Yang LT, Huang ZR, Chen LS (2018) Aluminum effects on photosynthesis, reactive oxygen species and methylglyoxal detoxification in two *Citrus* species differing in aluminum tolerance. *Tree physiology* 38:1548–1565. <https://doi.org/10.1093/treephys/tpy035>
- Habig WH, Jakoby WB (1981) [51] Assays for differentiation of glutathione S-Transferases. In: *Methods in Enzymology*. Elsevier, pp 398–405. [https://doi.org/10.1016/S0076-6879\(81\)77053-8](https://doi.org/10.1016/S0076-6879(81)77053-8)
- Havir EA, McHale NA (1987) Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol* 84: 450–455. <https://doi.org/10.1104/pp.84.2.450>
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. *Arch Biochem Biophys* 125:189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Horst WJ, Wang Y, Eticha D (2010) The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminum resistance of plants: a review. *Ann Bot* 106:185–197. <https://doi.org/10.1093/aob/mcq053>
- Kampfenkel K, Vanmontagu M, Inze D (1995) Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Anal Biochem* 225:165–167. <https://doi.org/10.1006/abio.1995.1127>
- Kar M, Mishra D (1976) Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol* 57:315–319. <https://doi.org/10.1104/pp.57.2.315>
- Kataoka T, Nakanishi TM (2001) Aluminum distribution in soybean root tip for a short time Al treatment. *J Plant Physiol* 158:731–736. <https://doi.org/10.1078/0176-1617-00163>
- Kato Y, Miura E, Matsushima R, Sakamoto W (2007) White leaf sectors in *yellow variegated2* are formed by viable cells with undifferentiated plastids. *Plant Physiol* 144:952–960. <https://doi.org/10.1104/pp.107.099002>
- Kochian LV, Piñeros MA, Liu J, Magalhaes JV (2015) Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annu Rev Plant Biol* 66:571–598. <https://doi.org/10.1146/annurev-arplant-043014-114822>
- Kollmeier M, Felle HH, Horst WJ (2000) Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? *Plant Physiol* 122:945–956. <https://doi.org/10.1104/pp.122.3.945>
- Lindeman W (1959) Observations on the behaviour of phosphate compounds in *Chlorella* at the transition from dark to light. Agricultural Univ., Wageningen, The Netherlands.
- Lu H, Dong G, Hua H, Zhao W, Li J, Xu R (2020) Method for initially selecting Al-tolerant rice varieties based on the charge characteristics of their roots. *Ecotoxicol Environ Saf* 187:109813. <https://doi.org/10.1016/j.ecoenv.2019.109813>
- Malta, P.G., Arcanjo-Silva, S., Ribeiro, C., Campos, N.V., Azevedo, A.A., 2016. *Rudaea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplasts. *Plant Soil*, 408, 369–384. <https://doi.org/10.1007/s11104-016-2926-x>
- Maphumulo S, Derera J, Sibiyi J, Mathew I (2021) Combining ability, gene action and heterosis analyses of maize lines that were developed for maize streak virus resistance and acidic soil tolerance. *Euphytica* 217:23. <https://doi.org/10.1007/s10681-020-02754-z>
- Mohammadi M, Karr AL (2001) Superoxide anion generation in effective and ineffective soybean root nodules. *J Plant Physiol* 158:1023–1029. [https://doi.org/10.1078/S0176-1617\(04\)70126-1](https://doi.org/10.1078/S0176-1617(04)70126-1)
- Murshed R, Lopez-Lauri F, Sallanon H (2008) Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. *Anal Biochem* 383:320–322. <https://doi.org/10.1016/j.ab.2008.07.020>
- Nagalakshmi N, Prasad MNV (2001) Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. *Plant Sci* 160:291–299. [https://doi.org/10.1016/S0168-9452\(00\)00392-7](https://doi.org/10.1016/S0168-9452(00)00392-7)
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880. <https://doi.org/10.1093/oxfordjournals.pcp.a076232>

