



Isotopic labelling reveals the efficient adaptation of wheat root TCA cycle flux modes to match carbon demand under ammonium nutrition

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Proper carbon (C) supply is essential for nitrogen (N) assimilation especially when plants are grown under ammonium (NH_4^+) nutrition. However, how C and N metabolic fluxes adapt to achieve so remains uncertain. In this work, roots of wheat (*Triticum aestivum* L.) plants grown under exclusive NH_4^+ or nitrate (NO_3^-) supply were incubated with isotope-labelled substrates ($^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, or [^{13}C]Pyruvate) to follow the incorporation of ^{15}N or ^{13}C into amino acids and organic acids. Roots of plants adapted to ammonium nutrition presented higher capacity to incorporate both $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ into amino acids, thanks to the previous induction of the NH_4^+ assimilative machinery. The ^{15}N label was firstly incorporated into [^{15}N]Gln via glutamine synthetase; ultimately leading to [^{15}N]Asn accumulation as an optimal NH_4^+ storage. The provision of [^{13}C]Pyruvate led to [^{13}C]Citrate and [^{13}C]Malate accumulation and to rapid [^{13}C]2-OG consumption for amino acid synthesis and highlighted the importance of the anaplerotic routes associated to tricarboxylic acid (TCA) cycle. Taken together, our results indicate that root adaptation to ammonium nutrition allowed efficient assimilation of N thanks to the promotion of TCA cycle open flux modes in order to sustain C skeleton availability for effective NH_4^+ detoxification into amino acids.

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