CHLORIDE NUTRITION: NOVEL FUNCTIONS IN WATER RELATIONS

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ABSTRACT

Although Cl^- has been characterized as a micronutrient, we have observed that when available in the millimolar range (e.g. 1-5 mM), higher plants accumulate Cl^- to levels that are typical of the content of a macronutrient (Plant Cell Env. 2010, 33: 2012-27). Since this requires a considerable cost of energy, we speculate whether Cl^- might play a poorly understood function in plants when accumulated to macronutrient levels. Given that Cl^- is a major osmotically active solute in the plant vacuole, we propose that this element alter plant water relation mechanisms. Besides promoting plant growth and dry weight, we observed that chloride nutrition in the millimolar range improved water parameters like the relative water content, leaf succulence and water use efficiency. Under conditions of water deficit chloride-treated plants exhibited an improved regulation of the water balance and drought-tolerance. According to the data obtained, we propose that critical factors behind these phenomena are an improved osmotic regulation, a reduced transpiration and developmental alterations.

INTRODUCTION

Chloride (Cl^-) is defined as a micronutrient, having crop plants a critical requirement around 0.2 mg Cl^- g^{-1} leaf tissue dry weight (mg g^{-1}) (Marschner, 1995). As an essential micronutrient, it's involved in the regulation of several cellular functions like: the stabilization of the water splitting system of photosystem II; regulation of some enzymes activity; and electrical charge balance of essential cations involved in regulation of intracellular pH and the plasma membrane potential (Reviewed in White and Broadley, 2001). When Cl^- is available (e.g. 1-5 mM), despite being a micronutrient, it is actively taken and accumulated into leaf tissues to levels that exceed the critical requirement concentration by two orders of magnitude (Marschner, 1995; Brumós et al, 2010). Since this accumulation requires a very high cost of energy (Brumós et al, 2010), we propose that Cl^- fulfil a poorly understood role when accumulated to such high levels. It is known that Cl^- is a major osmotically active solute in the vacuole involved in both turgor and osmoregulation processes (Flowers, 1988). Chloride is particularly well suited to fulfil these roles given its relatively low biochemical activity and its high mobility in the short and long distance in plants (Maas, 1986; Xu et al, 2000; Hänsch and Mendel, 2009). We propose that, when accumulated to levels that are

typical of the content of a macronutrient, Cl^- may have an impact in osmoregulation and water relations in higher plants.

MATERIALS AND METHODS

Tobacco plants (*Nicotiana tabacum* L. var. Light Habana) were grown under greenhouse conditions at 24 ± 2 ºC / 17 ± 2 ºC (day/night), and a relative humidity of 60 ± 10%, and a 16h/8 h photoperiod. Tobacco seeds were sown in a mixture of peat and coarse sand in flat trays. After 3 weeks, seedlings were transplanted to 3.5 L pots containing a mix of perlite:coarse sand:vermiculite (2:3:5). The nutrient basal solution (**BS**) is a ¼ dilution of full strength nutrient solution (Arteca, 2000). **BS** contained sufficient residual Cl^- (50-70 µM) to fulfill essential requirements. Plants were subjected to three different nutritional treatments, based in the **BS** supplemented with: 1) a 5 mM Cl^- salt mixture (**CL**; with 2.5 mM KCl, 0.625 mM $MgCl_2$, and 0.625 mM $CaCl_2$); 2) a 5 mM NO_3^- salt mixture (**N**); and 3) a 3.125 mM SO_4^{2-} and PO_3^{3-} solution (**SP**). Both N and SP controls contained the same cations balance of the Cl^- treatment. Pots were subjected for 6 weeks to two irrigation treatments like: 1) up to field capacity (**Well-irrigated plants**) and 2) up to 50% field capacity (**Water Deficit**). Pots were weighted each week at field capacity to estimate the evolution of plants fresh weight (FW) over time. After 6 weeks (72 Days after seeding, DAS), plants were harvested, FW measurements were obtained, and then samples were dried in a forced-air oven at 75ºC for 48 h, and the dry weight (DW) was recorded as grams per plant. Dry tissues were ground for subsequently analyses. Leaf succulence, relative water content, and osmolarity was determined as Franco-Navarro et al., 2012. Quantum yield were determined as described in the instruction manual of the Fluorometer (FP100 Fluorpen photonsystem instrument, Drasov, Czech Republic). Turgency were recorded with the non-invasive, online-monitoring LPCP probe as described in Rüger et al., 2010 (ZIM Plant Technology GmbH, Hennigsdorf, Germany). A representative group of plants were used to obtain different parameters like: the loss of FW in detached leaves, and the analysis of abaxial leaf cells (Gitz and Baker, 2009). Cells count was done through the Counterall® software. Data compiled were submitted to an analysis of variance (ANOVA) and the differences between the means were compared by Tukey's range test ($P < 0.05$). Values represent mean of six tobacco plants in each treatment

