

CHLORIDE NUTRITION AFFECTS ROOT ARCHITECTURE DURING EARLY VEGETATIVE DEVELOPMENT IN TOBACCO AND TOMATO PLANTS

Marta Lucas*^{1,2}, Procopio Peinado-Torrubia¹, Francisco J. Moreno-Racero¹,
Álvaro F. Garcia-Rodriguez¹, José M. Colmenero-Flores^{1,2}
and Miguel A. Rosales*^{1,2}

¹Group of Plant Ion and Water Regulation, Instituto de Recursos Naturales y Agrobiología (IRNAS-CSIC), Seville, SPAIN. ²Laboratory of Plant Molecular Ecophysiology, Instituto de Recursos Naturales y Agrobiología (IRNAS-CSIC), Seville, SPAIN.

**mlucas@irnas.csic.es and mrosales@irnas.csic.es*

Abstract:

Chloride (Cl⁻) is an anion that participates in key processes for plants, such as photosynthesis and osmotic regulation¹. Cl⁻ has recently been defined as a beneficial macronutrient for higher plants, due to the stimulation of plant growth, inducing morphological and cellular changes that include the stimulation of leaf cell size, reducing stomatal conductance and inducing additional osmolarity and turgor in adult tobacco plants. Thus, Cl⁻ generates bigger plants with better water and nitrogen use efficiency and, as a result, a better photosynthetic performance^{2,3}.

Roots are in charge of searching for water and nutrients which are essentials for plant development and growth. Nutrients distribute to the rest of plant organs via the vascular system, being regulated by complex hormonal mechanisms⁴. Although some proteins regulating Cl⁻ homeostasis (e.g. Cation-Chloride Cotransporter) have been reported to be important for root cell elongation⁵, little is known about the effect of Cl⁻ nutrition on root developmental processes during early vegetative growth.

To this aim, in this work tobacco and tomato seeds were sown under greenhouse conditions and seedlings were harvested at different developmental stages, from 8 to 35 days after sowing. A Cl⁻ treatment (5 mM of Cl⁻ salts) was supplemented to the basal nutrient solution, and a treatment with a mix of sulphate+phosphate salts (SP) with the same cationic content was used as a control. Afterwards, biomass and root system architecture (RSA) parameters (primary root length, number and length of lateral roots) were analysed by using 'ImageJ' and 'Optimas' software.

Results from this work showed that the Cl⁻ treatment generated bigger plants with longer primary roots and shorter lateral roots during the early vegetative development. These effects may be caused by higher cell size or division during the root development, with a possible effect on hormonal regulation. Therefore, these root architecture changes are particularly relevant during early vegetative development, allowing to Cl⁻-fertilized crops

with longer roots to reach deeper zones in the soil with more water and nutrients and, consequently, helping to withstand drought periods that are increasingly more frequent because of climate change.

Key words: Chloride, Beneficial macronutrient, Root architecture, Early vegetative stage

Acknowledgments: Funding for the present research was provided by the Spanish Ministry of Science Innovation and Universities-FEDER grant RTI2018-094460-B-I00, Spanish National Research Council grants CSIC-202040E266 and CSIC-202140E023, and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 895613. We would like to thank F.J. Durán for technical assistance.

References:

- ¹Colmenero-Flores, J.M., Franco-Navarro, J.D., Cubero-Font, P., Peinado-Torrubia, P. and Rosales, M.A., (2019). Chloride as a beneficial macronutrient in higher plants: New roles and regulation. *International Journal of Molecular Sciences*, 20, 4686.
- ²Franco-Navarro, J.D., Brumós, J., Rosales, M.A., Cubero-Font, P., Talón, M., and Colmenero-Flores, J.M. (2016). Chloride regulates leaf cell size and water relations in tobacco plants. *Journal of Experimental Botany*, 67(3), 873-891.
- ³Franco-Navarro, J.D., Rosales, M.A., Cubero-Font, P., Calvo, P., Álvarez, R., Diaz-Espejo, A., and Colmenero-Flores, J.M. (2019). Chloride as a macronutrient increases water-use efficiency by anatomically driven reduced stomatal conductance and increased mesophyll diffusion to CO₂. *The Plant Journal*, 99, 815-831.
- ⁴White, P.J., and Broadley, M.R. (2001). Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany*, 88, 967-988.
- ⁵Colmenero-Flores, J.M., Martínez, G., Gamba, G., Vázquez, N., Iglesias, D.J., Brumós, J., and Talón, M. (2007). Identification and functional characterization of cation-chloride cotransporters in plants. *The Plant Journal*, 50, 278-292