RIH Regulación Iónica e Hídrica en plantas

PHYSIOLOGICAL ANALYSIS OF CHLORIDE-INDUCED RESPONSES DURING NITROGEN DEFICIENCY IN TOMATO PLANTS

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INTRODUCTION

Chloride (Cl$^-$) has been traditionally **considered a harmful ion** for plants because of its accumulation in photosynthetic organs under high salinity conditions. In addition, the close relationship between the physical-chemical properties of **Cl$^-$ and nitrate** (NO$_3^-$) defined Cl$^-$ as a NO$_3^-$ antagonist for many years[1,2]. However, we recently demonstrated the opposite, defining **Cl$^-$ as a beneficial element for plants** when it's accumulated in **macronutrient levels**, capable of improving plant development and growth, as well as the efficiency in the use of water, carbon and nitrogen (N) [3,4].

Nowadays, the use of N fertilizers for a better crop production and yield is an ordinary practice in agriculture. High levels of N in soils can adversely affect N metabolism and reduce crop yield and its quality[5,6]. To avoid this, its content in fertilization must be significantly reduced through practices that allow a more efficient use of N.

Therefore, we propose **Cl$^-$ nutrition** as an important tool to improve current agricultural sustainability, which could be a potential strategy to **reduce N fertilization** and **deficiency symptoms,** improving growth, turgor maintenance and photosynthesis. In this work, we analyzed physiological traits in tomato plants grown under different Cl$^-$ and NO$_3^-$ treatments. Our results confirmed that the application of **5 mM Cl$^-$ allowed to reduce NO$_3^-$ supply** while maintaining the growth, photosynthesis and turgor of plants, and especially when N remains scarce in the substrate.

MATERIALS AND METHODS

PLANT MATERIAL AND NUTRITIONAL TREATMENTS: Tomato plants *(Solanum Lycopersicum* L.*)* were grown under greenhouse conditions for 28 and 90 days. Plants were subjected to two nutritional treatments: a supplement of 5 mM of sulphate (SO$_4^-$) and phosphate (PO$_4^-$) salts as a control (SP), and a supplement of 5 mM Cl$^-$ salts (CL). Different concentrations of NO$_3^-$ were added (from 3 to 12 mM), having a total of up to 12 treatments.

GROWTH PARAMETERS: Plant material was dried at 75 °C for 48 h and weighted. For the total leaf area, leaves were placed fully expanded on a filter paper and analyzed with ImageJ. Analysis of leaf cells was carried out in epidermal peels and epidermal impressions. Photographs were taken with a light microscope and analyzed with ImageJ.

GAS-EXCHANGE AND WATER PARAMETERS: Gas-exchange parameters were measured using a LI-6400 portable photosynthesis system in tomato plants. Water potentials were measured with a Scholander pressure chamber. Leaf osmotic potential (Ψπ) and turgor of individual detached leaves were determined as described by *Franco-Navarro et al., 2016*[7].

STRESS MARKERS: During the final stage of plant growth and development, two non-invasive methods were used to measure the stress level: SPAD and QUANTUM YIELD.

STATISTICAL ANALYSIS: Statistics was calculated through ANOVA, where mean values with different letters are significantly different according to Tukey's test at *P*≤0.05. Levels of significance: **P*≤0.05; ***P*≤0.01; ***P*≤0.001; and 'ns' *P*>0.05.

RESULTS

Cl$^-$ enhances plant growth and fruit production with less NO$_3^-$ fertilization