RESULTS AND DISCUSSION

Compared to plants treated with the basal nutrient solution (BS), growth responded positively to the addition of supplementary salts (Fig. 1A). The positive effect on growth showed the following order: N>CL>SP (Fig. 1A), confirming our previous observation that chloride in the millimolar range stimulates plant growth (Franco-Navarro et al., 2012). Since N plants presented the lowest Cl^- content (not shown) and exhibited the highest growth and biomass values (Fig. 1A), we can conclude that N, BS and SP plants, were not experiencing

nutritional deficiency of Cl^- for those biochemical functions specifically played by Cl^- as an essential micronutrient. The possibility that this effect was due to the accompanying cations was ruled out since SP plants contained the same cations balance as CL plants. Under optimal irrigation, plant water consumption was lower in CL plants compared to N, SP and BS plants (Fig. 1B), indicating that Cl^- was inducing a more efficient use of water since it stimulated growth while reducing water use. In order to ascertain whether Cl^- could reduce leaf transpiration, fresh weight loss was measured in detached leaves. The reduced fresh weight loss confirmed a lower transpiration rate of CL plants (Fig. 2A). This phenomenon could be linked to leaf physiological variables, such as water and osmotic potential, relative water content and turgor (Kramer & Boyer, 1995). Since we hypothesized an osmotic effect of Cl^- nutrition on plant tissues, we measured the leaf tissue osmolarity. Cl plants exhibited the highest osmolarity, whereas BS plants exhibited the lowest one, having SP and N plants intermediate values (Fig. 2B). Accordingly, CL plants also showed the highest relative water content and succulence values (Fig. 2C and 2D). When subjected to water deficit, CL plants were able to keep higher leaf turgor values compared to SP and N plants (Fig. 3A). Furthermore, CL plants presented significantly higher quantum yield values along the water deficit treatment (Fig. 3B), indicating a more favourable preservation of PS-II integrity and, therefore, better drought tolerance. These and other parameters will be presented and discussed in the congress communication to explain how Cl^- nutrition can improve plant water relations under control and water deficit conditions.

CONCLUSIONS

When Cl^- is available in the millimolar range, tobacco plants take and accumulate this nutrient to values that are typical of the content of a macronutrient, increasing the osmolarity and relative water content of leaves, and probably stimulating its growth. The reduced water consume and increased water content of CL plants indicates that Cl^- must have an impact in the regulation of the plant water use, which is evidenced through the maintenance of a greater leaf turgor under water deficit conditions and higher drought tolerance.

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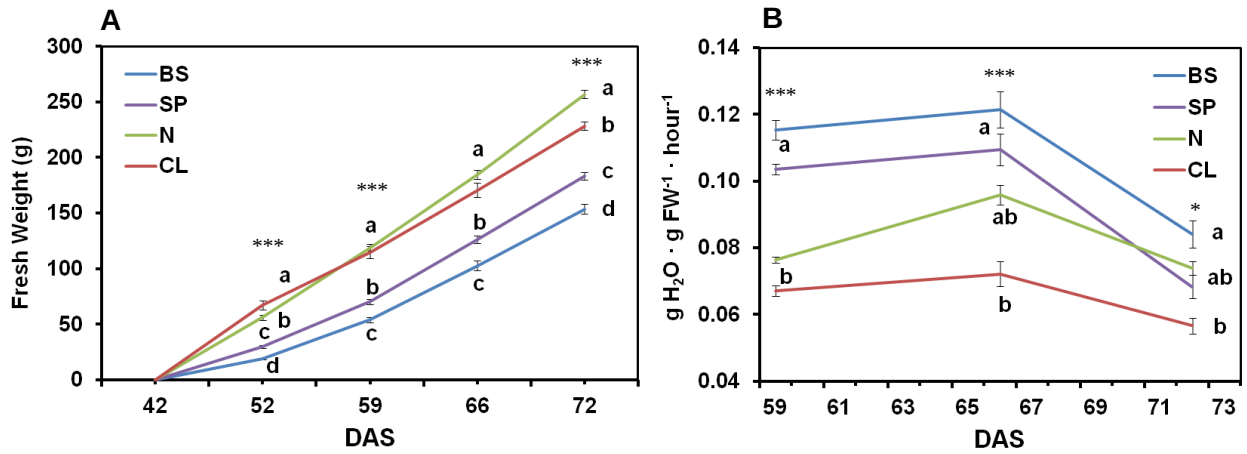

Figure 1. Growth and Water Use. (A) Evolution of plant fresh weight (FW) throughout the experiment; (B) Water consumed per hour and FW. Level of significance is represented by *** *P*<0.001 and * *P*<0.05.