- Nemat Alla MM, Badawi A-HM, Hassan NM, El-Bastawisy ZM, Badran EG (2008) Herbicide tolerance in maize is related to increased levels of glutathione and glutathione-associated enzymes. *Acta Physiol Plant* 30:371–379. <https://doi.org/10.1007/s11738-008-0134-x>
- Noctor G, Strohm M, Jouanin L, Kunert K.J, Foyer CH, Rennenberg H (1996) Synthesis of glutathione in leaves of transgenic poplar overexpressing  $\gamma$ -Glutamylcysteine Synthetase. *Plant Physiol* 112:1071–1078. <https://doi.org/10.1104/pp.112.3.1071>
- Paraginski RT, de Souza NL, Alves GH, Ziegler V, de Oliveira M, Elias MC (2016) Sensory and nutritional evaluation of popcorn kernels with yellow, white and red pericarps expanded in different ways. *J Cereal Sci* 69:383–391. <https://doi.org/10.1016/j.jcs.2016.05.013>
- Pinto VB, Almeida VC, Pereira-Lima ÁA, Vale EM, Araújo WL, Silveira V, Viana JMS (2021a) Deciphering the major metabolic pathways associated with aluminum tolerance in popcorn roots using label-free quantitative proteomics. *Planta* 254:132. <https://doi.org/10.1007/s00425-021-03786-y>
- Pinto VB, Ferreira PG, Vidigal PMP, Oliveira Mendes TA, Dal-Bianco M, de Magalhaes JV, Viana JMS (2021b) Uncovering the transcriptional response of popcorn (*Zea mays* L. var. everta) under long-term aluminum toxicity. *Sci Rep* 11:19644. <https://doi.org/10.1038/s41598-021-99097-z>
- Pirzadah TB, Malik B, Tahir I, Rehman RU, Hakeem KR, Alharby HF (2019) Aluminum stress modulates the osmolytes and enzyme defense system in *Fagopyrum* species. *Plant Physiol Biochem* 144:178–186. <https://doi.org/10.1016/j.plaphy.2019.09.033>
- Polle E, Konzak CF, Kattrick JA (1978) Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. *Crop Sci* 18:823–827. <https://doi.org/10.2135/cropsci1978.0011183X001800050035x>
- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2018. Available in: <https://www.R-project.org/>
- Rahim F, Almeida VC, Viana JMS, Ribeiro C, Risso LA, Ribeiro MP (2019) Identification of contrasting tropical popcorn inbreds for studying aluminum toxicity tolerance inheritance. *Euphytica* 215:47. <https://doi.org/10.1007/s10681-019-2372-y>
- Rahman I, Kode A, Biswas SK (2006) Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protoc* 1:3159–3165. <https://doi.org/10.1038/nprot.2006.378>
- Ramakrishna B, Rao SSR (2013) Preliminary studies on the involvement of glutathione metabolism and redox status against zinc toxicity in radish seedlings by 28-Homobrassinolide. *Environ Exp Bot* 96:52–58. <https://doi.org/10.1016/j.envexpbot.2013.08.003>
- Ramírez-Benítez JE, Chee-González L, Teresa Hernandez-Sotomayor SM (2008) Aluminum induces changes in organic acids metabolism in *Coffea arabica* suspension cells with differential Al-tolerance. *J Inorg Biochem* 102:1631–1637. <https://doi.org/10.1016/j.jinorgbio.2008.03.002>
- Ribeiro C, Cambraia J, Peixoto PHP, Fonseca Júnior ÉM da (2012) Antioxidant system response induced by aluminum in two rice cultivars. *Braz J Plant Physiol* 24:107–116. <https://doi.org/10.1590/S1677-04202012000200004>
- Sarruge, J.R., Haag, H.P., 1974. Análises químicas em plantas. Esalq, Piracicaba, p. 56.
- Schmohl N, Pilling J, Fisahn J, Horst WJ (2000) Pectin methylesterase modulates aluminum sensitivity in *Zea mays* and *Solanum tuberosum*. *Physiol Plant* 109:419–427. <https://doi.org/10.1034/j.1399-3054.2000.100408.x>
- Silva CO, Brito DS, da Silva AA, do Rosário Rosa V, Santos MFS, de Souza GA, Azevedo AA, Dal-Bianco M, Oliveira JA, Ribeiro C (2020). Differential accumulation of aluminum in root tips of soybean seedlings. *Braz J Bot* 43:99–107. <https://doi.org/10.1007/s40415-020-00593-9>
- Singh S, Tripathi DK, Singh S, Sharma S, Dubey NK, Chauhan DK, Vaculík M (2017) Toxicity of aluminum on various levels of plant cells and organism: A review. *Environ Exp Bot* 137:177–193. <https://doi.org/10.1016/j.envexpbot.2017.01.005>