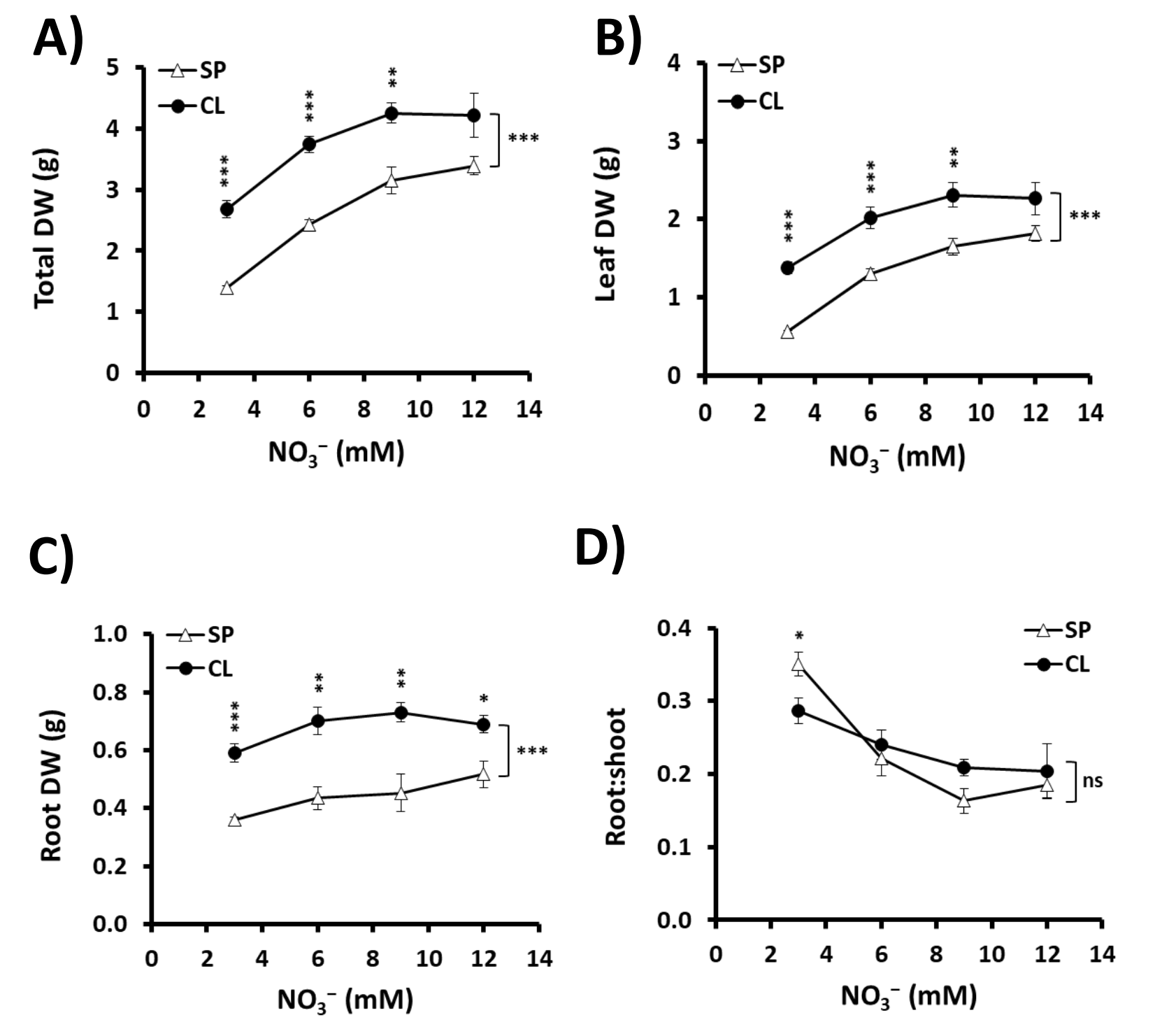

Fig. 1.: **Effect of different Cl$^-$ and NO$_3^-$ treatments on plant growth at 28 days after sowing (DAS).** Tomato plants were treated with increasing NO$_3^-$ concentrations (from 3 to 12mM) and alternatively with two different treatments: SP (as a control) and CL (5mM), maintaining the same balance of cations. **(A)** Total dry weight (DW); **(B)** Total leaf DW; **(C)** Total root DW; **(D)** Root:shoot ratio.

