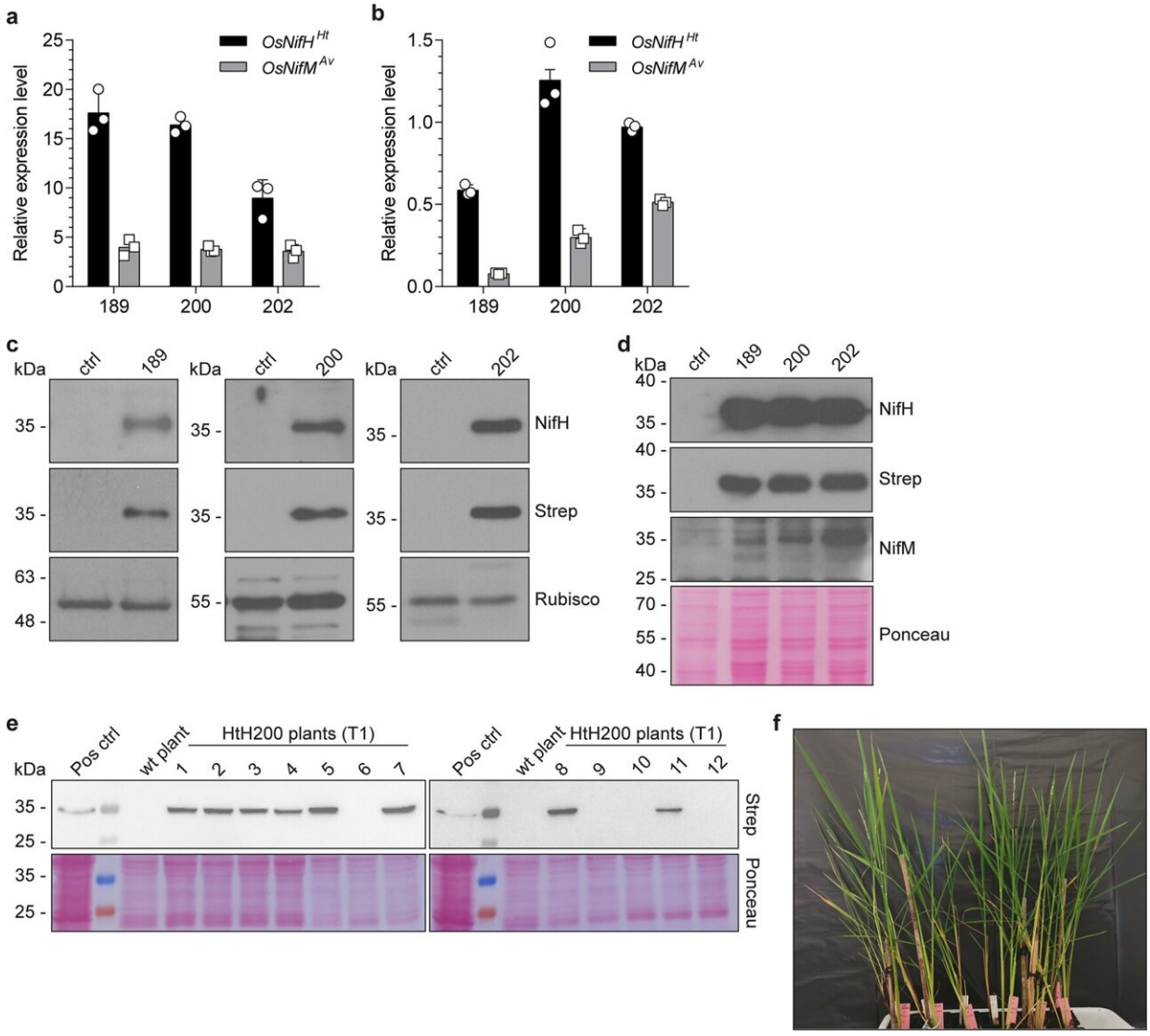
New advance in the biological fixation of nitrogen in rice