- Sivaguru M, Horst WJ (1998) The Distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. *Plant Physiol* 116:155–163. <https://doi.org/10.1104/pp.116.1.155>
- Sosa-Morales ME, Andaluz-Mejía LM, Cardona-Herrera R, Castañeda-Rodríguez LR, Ochoa-Montes DA, Santiesteban-López NA, Rojas-Laguna R (2022) Quality evaluation of yellow corn (*Zea mays* cv. Everta) subjected to 27.12-MHz radio frequency treatments for popcorn production. *Int J of Food Sci Tech* 57:3263–3267. <https://doi.org/10.1111/ijfs.15614>
- Sousa A, Saleh AM, Habeeb TH, Hassan YM, Zrieq R, Wadaan MAM, Hozzein WN, Selim S, Matos M, Abdelgawad H (2019) Silicon dioxide nanoparticles ameliorate the phytotoxic hazards of aluminum in maize grown on acidic soil. *Sci Total Environ* 693:133636. <https://doi.org/10.1016/j.scitotenv.2019.133636>
- Sun C, Liu L, Yu Y, Liu W, Lu L, Jin C, Lin X (2015) Nitric oxide alleviates aluminum-induced oxidative damage through regulating the ascorbate-glutathione cycle in roots of wheat: NO enhances wheat tolerance to Al toxicity. *J Integr Plant Biol* 57:550–561. <https://doi.org/10.1111/jipb.12298>
- Taylor GJ, McDonald-Stephens JL, Hunter DB, Bertsch PM, Elmore D, Rengel Z, Reid RJ (2000) Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina*. *Plant Physiol* 123:987–996. <https://doi.org/10.1104/pp.123.3.987>
- Vittorazzi C, Júnior ATA, Guimarães AG, Viana AP, Silva FHL, Pena GF, Daher RF, Gerhardt IFS, Oliveira GHF, Pereira MG (2017) Research Article Indices estimated using REML/BLUP and introduction of a super-trait for the selection of progenies in popcorn. *Genet Mol Res* 16: 1–10. <https://doi.org/10.4238/gmr16039769>
- Wu D, Shen H, Yokawa K, Baluška F (2015) Overexpressing *OsPIN2* enhances aluminum internalization by elevating vesicular trafficking in rice root apex. *J Exp Bot* 66:6791–6801. <https://doi.org/10.1093/jxb/erv385>
- Xiao M, Li Z, Zhu L, Wang J, Zhang B, Zheng F, Zhao B, Zhang H, Wang Y, Zhang Z (2021) The multiple roles of ascorbate in the abiotic stress response of plants: antioxidant, cofactor, and regulator. *Front Plant Sci* 12:598173. <https://doi.org/10.3389/fpls.2021.598173>
- Yang TY, Huang WT, Zhang J, Yang LT, Huang ZR, Wu BS, Lai NW, Chen LS (2021) Raised pH conferred the ability to maintain a balance between production and detoxification of reactive oxygen species and methylglyoxal in aluminum-toxic *Citrus sinensis* leaves and roots. *Environmental Pollution* 268:115676. <https://doi.org/10.1016/j.envpol.2020.115676>
- Zhao X, Chen Q, Wang Y, Shen, Z, Shen W, Xu X (2017) Hydrogen-rich water induces aluminum tolerance in maize seedlings by enhancing antioxidant capacities and nutrient homeostasis. *Ecotoxicol Environ Saf* 144:369–379. <https://doi.org/10.1016/j.ecoenv.2017.06.045>
- Zhu XF, Lei GJ, Wang ZW, Shi YZ, Braam J, Li GX, Zheng SJ (2013) Coordination between apoplastic and symplastic detoxification confers plant aluminum resistance. *Plant Physiol* 162:1947–1955. <https://doi.org/10.1104/pp.113.219147>
- Zobel RW (2008) Hardware and software efficacy in assessment of fine root diameter distributions. *Comput Electron Agric* 60:178–189. <https://doi.org/10.1016/j.compag.2007.08.002>