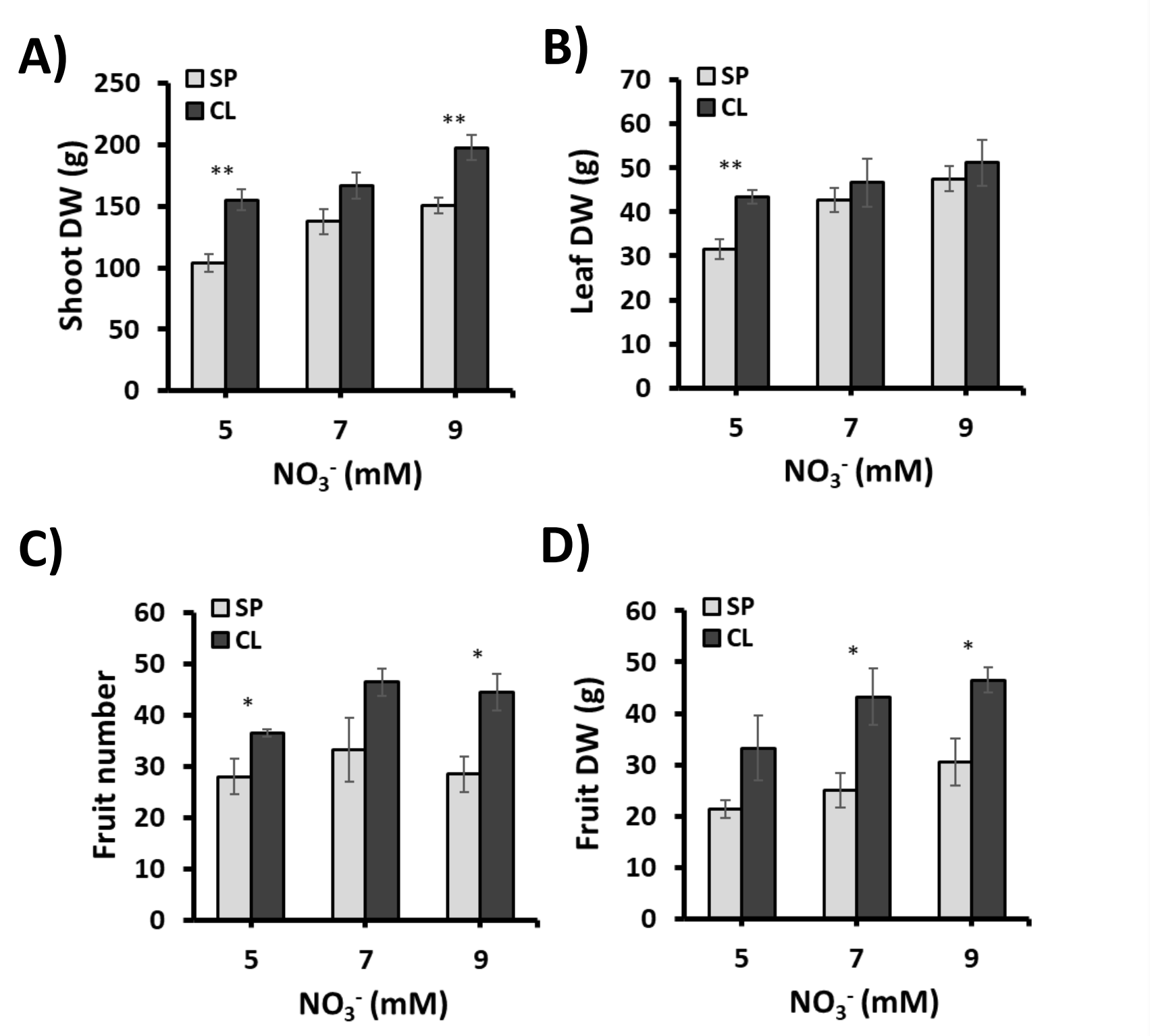

Fig. 2.: **Effect of different Cl$^-$ and NO$_3^-$ treatments on plant growth and fruit production at 90 DAS.** Tomato plants were treated with the most relevant increasing concentrations of NO$_3^-$ and alternatively with two different treatments: SP (as control) and CL(5mM), maintaining the same balance of cations. **(A)** Leaf DW; **(B)** Shoot DW; **(C)** Fruit number and **(D)** Fruit DW.

