CHLORIDE NUTRITION REGULATES DEVELOPMENT, WATER BALANCE AND DROUGHT RESISTANCE IN PLANTS

Franco-Navarro, J.D.[1], Brumós, J.[2], Rosales, M.A.[1], Vázquez-Rodríguez, A.[1], Sañudo, B.J.[1], Díaz-Rueda, P.[1], Rivero, C.[1], Talón, M.[2], Colmenero-Flores, J.M.[1]*

[1*] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS). Consejo Superior de Investigaciones Científicas (CSIC). Avda. Reina Mercedes 10, 41012-Sevilla. chemacf@irnase.csic.es

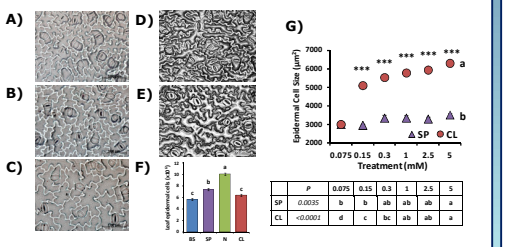
[2] Instituto Valenciano de Investigaciones Agrarias (IVIA). Centro de Genómica. Carretera Moncada - Náquera, Km. 4,5. 46113-Moncada (Valencia).

INTRODUCTION. Chloride (Cl^-) is considered a micronutrient because it is supposed to be needed in a small quantity for a healthy growth in higher plants (<50-100 mM in the nutrient media, *Johnson et al., 1957; Terry, 1977*). However, Cl^- is a strange micronutrient since actual Cl^- concentration in plants is typical of the content of a macronutrient (about 50-300 times higher than the content required as essential micronutrient, *Marschner, 1995*). This accumulation requires a very high cost of energy (*Brumós et al., 2010*), and because of Cl^- is the major osmotically active solute in the vacuole (*Flowers, 1998*), we hypothesize that when it is accumulated to levels that are typical of the content of a macronutrient, Cl^- may fulfill a poorly understood biological role when accumulated to such high levels, and it may have an impact in osmoregulation, water relations and drought resistance in higher plants.

OBJETIVES. We aimed to elucidate the **involvement of Cl^- in the development, water balance and drought resistance** of tobacco plants in response to increasing concentration of anions and the correlations to different water parameters, including a complete leaf water/osmotic/turgor potential measurement.

EXPERIMENTAL DESIGN. Tobacco plants were grown subjected to different treatments: basal nutrient solution (BS); BS supplemented with different concentrations of Cl^- salts (CL); BS supplemented with different concentrations of NO_3^- salts (N); BS supplemented with different concentrations of SO_4^{2-} + PO_4^{3-} salts (SP). All treatments (CL, N and SP) contained the same concentration of charge-balancing cations. Plants were subjected to two irrigation treatments: **optimal irrigation** (Control, at 100% of field capacity), and **water deficit** (drought), in which pots were irrigated every two days to 60% of field capacity. As it was shown before (*Franco-Navarro et al., 2013a,b*), no deficiency symptoms were observed in BS, N or SP treatments, and no differences were observed in three of the main leaf cation content (Ca^{2+}, Mg^{2+} and K^+).

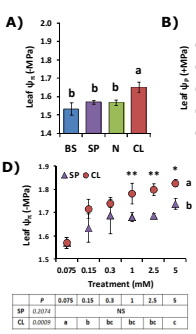
1. Cl^- INCREASED LEAF EPIDERMAL CELL ELONGATION



	P	0.075	0.15	0.3	1	2.5	5
SP	0.0035	b	b	ab	ab	ab	a
CL	<0.0001	d	c	bc	ab	ab	a

Fig. 1. Effect of Cl^- nutrition on epidermal cell elongation. Microscopy (20X) of abaxial leaf epidermal impressions at BS **(A)**, 5 mM N **(B)**, 5 mM SP **(D)** and 5 mM CL **(C, E)** treatments. **(F)** Cell division rate, quantified as the number of epidermal cells per leaf. **(G)** Epidermal Cell Size. Mean values ± SE, n = 4 - 6. Levels of significance (ANOVA, MANOVA): $P \leq 0.01$ (**) and $P \leq 0.001$ (***). "homogeneous group" statistics was calculated through Tukey's HSD.