## Article II

### **Histochemical, microscopic and metabolic responses of silicon as a mitigating agent of aluminum toxicity in popcorn seedling root tips**

#### **ABSTRACT**

The toxicity of aluminum (Al) in acid soils impairs plant growth, however silicon (Si) can attenuate this toxic effect. the aim of this study was to investigate the short-term effects of Si as an attenuator of Al toxicity in popcorn (*Zea Mays* L. var. *Everta*) root tips. To achieve this, the mechanisms through which Si could alleviate the detrimental effects of Al were investigated, encompassing physiological, metabolic, and morphological traits. The treatments consisted of exposing the plants to the absence and presence of Al (200  $\mu$ M) and the absence and presence of Si (2 mM). The results showed that the presence of Al reduced the root growth rate, evidencing its toxic effect. However, Si was able to mitigate this negative effect, induced a lower rate of root elongation. Furthermore, Si reduced the Al concentration in the roots, indicating its ability to bind Al in the nutrient solution, and consequently reducing the absorption of this toxic metal by the plants. The morphoanatomical analysis of the roots revealed that Si mitigated the alterations induced by Al, such as the increase in the radial diameter of the root tip, desquamation of the root apex cells and disorganization of the epidermal cells. These changes suggest that Si benefits the morphology of roots exposed to Al. In the metabolism of plants, the addition of Si resulted in increases in the concentrations of fructose, sucrose, total amino acids and nitrate. However, total protein concentrations decreased in plants treated with Si in the presence of Al. In summary, this study demonstrated that Si attenuates the toxic effects of Al in popcorn plants. These results highlight the beneficial potential of Si as a strategy to improve crop development in acidic soils and mitigate the negative effects of Al toxicity.

**Keywords:** Al and Si uptake; oxidative stress; root growth; C and N metabolism.

## References

- Ahmed, K. B. M., Khan, M., Shabbir, A., Ahmad, B., Uddin, M., & Azam, A. (2023). Comparative effect of foliar application of silicon, titanium and zinc nanoparticles on the performance of Vetiver- a medicinal and aromatic plant. *Silicon*, 15, 153-166. 1–14. <https://doi.org/10.1007/s12633-022-02007-9>
- American Public Health Association. (2005). *Standard Methods for the Examination of Water and Wastewater*. (21st ed.). [American Water Works Association/Water Environment Federation]
- Bieleski, R. I., & Turner, N. A. (1966). Separation and estimation of aminoacids in crude plants extracts by thin-layer electrophoresis and chromatography. *Anal Biochemistry*, 17, 278–293. [https://doi.org/10.1016/0003-2697\(66\)90206-5](https://doi.org/10.1016/0003-2697(66)90206-5)
- Bilal, S., Khan, A., Imran, M., Khan, A. L., Asaf, S., Al-Rawahi, A., ... & Lee, I. J. (2022). Silicon-and boron-induced physio-biochemical alteration and organic acid regulation mitigates aluminum phytotoxicity in date palm seedlings. *Antioxidants*, 11(6), 1063. <https://doi.org/10.3390/antiox11061063>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cai, M. Z., Wang, F. M., Li, R. F., Zhang, S. N., Wang, N., & Xu, G. D. (2011). Response and tolerance of root border cells to aluminum toxicity in soybean seedlings. *Journal of Inorganic Biochemistry*, 105(7), 966-971. <https://doi.org/10.1016/j.jinorgbio.2011.04.004>
- Cai, M., Wang, N., Xing, C., Wang, F., Wu, K., & Du, X. (2013). Immobilization of aluminum with mucilage secreted by root cap and root border cells is related to aluminum resistance in *Glycine max* L. *Environmental Science and Pollution Research*, 20, 8924-8933. <https://doi.org/10.1007/s11356-013-1815-6>
- Cai, M., Zhang, S., Xing, C., Wang, F., Ning, W., & Lei, Z. (2011). Developmental characteristics and aluminum resistance of root border cells in rice seedlings. *Plant science*, 180(5), 702-708. <https://doi.org/10.1016/j.plantsci.2011.01.017>
- Carillo, P., & Gibon, Y. (2011). Protocol: Extraction and determination of proline. *PrometheusWiki*, 1-5.
- Chandra, J., Keshavkant, S. (2021). Mechanisms underlying the phytotoxicity and genotoxicity of aluminum and their alleviation strategies: A review. *Chemosphere*, 278, 130384. <https://doi.org/10.1016/j.chemosphere.2021.130384>
- Clark, R. B. (1975). Characterization of phosphatase of intact maize roots. *Journal of Agricultural and Food Chemistry*, 23(3), 458-460. <https://doi.org/10.1021/jf60199a002>
- Doncheva, S., Amenos, M., Poschenrieder, C., & Barcelo, J. (2005). Root cell patterning: A primary target for aluminium toxicity in maize. *Journal of experimental botany*, 56(414), 1213-1220. <https://doi.org/10.1093/jxb/eri115>



