

From root to grain: multi-omics reveals how nitrogen, CO₂, and microbes tune wheat drought resilience and grain quality

Utpal Bose ⁽¹⁾ - **Jennifer Michel** ⁽²⁾ - **Claire Leon** ⁽³⁾ - **Cassandra Pegg** ⁽⁴⁾ - **David J. Beale** ⁽⁵⁾ - **Bhabananda Biswas** ⁽⁶⁾ - **Mohammad Mahmud Rahman** ⁽⁶⁾ - **Biswajit Biswas** ⁽⁷⁾ - **Shahida A Mitu** ⁽⁸⁾ - **Sally Stockwell** ⁽⁴⁾ - **Keren Byrne** ⁽⁴⁾ - **Frédéric Serre** ⁽⁹⁾ - **David Cormier** ⁽⁹⁾ - **Boris Adam** ⁽⁹⁾ - **Marie Leguillon** ⁽⁹⁾ - **Sibille Perrochon** ⁽³⁾ - **Michelle L Colgrave** ⁽¹⁰⁾ - **BIOFAIR Consortium** ⁽²⁾ - **Hervé Vanderschuren** ⁽¹¹⁾ - **Cécile Thonar** ⁽¹²⁾ - **Pierre Delaplace** ⁽²⁾ - **Angela Juhasz** ⁽¹³⁾ - **Jacques Le Gouis** ⁽³⁾

⁽¹⁾ CSIRO Agriculture and Food, Edith Cowan University, ARC CoE CIPPS, CHANCE, School of Science, St Lucia, Australia - ⁽²⁾ University of Liège, Plant Sciences, TERRA teaching and research centre, Gembloux Agro-Bio Tech, Gembloux, Belgium - ⁽³⁾ INRAE, UMR GDEC, Clermont-Ferrand, France - ⁽⁴⁾ CSIRO, Agriculture and Food, St Lucia, Australia - ⁽⁵⁾ CSIRO, Environment, Ecosciences Precinct, Dutton Park, Australia - ⁽⁶⁾ The University of Newcastle, Global Centre for Environmental Remediation (GCER), College of Engineering, Science and Environment, Callaghan Campus, Australia - ⁽⁷⁾ The University of Queensland, Institute for Molecular Bioscience, Brisbane, Australia - ⁽⁸⁾ Edith Cowan University, ARC CoE CIPPS, School of Science, Joondalup, Australia - ⁽⁹⁾ INRAE, UE PHACC, Clermont-Ferrand, France - ⁽¹⁰⁾ CSIRO Agriculture and Food, Edith Cowan University, ARC CoE CIPPS, CHANCE, School of Science, St Lucia, Australia - ⁽¹¹⁾ KULeuven, Laboratory for Tropical Crop Improvement, Crop Biotechnics, Faculty of Bioscience Engineering, Leuven, Belgium - ⁽¹²⁾ Université Libre de Bruxelles, Agroecology lab, Sciences Faculty, Bruxelles, Belgium - ⁽¹³⁾ Edith Cowan University, ARC CoE CIPPS, CHANCE, School of Science, Joondalup, Australia

Abstract

Plant's available nitrogen status, atmospheric CO₂ concentration and soil microbiome composition interact to modulate plant drought responses, yet their combined effects on grain yield and grain composition remain poorly characterised. Here, four bread wheat cultivars grown across two consecutive seasons (2022-2023) at the INRAE field-trial site in Clermont-Ferrand, France, under four treatment combinations - contrasting water supply and CO₂ levels with differing soil nitrogen levels to simulate current and future agroclimatic scenarios. Using quantitative and targeted proteomics, metabolomics and micronutrient composition analysis of the harvested grains, we investigated how water availability and atmospheric CO₂ concentration combinedly affect grain protein concentration and composition. We identified substantial treatment-driven changes in grain protein accumulation, with water availability as the primary driver, further modulated by CO₂ concentration and soil nitrogen availability in a cultivar-dependent manner. Drought stress primarily altered proteins associated with nitrogen allocation, osmotic adjustment and energy metabolism, indicating a shift in nutrient reservoir reserves toward grain filling. Elevated CO₂ levels, by contrast, shifted the plant carbon-nitrogen-sulphur balance, increasing proteins levels related to nutrient uptake and energy metabolism, prioritising plant growth over stress defence. In parallel, targeted metabolomics profiling of central carbon pathway metabolites revealed shifts in osmolyte and vitamin production, production and scavenging of reactive oxygen species and hormonal signalling, each closely associated with rhizosphere microbiome composition and nitrogen availability. Phenomics data, including root N and C levels measured across several developmental timepoints, heading and flowering dates, yield components, biomass partitioning, grain nitrogen and end-use quality measurements, were integrated with proteomics, metabolomics and microbiome profiling of the rhizosphere and bulk soil across all four conditions using a multiomics integration approach. The multi-omics integration analysis revealed cultivar-specific molecular signatures, indicating that protein-yield trade-off under drought stress is environmentally plastic rather than genetically fixed, mitigated by atmospheric CO₂, soil N levels and soil microbiome.