Cl$^-$ generates bigger leaves with larger epidermal and stomatal cells, particularly under low N supply (deficiency)

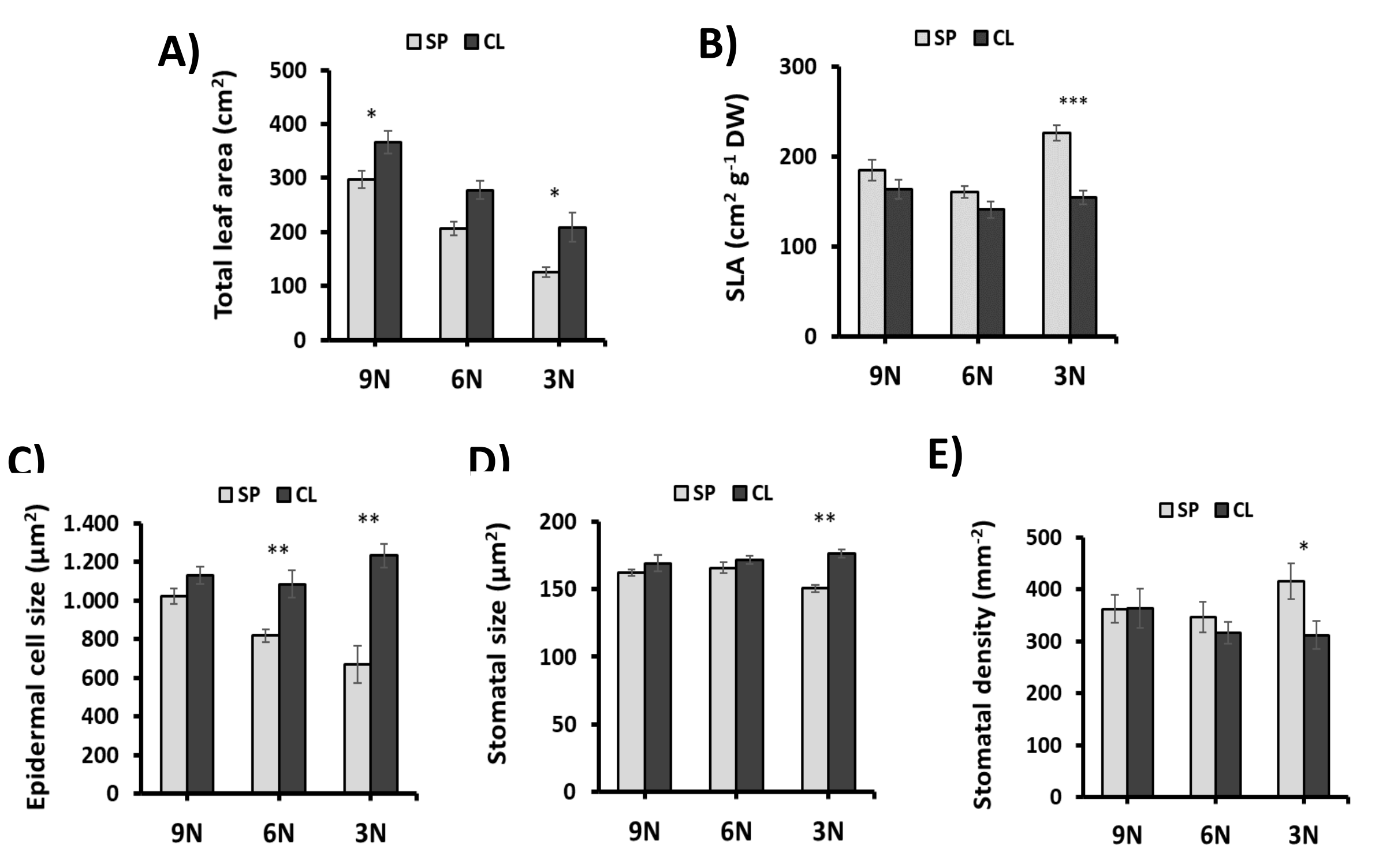

Fig. 3.: **Effect of Cl$^-$ and NO$_3^-$ treatments on leaf anatomy of tomato plants at 28 DAS.** Tomato plants were treated with the most relevant increasing concentrations of NO$_3^-$ and alternatively with two different treatments: SP (as control) and CL(5mM) , maintaining the same balance of cations. Effect on **(A)** total leaf area and **(B)** Specific leaf area. **(C)** Epidermal cell size; **(D)** Stomatal size and **(E)** Stomatal cell density .