- Dong, Z., Li, Y., Xiao, X., Chen, Y., & Shen, X. (2018). Silicon effect on growth, nutrient uptake, and yield of peanut (*Arachis hypogaea* L.) under aluminum stress. *Journal of Plant Nutrition*, 41(15), 2001-2008. <https://doi.org/10.1080/01904167.2018.1485163>
- Fernie, A. R., Roscher, A., Ratcliffe, R. G., & Kruger, N. J. (2001). Fructose 2,6- biphosphate activates pyrophosphate: Fructose-6-phosphate 1-phosphotranferase and increase triose phosphate to hexose phosphate cycling in heterotrophic cells. *Plants*, 212(2), 250-263.
- Freitas, L. B., Fernandes, D. M., Maia, S. C. M., Fernandes, A. M. (2017). Effects of silicon on aluminum toxicity in upland rice plants. *Plant Soil*, 420, 263–275. <https://doi.org/10.1007/s11104-017-3397-4>
- Gay, C., & Gebicki, J. M. A. (2000). Critical evaluation of the effect of sorbitol on the ferric–xynol orange hydroperoxide assay. *Analytical biochemistry*, 284(2), 217-220. <https://doi.org/10.1006/abio.2000.4696>
- Giannakoula, A., Moustakas, M., Syros, T., & Yupsanis, T. (2010). Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. *Environmental and Experimental Botany*, 67(3), 487-494. <https://doi.org/10.1016/j.envexpbot.2009.07.01>
- Guzmán-Rangel, G., Montalvo, D., & Smolders, E. (2018). Pronounced antagonism of zinc and arsenate on toxicity to barley root elongation in soil. *Environments*, 5(7) 83. <https://doi.org/10.3390/environments5070083>
- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125(1), 189-198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Jiang, D., Wu, H., Cai, H., & Chen, G. (2022). Silicon confers aluminium tolerance in rice via cell wall modification in the root transition zone. *Plant, Cell & Environment*, <https://doi.org/10.1111/pce.14307>
- Kataoka, T., & Nakanishi, T. M. (2001). Aluminium distribution in soybean root tip for a short time Al treatment. *Journal of Plant Physiology*, 158(6), 731-736. <https://doi.org/10.1078/0176-1617-00163>
- Kato, Y., Miura, E., Matsushima, R., & Sakamoto, W. (2007). White leaf sectors in yellow variegated are formed by viable cells with undifferentiated plastids. *Plant Physiology*, 144(2), 952-960. <https://doi.org/10.1104/pp.107.099002>
- Kopittke, P. M., Gianoncelli, A., Kourousias, G., Green, K., & Mckenna, B. A. (2017). Alleviation of Al toxicity by Si is associated with the formation of Al–Si complexes in root tissues of sorghum. *Frontiers in plant science*, 8, 2189. <https://doi.org/10.3389/fpls.2017.02189>
- Kováčik, J., Štork, F., Klejdus, B., Grúz, J., & Hedbavny, J. (2012). Effect of metabolic regulators on aluminium uptake and toxicity in *Matricaria chamomilla* plants. *Plant Physiology and Biochemistry*, 54, 140-148. <https://doi.org/10.1016/j.plaphy.2012.02.018>
- Li, H., He, J., Yang, X., Li, X., Luo, D., Wei, C., Ma, J., Zhang, Y., Yang, J., & Zhang, X. (2016). Glutathione-dependent induction of local and systemic defense against oxidative

