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# FROM MICRONUTRIENT TO BENEFICIAL MACRONUTRIENT: THE IMPACT OF CHLORIDE ON NITROGEN METABOLISM

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## Abstract

Chloride ($Cl^-$) is an essential micronutrient recently defined as a beneficial macronutrient for plants, with new specific functions that improve the efficiency in the use of water (WUE), nitrogen (NUE) and $CO_2$. Nitrate ($NO_3^-$) and $Cl^-$ are the most abundant inorganic anions in plants, sharing physical and osmoregulatory properties, transport mechanisms, dealing to strong dynamic interactions between the two anions[1]. $Cl^-$ has been traditionally considered harmful for agriculture due to the belief of its competitive interaction with $NO_3^-$ and the toxicity generated by excessive Cl- accumulation in sensitive crops under salt-stress conditions[2,3]. However, when $Cl^-$ is accumulated in leaves at beneficial macronutrient levels (1-5 mM), the $NO_3^-$ content is reduced while plant growth and NUE increased[4]. Therefore, the objective of this work was to determine how $Cl^-$ nutrition affects nitrate accumulation by studying different nitrogen metabolic pathways.

To this aim, tobacco plants were grown under experimental greenhouse conditions and watered with a basal nutrient solution supplemented with 6 mM $Cl^-$ and a control treatment containing a mix of sulphate+phosphate salts (SP) to keep identical concentration of cations. After 60 days of nutritional treatments, mature tobacco leaves were collected and, subsequently, different nitrogen forms, key enzyme activities involved in nitrogen metabolism and protein content were spectrophotometrically analysed. In addition, a free amino acids profile was determined by HPLC.

Our results showed that the Cl- treatment stimulated plant growth and increased the content of total nitrogen and ammonium in leaves, whereas the content of nitrate was reduced. The analysis of enzymes showed increased activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT) and aspartate aminotransferase (AAT) in $Cl^-$-treated plants, supporting the $Cl^-$-dependent stimulation of nitrate assimilation.

Interestingly, the analysis of free amino acids content showed a reduction of aspartate, glutamate, glutamine, glycine and threonine, whereas serine and asparagine significantly increased. These results suggest the implication of other metabolic pathways, such as photorespiration or urea metabolism, in the $Cl^-$-specific response. Further studies are required to confirm the regulation of photorespiration by $Cl^-$, a pathway which is closely linked to $NO_3^-$ assimilation and protein synthesis in plants.

**Key words:** Chloride, Nitrate, Amino acids, Nitrogen metabolism, Tobacco.

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