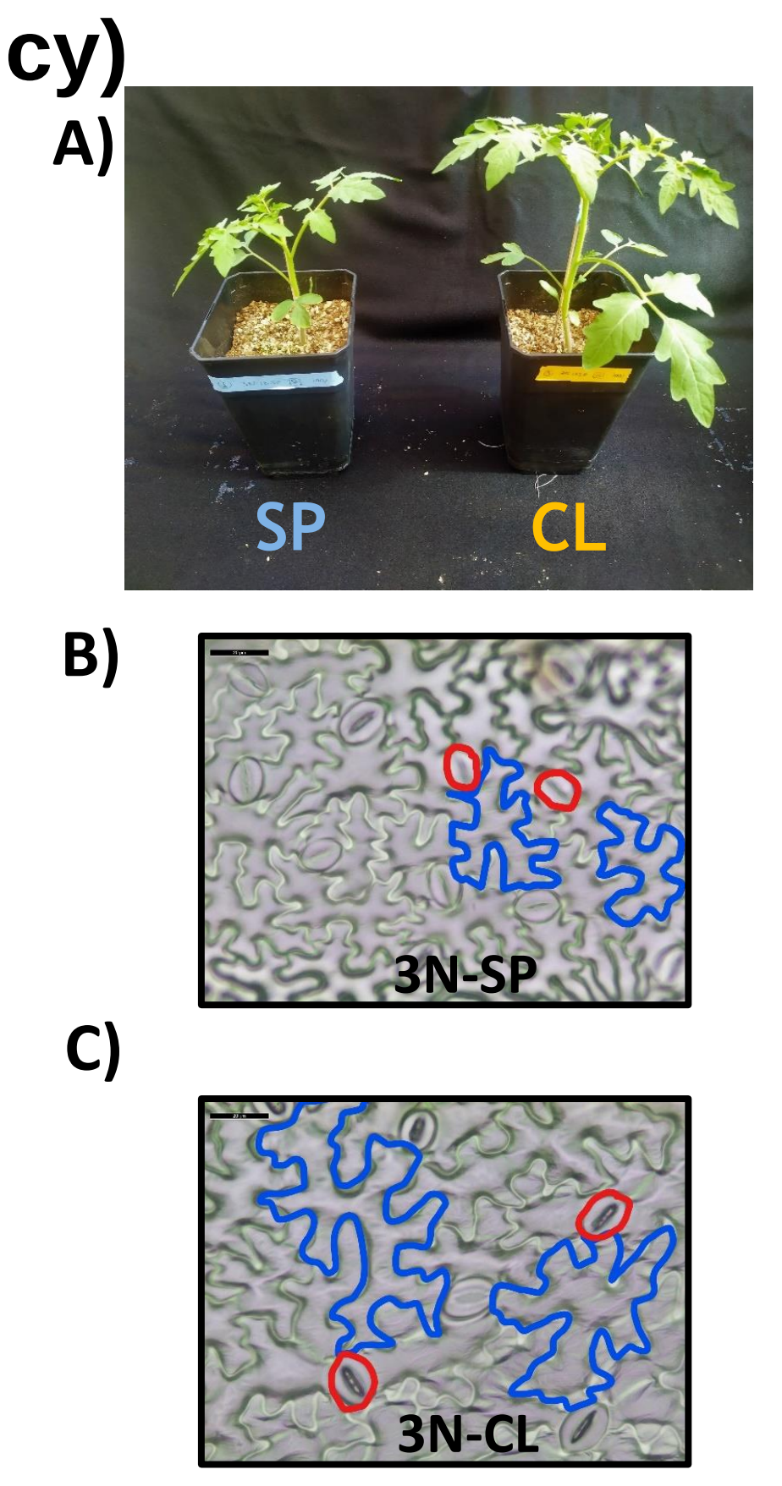

Fig. 4.: **Plant size at 28 DAS and microscopy images for leaf cell analysis.** **(A)** Left: 3N:SP; right: 3N:CL; **(B,C)** Images of epidermal cells in blue and stomata in red, in teatments with N deficiency without **(B)**; and with **(C)** Cl$^-$.