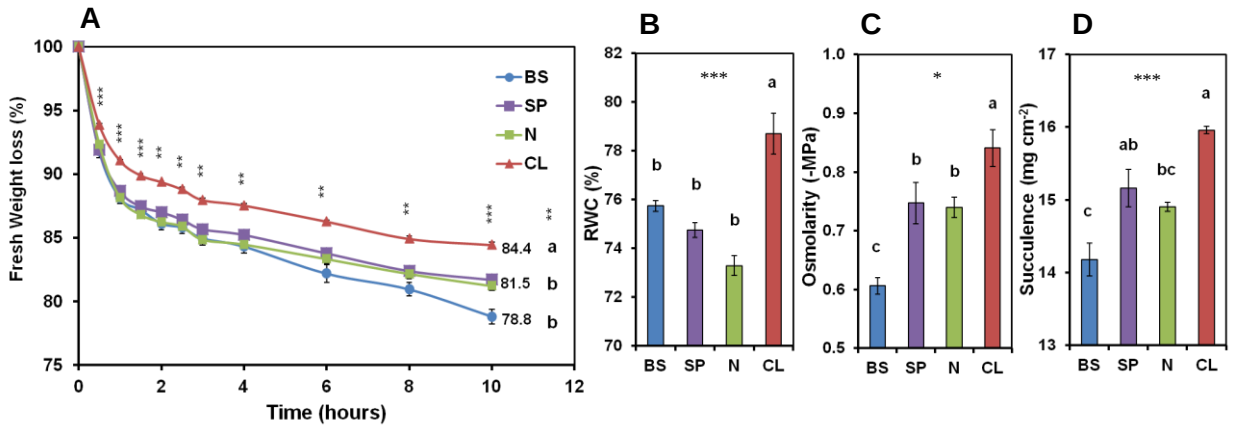

Figure 2. Water parameters under control irrigation. (A) Fresh weight loss in detached leaves; (B) Leaf Relative Water Content; (C) Leaf Osmolarity; (D) Leaf Succulence. Level of significance is represented by *** *P*<0.001, ** *P*<0.01, and * *P*<0.05.

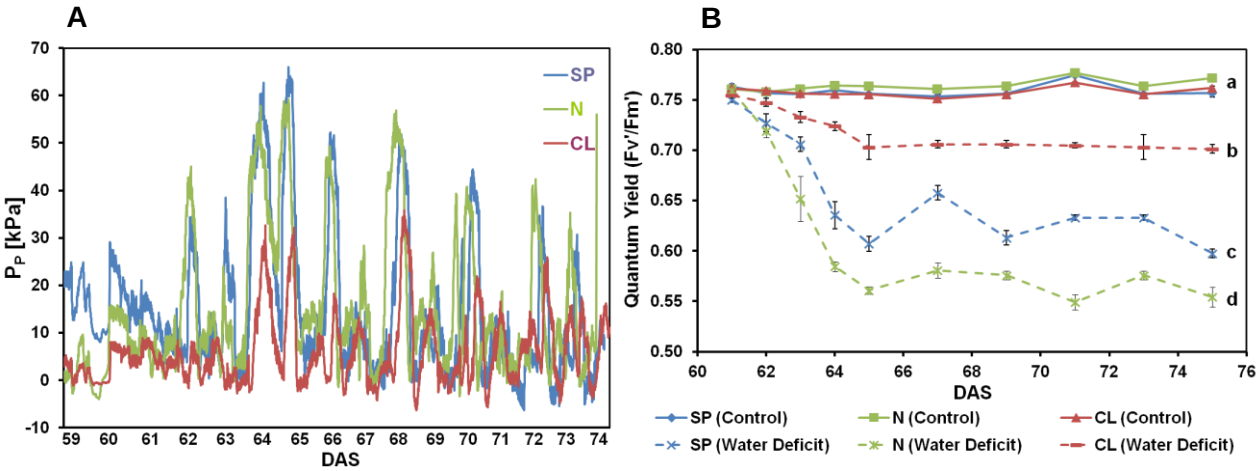

Figure 3. Water deficit resistance. (A) Leaf patch clamp pressure probe measurement (inversely proportional to the leaf turgor) registered during the water deficit period (50% field capacity); (B) PSII efficiency (Quantum Yield) recorded on control (field capacity) and water deficit conditions (50% field capacity). *P*<0.001.