stress by exogenous melatonin in cucumber (*Cucumis sativus* L.). *J. Pineal Res.*, 60(2), 206-216. <https://doi.org/10.1111/jpi.12304>

Liang, Y., Nikolic, M., Bélanger R., Gong, H., & Song, A. (2015). *Silicon in agriculture from theory to practice*. Springer Dordrecht.

Lin, C. C., & Kao, C. H. (2001). Absciscic acid induced changes in cell wall peroxidase activity and hydrogen peroxide level in roots of rice seedlings. *Plant Science*, 160(2), 323-329. [https://doi.org/10.1016/S0168-9452\(00\)00396-4](https://doi.org/10.1016/S0168-9452(00)00396-4)

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nature protocols*, 1(1), 387-396. <https://doi.org/10.1038/nprot.2006.59>

Liu, W., Xu, F., Lv, T., Zhou, W., Chen, Y., Jin, C., Lu, L., & Lin, X. (2018). Spatial responses of antioxidative system to aluminum stress in roots of wheat (*Triticum aestivum* L.) plants. *Science of the Total Environment*, 627, 462-469. <https://doi.org/10.1016/j.scitotenv.2018.01.021>

Ma, B., Gao, L., Zhang, H., Cui, J., & Shen, Z. (2012). Aluminum-induced oxidative stress and changes in antioxidant defenses in the roots of rice varieties differing in Al tolerance. *Plant cell reports*, 31, 687-696. <https://doi.org/10.1007/s00299-011-1187-7>

McCullough, H. (1967). The determination of ammonia in whole blood by a direct colorimetric method. *Clínica chimica acta*, 17(2), 297-304. [https://doi.org/10.1016/0009-8981\(67\)90133-7](https://doi.org/10.1016/0009-8981(67)90133-7)

Mehrabanjoubani, P., Abdolzadeh, A., Sadeghipour, H. R., Aghdasi, M., Bagherieh-Najjar, M. B., & Barzegargolchini, B. (2019). Silicon increases cell wall thickening and lignification in rice (*Oryza sativa*) root tip under excess Fe nutrition. *Plant Physiology and Biochemistry*, 144, 264-273. <https://doi.org/10.1016/j.plaphy.2019.09.047>

Mukarram, M., Khan M. M. A., & Corpas, F. J. (2021). Silicon nanoparticles elicit an increase in lemongrass (*Cymbopogon flexuosus* (Steud.) Wats) agronomic parameters with a higher essential oil yield. *Journal of Hazardous Materials*, 412, 125254. <https://doi.org/10.1016/j.jhazmat.2021.125254>

Navascués, J., Pérez-Rontomé, C., Sánchez, D. H., Staudinger, C., Wienkoop, S., Rellán-Álvarez, R., & Becana, M. (2012). Oxidative stress is a consequence, not a cause, of aluminum toxicity in the forage legume *Lotus corniculatus*. *New Phytologist*, 193(3), 625-636. <https://doi.org/10.1111/j.1469-8137.2011.03978.x>

Nunes-Nesi, A., Carrari, F., Gibon, Y., Sulpice, R., Lytovchenko, A., Fisahn, J., Graham, J., Ratcliffe, R. G., Sweetlove, L. J., & Fernie, A. R. (2007). Deficiency of mitochondrial fumarate hydratase activity in tomato plants impairs photosynthesis via an effect on stomatal function. *The Plant Journal*, 50, 1093-1106. <https://doi.org/10.1111/j.1365-313X.2007.03115.x>

