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^{15}N depletion in plant DNA: a heavy isotopic labelling experiment on Brassica napus L.

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In last decades, a large body of evidence clarified nitrogen isotope composition (δ ^{15}N) patterns in plant leaves and roots, showing isotopic fractionation along nitrogen absorption and assimilation pathways, in relation to N source and use efficiency. Nowadays, nitrogen isotope composition (δ ^{15}N), assessed by Isotopic Ratio Mass Spectrometry (IRMS), is often used in labelling experiments to investigate the occurrence of fractionation in metabolic reactions, which can cause substantial differences among different molecular N pools of leaves and roots. In a recent study on *Brassica napus*, substantial differences in δ ^{15}N among different nitrogenous metabolites (Chlorophyll, amino acids and DNA) were observed and related to both biochemical pathways and nitrogen sources, also suggesting a depletion of ^{15}N in DNA. In order to test such hypothesis, we set up a manipulative experiment on the same target species, with pre-germinated seeds potted in N-free sand substrate, fertilized using a modified Hoagland solution including ammonium nitrate as the only N source, supplied once in a total dose (470 mg N per plant) 14 days after germination. Three heavy labelling treatments, differing by the labelled chemical species ($^{15}NH_4^{15}NO_3$, $^{15}NH_4^{14}NO_3$, $^{14}NH_4^{15}NO_3$) but with the same ^{15}N/^{14}N ratio (δ ^{15}N = 2000 ‰) were considered, and obtained by mixing unlabeled (δ ^{15}N = 0.7 ‰) and labelled NH_4NO_3 (Cambridge Isotope Labs, labelled atoms 98% for all treatments) in the opportune ratios. Plants (1 plant per pot, 30 replicated pots per treatment, randomly placed and daily moved within the same growing room) were kept in controlled optimal conditions (T=22/20 °C day/night, photoperiod 12h, RH=50%, PAR 1000 μmol photons m^{-1} s^{-1}, watered every two-days to field capacity) for 120 days after treatments. Starting at day 60, six plants per treatment were harvested every 15 days, for a total of 5 sampling dates. At each destructive sampling, we separate root, stem and leaf materials of each plant, then assessing dry biomass, morphological traits, total N content (Elementar Vario Micro) and δ ^{15}N by IRMS (Elementar Isoprime 100) and extracted DNA from a 5 g fresh aliquot following a Doyle & Doyle modified protocol. Extracted, suspended DNA pellets were lyophilized and submitted to IRMS analysis for comparison to source materials. N concentration and δ ^{15}N of the fertilizer solution in each harvested pot was also assessed on solution samples collected by rhizosphere micro-sampler (Rhizon SMS, Ø 2,5 mm).

Results confirmed known dynamics of plant material labelling, strictly dependent on the different timing NH_4 vs. NO_3 absorption and transport. A remarkable isotopic fractionation in DNA, consistent across treatments and source material was observed. By an application perspective, our study not only confirms the possibility of using heavy isotopic labelling techniques to assess fractionation processes at detailed molecular level, but also, interestingly, suggest a possible sustainable approach to produce heavy labelled nucleic acids by labelled fertilizing coupled to direct massive extraction by classic molecular biology protocols. Pros and cons of such approach compared to in vitro synthesis by PCR-based protocols using labelled nucleotides as substrate are briefly discussed.