October 6 2022



Expression of rice-derived OsNifH^{Ht} and OsNifM^{Av}. Relative mRNA expression levels of OsnifH^{Ht} and OsnifM^{Av} in three different (independent biological

replicates) rice plant lines (a) and the corresponding callus lines (b). Data (normalized to OsActin mRNA) are means ± SD (n = 3 technical replicates). Immunoblot analysis of soluble protein extracts from rice leaves (c) and callus (d) probed with antibodies against NifH, NifM, and the Strep-tag. Antibodies against RuBisCO were used as loading control for plant lines. Ponceau staining was used as loading control for callus extracts due to the low expression of RuBisCO. Ctrl lane shows non-transformed callus and plant lines. e Stable expression of $OsNifH^{Ht}$ in the T1 segregating generation of rice plant line HtH200. Protein extract from callus expressing $OsNifH^{Ht}$ (line HtH206) was used as positive control (Pos ctrl). Uncropped immunoblots are shown in Supplementary Figs. 6–10. f Phenotype of $OsNifH^{Ht}$ expressing T1 progeny showing normal growth and development. Credit: *Communications Biology* (2022). DOI: 10.1038/s42003-022-03921-9

Researchers from the Center for Plant Biotechnology and Genomics (CBGP, UPM-INIA), in collaboration with the University of Lleida-Agrotecnio and the Catalan Institution for Research and Advanced Studies (ICREA), have succeeded in producing the first transgenic cereals that express two key components of nitrogenase, the enzyme that fixes atmospheric nitrogen by converting it into ammonia.

Each component was produced in a separate transgenic plant line and was shown to be biologically active in vitro or in living plants. These transgenic plants cannot yet fix their own nitrogen because additional components are needed to reconstruct the complete nitrogenase enzyme, but the work is nevertheless groundbreaking because it demonstrates for the first time that it is possible to express these highly oxygen sensitive proteins stably in plants, and that the proteins retain their activities.

Crops require nitrogen for growth and productivity because it is a major component of DNA, proteins, chlorophyll and energy-storage molecules such as adenosine triphosphate (ATP). Most crops depend on supplies of

nitrate and ammonium from industrial synthetic fertilizers, but more than half of these inputs remain unassimilated, spilling over or leaching into rivers and lakes as a major source of pollution.

Leguminous crops such as peas and beans harbor bacteria that convert nitrogen gas directly into ammonia using an enzyme called nitrogenase. This process is known as biological nitrogen fixation. The introduction of nitrogenase genes into crop plants would provide the machinery needed to fix nitrogen independently. However, the process is extremely complex because many different individual proteins are required not only as the direct structural components of nitrogenase but also accessory proteins needed for its assembly and the provision of energy. The major protein components are also extremely oxygen sensitive.

The researchers overcame this critical bottleneck by producing functional dinitrogenase reductase (Fe protein, NifH) and the nitrogenase cofactor maturase (NifB) in separate transgenic rice lines. Research on nitrogenase expression is usually conducted on laboratory model plants. However, by focusing on rice, an important staple crop that provides the main or only source of calories for more than 2.5 billion people in developing countries, the importance and impact of the results of the studies are substantially increased.

The project's principal investigator, Dr. Luis Rubio, says, "This is a major bioengineering advance as it tears down two technical roadblocks and shows the path to make nitrogen-fixing cereals." The achievement removes one of the major constraints hindering biological nitrogen fixation in crops and sets the stage for the assembly of a complete and functional nitrogenase complex in plants.

Further work to establish plants containing the full nitrogenase would have a lasting impact on global food security. Dr. Paul Christou, ICREA research professor and project lead at the University of Lleida-

Agrotecnio Center, says, "One of the major impacts of the work in the long term will be in low- and middle-income countries, which cannot afford expensive nitrogen fertilizers."

The related research has been published in *Communications Biology* and *ACS Synthetic Biology*.

More information: Can Baysal et al, Functional expression of the nitrogenase Fe protein in transgenic rice, *Communications Biology* (2022). DOI: 10.1038/s42003-022-03921-9

Wenshu He et al, Nitrogenase Cofactor Maturase NifB Isolated from Transgenic Rice is Active in FeMo-co Synthesis, *ACS Synthetic Biology* (2022). DOI: 10.1021/acssynbio.2c00194

Provided by Universidad Politécnica de Madrid