Palma, J. M., Mateos, R. M., López-Jaramillo, J., Rodríguez-Ruiz, M., González-Gordo, S., Lechuga-Sancho, A. M., & Corpas, F. J. (2020). Plant catalases as NO and H<sub>2</sub>S targets. *Redox Biology*, 34, 101525. <https://doi.org/10.1016/j.redox.2020.101525>

Pereira, A. S., Dorneles, A. O. S., Bernardy, K., Sasso, V. M., Bernardy, D., Possebom, G., Rossato, L. V., Dressler, V. L. & Tabaldi, L. A. (2018). Selenium and silicon reduce cadmium



uptake and mitigate cadmium toxicity in *Pfaffia glomerata* (Spreng.) Pedersen plants by activation antioxidant enzyme system. *Environmental Science and Pollution Research*, 25(19), 18548–18558. <https://doi.org/10.1007/s11356-018-2005-3>

Pinto, V. B., Almeida, V. C., Pereira-Lima, Í. A., Vale, E. M., Araújo, W. L., Silveira, V., & Viana, J. M. S. (2021). Deciphering the major metabolic pathways associated with aluminum tolerance in popcorn roots using label-free quantitative proteomics. *Planta*, 254, 1-14. <https://doi.org/10.1007/s00425-021-03786-y>

Polle, E. K. C. F., Konzak, C. F., & Kattrick, J. A. (1978). Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. *Crop Science*, 18(5), 823-827. <https://doi.org/10.2135/cropsci1978.0011183X001800050035x>

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/4>

Rahman, R., & Upadhyaya, H. (2021). Aluminium toxicity and its tolerance in plant: A review. *Journal of Plant Biology*, 64(2), 101-121. <https://doi.org/10.1007/s12374-020-09280-4>

Ren, J., Yang, X., Zhang, N., Feng, L., Ma, C., Wang, Y., Yang, Z., & Zhao, J. (2022). Melatonin alleviates aluminum-induced growth inhibition by modulating carbon and nitrogen metabolism, and reestablishing redox homeostasis in *Zea mays* L. *Journal of Hazardous Materials*, 423, 127159. <https://doi.org/10.1016/j.jhazmat.2021.127159>

Ribeiro, C., Lapaz, A. M., Freitas-Silva, L., Ribeiro, K. V. G., Yoshida, C. H. P., Dal-Bianco, M., & Cambraia, J. (2022). Aluminum promotes changes in rice root structure and ascorbate and glutathione metabolism. *Physiology and Molecular Biology of Plants*, 28(11-12), 2085-2098. <https://doi.org/10.1007/s12298-022-01262-9>

Sarruge, J.R., & Haag, H.P. (1974). *Análises químicas em plantas*. Esalq.

Shetty, R., Vidya, C. S. N., Prakash, N. B., Lux, A., & Vaculik, M. (2021). Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. *Science of the Total Environment*, 765, 142744. <https://doi.org/10.1016/j.scitotenv.2020.142744>

Silva, C. O., Brito, D. S., Silva, A. A., Rosa, V. R., Santos, M. F. S., Souza, G. A., Azevedo, A. A., Dal-Bianco, M., Oliveira, J. A., & Ribeiro, C. (2020). Differential accumulation of aluminum in root tips of soybean seedlings. *Brazilian Journal of Botany*, 43(1), 99-107. <https://doi.org/10.1007/s40415-020-00593-9>

Soares, C., Carvalho, M. E., Azevedo, R. A., & Fidalgo, F. (2019). Plants facing oxidative challenges - A little help from the antioxidant networks. *Environmental and Experimental Botany*, 161, 4-25. <https://doi.org/10.1016/j.envexpbot.2018.12.009>

Sousa, A., Saleh, A. M., Habeeb, T. H., Hassan, Y. M., Zrieq, R., Wadaan, M. A., ... & Abdelgawad, H. (2019). Silicon dioxide nanoparticles ameliorate the phytotoxic hazards of aluminum in maize grown on acidic soil. *Science of the Total Environment*, 693, 133636. <https://doi.org/10.1016/j.scitotenv.2019.133636>

Souza, L. C., Nogueira, D. C. S., Machado, L. C., Costa, T. C., Martins, J. T. S., Mendes, C. A. P., Pires, N. M. C., Conceição, S. S., Brito, A. E. A., & Oliveira Neto, C. F. (2016).