2. Cl^- PROVIDED ADITIONAL LEAF OSMOLARITY AND TURGOR



	P	0.075	0.15	0.3	1	2.5	5
SP	0.0074	NS					
CL	0.0009	a	b	bc	bc	bc	c

Fig. 2. Effect of Cl^- nutrition on leaf osmotic potential and turgor. **(A, D)** Mature Leaf Osmotic potential; **(B, C)** Mature Leaf Turgor potential; **(A, B, D)** Pre-dawn values; **(C)** Midday values. Mean values ± SE, n = 4 - 6. Levels of significance (ANOVA and MANOVA test): P> 0.05 (NS, Not Significant), $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

3. Cl^- REDUCED STOMATAL CONDUCTANCE

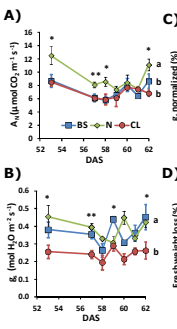


Fig. 3. Effect of Cl^- nutrition on Leaf Transpiration. **(A)** A_N, Photosyntetic Rate and **(B)** g_s, Stomatal Conductance; **(C)** Effect on stomatal conductance normalized according to percentage of SP g_s activity. **(D)** Plant transpiration measured as fresh weight loss over time of detached leaves. Days After Sowing (DAS). Mean values ± SE, n = 4 - 6. Levels of significance (ANOVA and MANOVA test): $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

4. Cl^- IMPROVED WATER USE EFFICIENCY

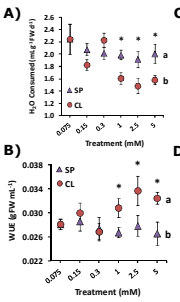


Fig. 4. Effect of Cl^- nutrition on Water Use Efficiency. (A, C) Water consumption in relation to plant biomass; **(B)** WUE, integrated water use efficiency obtained from total biomass produced in relation to total water consumed; **(D)** Total water consumption. Days After Sowing (DAS). Mean values ± SE, n = 4 - 6. Levels of significance (ANOVA and MANOVA test): $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***).

5. LEAF Cl^- CONTENT CORRELATED TO ANATOMICAL AND WATER PARAMETERS

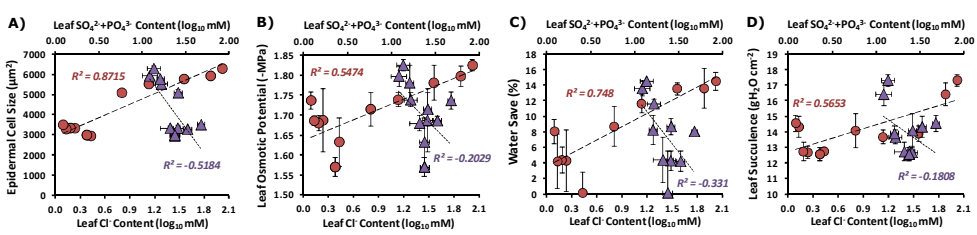


Fig. 5. Correlations between leaf anions content *vs.* anatomical and leaf water parameters. (A) Correlation to Epidermal Leaf cell size. **(B)** Correlation to Leaf Osmotic Potential (Ψπ). **(C)** Correlation to Leaf Water Save (calculated from detached leaves weighted at 6 hours). **(D)** Correlation to Leaf Succulence. Mean values ± SE, n = 6. Levels of significance represented by the Pearson's R-squared linear correlation test (R^2).

