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Nitrate and ammonium uptake and assimilation in radish (*Raphanus sativus* L.) grown under nitrate and ammonium co-provision

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Nitrate and ammonium are primary nitrogen sources for plants. The reduction of nitrate to ammonium requires substantial amounts of reducing equivalents. Therefore, ammonium should be considered the preferred nitrogen source for plant nutrition due to its lower energetic cost associated with its utilization. However, ammonium tends to be toxic to plants, especially when supplied as the sole nitrogen source, while the co-provision of nitrate alleviates ammonium toxicity, suggesting that an optimal balance between the two nitrogen forms is crucial for optimizing plant biomass and nitrogen assimilation.

Nitrogen mass balance performed on radish plants grown in $^{15}\text{N}\text{-NO}_3^-$ enriched hydroponic solutions containing 3.5 mM NO_3^- as the sole nitrogen source or $\text{NH}_4^+/\text{NO}_3^-$ combinations (1, 4, 8) with 3.5 mM final nitrogen concentration indicated that plants prioritize nitrate uptake over ammonium uptake. Conversely, under the most balanced ammonium to nitrate ratios ($\text{NH}_4^+/\text{NO}_3^- = 1$ and 4), the $^{15}\text{N}/^{14}\text{N}$ isotope ratio in plant proteins was significantly lower than that measured in total plant nitrogen, suggesting that adequate amounts of nitrate favored the preferential assimilation of the ammonium ions directly absorbed from the solution.

The effects of adequate nitrate supplies on the assimilation of ammonium directly absorbed by plant roots were further investigated by hypothesizing the involvement of plant nitrate reductase activity in this behavior. The study was conducted at a transcriptional level and included *in silico* analysis to identify radish genes encoding various nitrate reductase isoforms. The analysis led to the identification of three clones – designated as *RsNR1*, *RsNR2*, and *RsNR3* – whose expressions were analyzed in roots, hypocotyls, and leaves of plants grown under nitrate as the sole N source or nitrate and ammonium co-provision. The main results show that with increasing availability of ammonium, there is a reduction in the transcript accumulation of the most highly expressed nitrate reductase genes in roots and leaves. This effect may be directly related to the preferential assimilation of ammonium observed in various plant organs.

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