Cl$^-$ nutrition alleviates stress symptoms and maintains photosynthesis under N deficiency

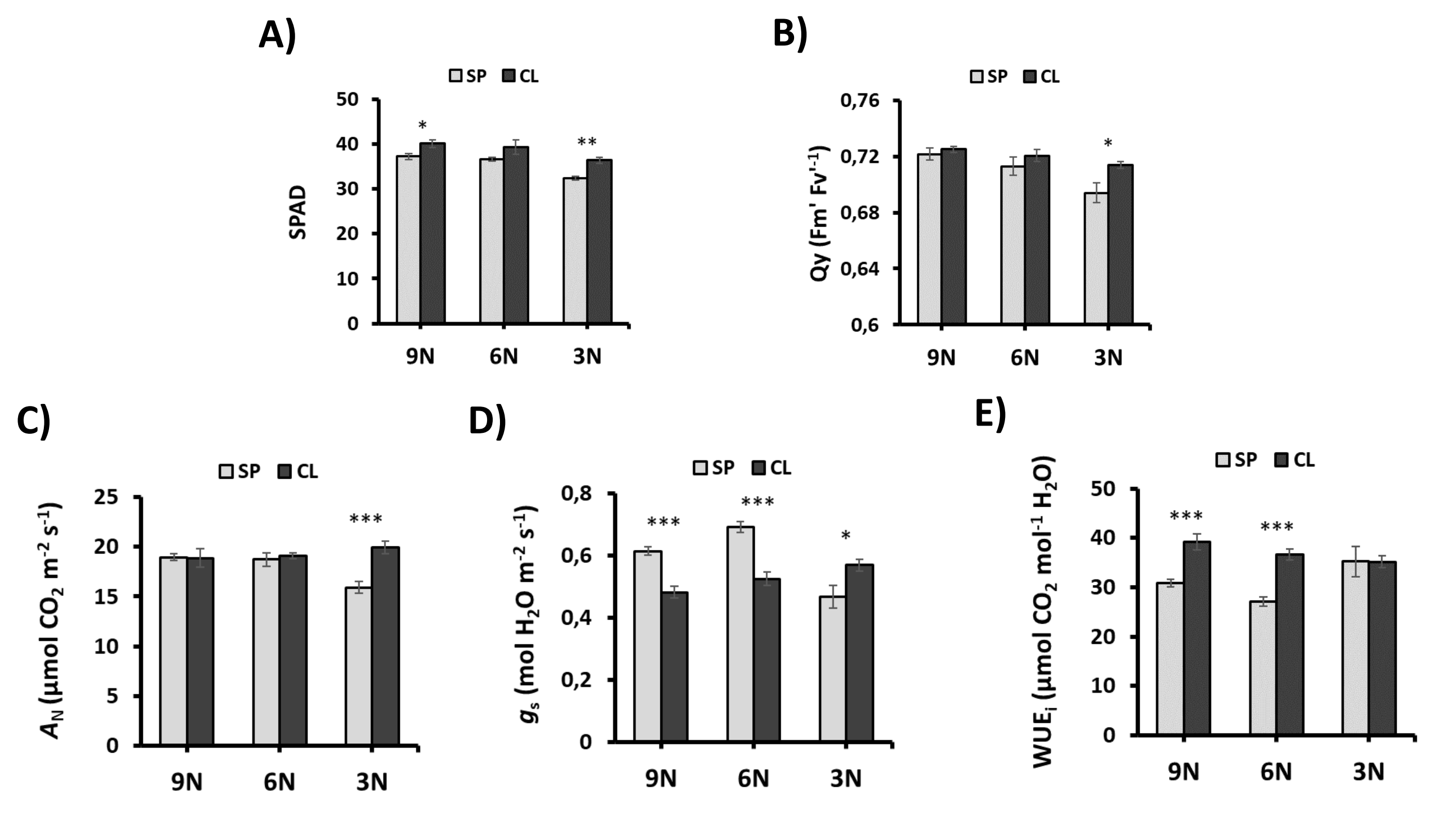

Fig. 5.: **Effect of Cl$^-$ and NO$_3^-$ treatments on gas exchange parameters, water-use efficiency, and non-invasive stress markers at 28 DAS.** Tomato plants were treated with the most relevant increasing concentrations of NO$_3^-$ and alternatively with two different treatments: SP (as control) and CL (5 mM)), maintaining the same balance of cations. Effect on **(A)** Stomatal conductance (g_s); **(B)** Net photosynthetic rate (A_N) and **(C)** Photosynthetic or instantaneous water-use efficiency (WUEi). Marker of plant stress that measures the amount of chlorophyll in leaves (SPAD) **(D)** and marker of plant stress that quantifies the efficiency of PSII (Qy) **(E)**, both measured in fully expanded photosynthetically active leaves.

Under N deficiency, Cl$^-$ allows to maintain or even increase water potential and turgor in leaves, retaining more water

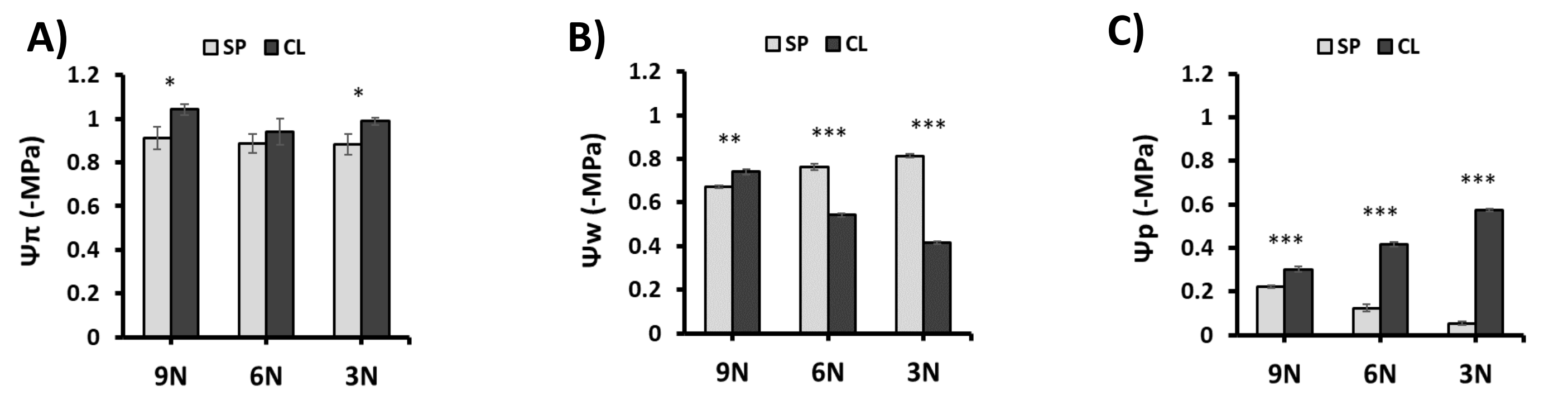