6. Cl^- IMPROVED WATER DEFICIT RESISTANCE

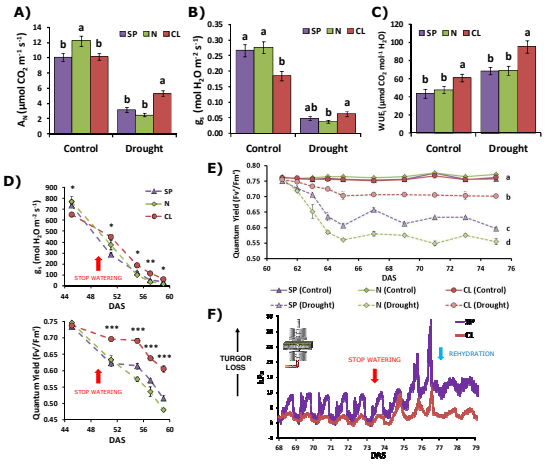


Fig. 6. Effect of Cl^- nutrition on drought resistance / avoidance. (A-C) 3-days averaged data of photosynthetic parameters (between 53-62 Days After Sowing -DAS-), measured from plants subjected to 2 irrigation treatments: well-irrigated plants (Control) and plants subjected to 60% field capacity (Drought): **(A)** A_N, Photosyntetic Rate; **(B)** g_s, Stomatal Conductance; **(C)** photosynthetic or instantaneous Water Use Efficiency (WUE$_i$). **(D)** g_s, Stomatal Conductance and efficiency of photosystem II (Quantum Yield). Red arrow indicates stop watering. **(E)** Efficiency of photosystem II in control and drought plants. **(F)** Leaf turgor measurement using the magnetic leaf patch clamp pressure probe (*Zimmermann et al., 2008*). Blue arrow indicates rehydration. Mean values ± SE, n = 4 - 6. Levels of significance (ANOVA and MANOVA test): $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

CONCLUSIONS

1. When fed with Cl^- levels in the millimolar range (1-5 mM), plants take up Cl^- to levels which are typical of the content of a macronutrient (*Franco-Navarro et al., 2012*), and its specific biological role cannot be induced by anionic macronutrient (NO_3^-, SO_4^{2-} or PO_4^{3-}).
2. Leaf cations content (K^+, Ca^{2+}, Mg^{2+}) was similar in plants treated with CL, N, and SP supplements (*Franco-Navarro et al., 2013a,b*).
3. Cl^- nutrition in contrast to SO_4^{2-}+PO_4^{3-} nutrition promotes adult plant growth through leaf cell elongation and leaf expansion (**Fig. 1**).
4. Cl^- provides additional osmolarity that decreases osmotic potential and increases water (not shown) and turgor potential (**Fig. 2**), leading plants to a greater hydration state (**Fig. 3**) that probably stimulates leaf epidermal cells growth. In well-watered plants, a reduction of stomatal conductance (Gs) and stomatal frequency is observed (**Fig. 3**; *Franco-Navarro et al., 2012; Franco-Navarro et al., 2013a*), and results in a reduction of water consumption and in an increase of both photosynthetic and integrated WUE parameters, (**Fig. 4**). Correlations of different parameters to Cl^- are positive correlations, and in all cases correlations to SO_4^{2-}+PO_4^{3-} are negative (**Fig. 5**).
5. Drought plants treated with Cl^- shows better plant growth, higher efficiency of photosystem II and improved photosynthetic and water parameters in contrast to BS, SP or N-treated plants (**Fig. 6**).
6. Biological functions indicated in the scheme (**Fig. 7**) summarize the results obtained in this work.

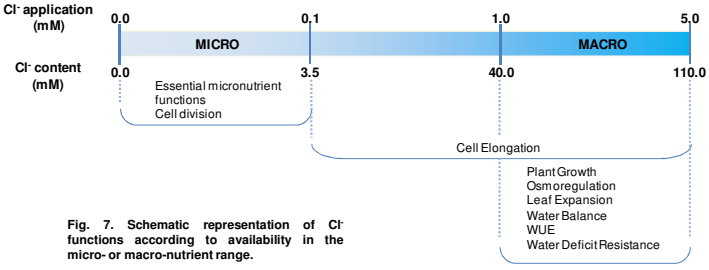


Fig. 7. Schematic representation of Cl^- functions according to availability in the micro- or macro-nutrient range.

REFERENCES

Brumós, J., et al. (2010). *Plant, Cell & Environment*, **33**, 2012-2027.
Flowers, T.J. (1988). *Advances in plant nutrition* (ed. L.A. Tinker B), pp. 55-78. Praeger, New York.
Franco-Navarro, J.D., et al. (2012). *X Simposio hispano-portugués de nutrición mineral en las plantas*. Madrid.
Franco-Navarro, J.D., et al. (2013a). *XI Simposio hispano-portugués de relaciones hídricas en las plantas*. IRNASE-CSIC, Sevilla.
Franco-Navarro, J.D., et al. (2013b). *Enviroment Workshop 2013*. UNIA-UMA-CSIC, Baeza.
Johnson, C.M., et al. (1957). *Plant Soil*, **8**, 337-353.
Marschner, H. (1995). *Mineral Nutrition of Higher Plants, 2nd ed.* (Second Edition ed.). Academic Press, London.
Terry, N. (1977). *Plant Physiology*, **60**, 69-75.
Zimmermann, D., et al. (2008). Journal of Experimental Botany, **59**, 3157-3167.