Nitrogen compounds, proteins and amino acids in corn subjected to doses of aluminum. *African Journal of Agricultural Research*, 11(17), 1519-1524.  
<https://doi.org/10.5897/AJAR2015.10758>

Tabuchi, A., Kikui, S., & Matsumoto, H. (2004). Differential effects of aluminium on osmotic potential and sugar accumulation in the root cells of Al-resistant and Al-sensitive wheat. *Physiologia Plantarum*, 120(1), 106-112. <https://doi.org/10.1111/j.0031-9317.2004.0206.x>

Thornton, B. (1998). Influence of pH and aluminum on nitrogen partitioning in defoliated grasses. *Grass Forage Sci.* 53, 170–178. <https://doi.org/10.1046/j.1365-2494.1998.5320170.x>

Todd, C. D., Tipton, P. A., Blevins, D. G., Piedras, P., Pineda, M., & Polacco, J. C. (2006). Update on ureide degradation in legumes. *Journal of Experimental Botany*, 57(1), 5-12.  
<https://doi.org/10.1093/jxb/erj013>

Vaculík, M., Pavlovič, A., & Lux, A., (2015). Silicon alleviates cadmium toxicity by enhanced photosynthetic rate and modified bundle sheath's cell chloroplasts ultrastructure in maize. *Ecotoxicol. Environ. Saf.* 120, 66–73. <https://doi.org/10.1016/j.ecoenv.2015.05.026>

Vega, I., Nikolic, M., Pontigo, S., Godoy, K., Mora, M. D. L. L., & Cartes, P. (2019). Silicon improves the production of high antioxidant or structural phenolic compounds in barley cultivars under aluminum stress. *Agronomy*, 9(7), 388.  
<https://doi.org/10.3390/agronomy9070388>

Wang, Y., Stass, A., Horst, W. J. (2004). Apoplastic binding of aluminum is involved in silicon-induced amelioration of aluminum toxicity in maize. *Plant physiology*, 136( 3), 3762-3770, <https://doi.org/10.1104/pp.104.045005>

Xiao, Z., & Liang, Y. (2022). Silicon prevents aluminum from entering root tip by promoting formation of root border cells in rice. *Plant Physiology and Biochemistry*, 175, 12-22.  
<https://doi.org/10.1016/j.plaphy.2022.02.003>

Xu, Q., Wang, Y., Ding, Z., Song, L., Li, Y., Ma, D., ... & Zhang, H. (2016). Aluminum induced metabolic responses in two tea cultivars. *Plant Physiology and Biochemistry*, 101, 162-172. <https://doi.org/10.1016/j.plaphy.2016.02.001>

Yokosho, K., Yamaji, N., Fujii-Kashino, M., & Ma J. F. (2016). Functional analysis of a MATE gene *OsFRDL2* revealed its involvement in Al-induced secretion of citrate, but a lower contribution to Al tolerance in rice. *Plant Cell Physiol*, 57, 976–985.  
<https://doi.org/10.1093/pcp/pcw026>

Yoshida, C. H. P., Pacheco, A. C., Lapaz, A. D. M., Gorni, P. H., Vítolo, H. F., & Bertoli, S. C. (2020). Methyl jasmonate modulation reduces photosynthesis and induces synthesis of phenolic compounds in sweet potatoes subjected to drought. *Bragantia*, 79, 319-334.  
<https://doi.org/10.1590/1678-4499.20200203>

Yoshida, C. H. P., Pacheco, A. C., Marcos Lapaz, A., Ferreira, C. S., Dal-Bianco, M., Viana, J. M. S., & Ribeiro, C. (2023). Tolerance mechanisms to aluminum in popcorn inbred lines involving aluminum compartmentalization and ascorbate–glutathione redox pathway. *Planta*, 257(2), 28. <https://doi.org/10.1007/s00425-022-04062-3>

Zhao, X. Q., & Shen, R. F. (2018). Aluminum–nitrogen interactions in the soil–plant system. *Frontiers in plant science*, 9, 807. <https://doi.org/10.3389/fpls.2018.00807>