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Review

The Chloroplast of *Chlamydomonas reinhardtii* as a Testbed for Engineering Nitrogen Fixation into Plants

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Supplementary Methods

1. Bioinformatic analyses

We performed an *in silico* study to look for potential *nifU* and *nifS* homologs in the *C. reinhardtii* nuclear genome. Similarity searches, using *K. oxytoca* NifU (WP_109213089.1) and NifS (CAB1215886.1) protein sequences as queries, were performed using PSI-Blast [1] against RefSeq databases; searches were limited to *C. reinhardtii* and excluded partial and predicted sequences. The NCBI CD-search tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and the ChloroP 1.1 predictor server [2] were used for conserve domain searching and subcellular localization prediction, respectively. Multiple sequence alignment was carried out using the CLUSTALW program [3]. Percentages of similarities between ChlL (ASF83651.1), NifH (VTS50234.1) and AnfH (SFW98605.1), were produced using the BLAST tool (https://blast.ncbi.nlm.nih.gov) [4].

2. pNifV chloroplast expression vector

The gene sequence encoding the homocitrate NifV from *K. oxytoca* (WP 023322951.1) was codon-optimised according to the codon bias found in the *C. reinhardtii* plastome (sequence given below) and cloned into the pSRSapI [5] vector using SapI and SphI sites. The resulting plasmid was named pNifV.

>crnifV: 1,176 bp

ATGATGGGTCGTGTATTAATTAACGATACTACTTTACGTGACGGTGAACAATCTCCAGGTGTAG
CTTTCCAAGCTTCAGAAAAAATTGCTATTGCTGAAGCTTTATACGCTGCTGGTGTTGAAGCTAT
GGAAGTAGGTACTCCAGCTATGGGTGAAGAAGAATGTGCTCGTATTCGTCAAGTTCGTCGTCAA
TTACCAGGTGCTACTTTAATGACATGGTGTCGTATGCAAGCTGGTGAAATTCGTCAATCAGCTG
ATTTAGGTATGGACTGGGTTGATATTTCTATTCCAGCTTCAGACAAATTACGTCAATACAAATT
ACGTGAAGGTTTACCATTATTATTAGAACGTTTAGCTGCTTTAATTCACTTAGCTCACACTTTA
GGTTTAAAAGTATGTATTGGTTGTGAAGATGCTTCACGTGCTTCTGATGCTACTTTACAAGATA
TTGCTCGTTTAGCTCGTGAAGCTGGTGCTACTCGTTTACGTTACGCTGATACTGTAGGTATTTT
AGATCCATTTACTACAGCTGCTCAAATTGCTGCTTTACGTCGTGTTTGGCCAGGTGAATTAGAA
ATGCACGCTCACAACGATTTAGGTTTAGCTACTGCTAACACTTTAGCTGCTGTTCGTGCTGGTG
CTACTTCTGTAAACACAACAGTATTAGGTTTAGGTGAACGTGCTGGTAACGCTGCTTTAGAAAC
AGTAGCTTTAGGTTTAGAACGTTGTTTAGAAGTTAAAACAGGTGTTCGTTTCACTGCTTTACCA
GCTTTATGTGAACAAGTAGCTTTAGCTACTCGTCGTCCAGTAGATCCACAACAACCATTAGTAG
GTGAATTAGTATTTACTCACGAATCAGGTGTTCACGTAGCTGCTTTATTACGTGACAGTGAATC
ATACCAAGCTATTGATCCAGCTTTATTAGGTCGTGGTTACCGTTTAGTTTTAGGTAAACACTCT
GGTCGTCAAGCTGTTAACGGTGTTTTTGACCGTATGGGTTACCACTTAACATCAGCTCAAATTG
ACCAATTATTACCAGCTTTACGTCGTTTCGCTGAAAACGGTAAACGTTCTCCACGTGATGATGA
ATTAGCTGCTATTTACCACGCTTTATGTTCAGCTGAAACTTTACAAGCTCGTGGTTACCCATAC
GATGTTCCAGATTACGCTTAATAA

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