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Analysis of Nitrogenase Fe Protein Activity in Transplastomic Tobacco

Authors : Jose A. Aznar-Moreno, Xi Jiang, Stefan Burén, Luis M. Rubio

Abstract : Integration of prokaryotic nitrogen fixation (nif) genes into the plastid genome for expression of functional nitrogenase components could render plants capable of assimilating atmospheric N2 making their crops less dependent of nitrogen fertilizers. The nitrogenase Fe protein component (NifH) has been used as proxy for expression and targeting of Nif proteins within plant and yeast cells. Here we use tobacco plants with the Azotobacter vinelandii nifH and nifM genes integrated into the plastid genome. NifH and its maturase NifM were constitutively produced in leaves, but not roots, during light and dark periods. Nif protein expression in transplastomic plants was stable throughout development. Chloroplast NifH was soluble, but it only showed in vitro activity when isolated from leaves collected at the end of the dark period. Exposing the plant extracts to elevated temperatures precipitated NifM and apo-NifH protein devoid of [Fe4S4] clusters, dramatically increasing the specific activity of remaining NifH protein. Our data indicate that the chloroplast endogenous [Fe-S] cluster biosynthesis was insufficient for complete NifH maturation, albeit a negative effect on NifH maturation due to excess NifM in the chloroplast cannot be excluded. NifH and NifM constitutive expression in transplastomic plants did not affect any of the following traits: seed size, germination time, germination ratio, seedling growth, emergence of the cotyledon and first leaves, chlorophyll content and plant height throughout development.

Keywords : NifH, chloroplast, nitrogen fixation, crop improvement, transplastomic plants, fertilizer, biotechnology
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