Fig.6.: **Effect of Cl$^-$ and NO$_3^-$ treatments on water status at 28 DAS.** Tomato plants were treated with the most relevant increasing concentrations of NO$_3^-$ and alternatively with two different treatments: SP (as control) and CL(5mM) , maintaining the same balance of cations. **(A)** Leaf osmotic potential (Ψπ)**;** **(B)** Leaf water potential (Ψw) and **(C)** Leaf turgor (or pressure) potential (Ψp).

CONCLUSIONS

- **Cl$^-$ nutrition increases growth and fruit production, achieving to reduce up to 3-times the application of NO$_3^-$ in the nutrient solution. In addition, the increase in the root:shoot ratio points to the appearance of stress symptoms in SP plants under low N supply, generating N deficiency stress.**
- **Plants treated with Cl$^-$ stimulate leaf area, especially during N deficiency, presenting larger stomata and epidermal cells size.**
- **Cl$^-$ nutrition promotes a reduction in g_s but similar A_N than SP plants during optimal N supply. Under N deficiency, SP plants manifested stronger stress symptoms compared to CL plants, showing a decrease in g_s, A_N, SPAD and Qy values and an increase WUEi.**
- **Cl$^-$ plants improve water retention (Ψπ) and turgor maintenance (Ψp) during N deficiency.**

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