



## INSIGHTS INTO PEARL MILLET (*Pennisetum glaucum* (L.) R. BR.) PHENOTYPIC DIVERSITY BY PREDICTIVE METABOLOMICS

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Milletts are an alternative to major cereal crops, due to their nutritional value, gluten-free nature, and resistance to environmental challenges (e.g. low soil fertility, heat, and drought). Despite this, little is known about how the genetic diversity of pearl millet impacts physiological functions, specifically metabolic responses. Predictive metabolomics combines omics with artificial intelligence, particularly machine learning, allowing the comprehensive top-down modelling of various agronomical traits based on metabolic data. This study aimed to find predictive biomarkers of agronomic traits in pearl millet grains from the active germplasm bank (Brazil) using predictive metabolomics via machine learning. UHPLC-LTQ-Orbitrap mass spectrometry was used to analyze grains from 203 accessions of pearl millet (*Pennisetum glaucum* (L.) R. Br) from the core collection. The raw data were processed and annotated using MS-DIAL, resulting in 7790 RT-*m/z* features. After data curation, 2767 RT-*m/z* features were used to predict 21 agronomic traits using generalised linear models (GLM), and supervised machine learning. Linear models were constructed using the glmnet package. To train the models, 50% of the individuals were employed, and 50% were reserved for testing prediction quality. GLM successfully predicted eight qualitative agronomic traits from the pearl millet metabolome (accuracy > 70%). From this, the top metabolic predictors were established based on 80% occurrence in the models (624 known-ion features and 258 unknowns). Remarkably, 492 known-ion features were considered unique and classified by the subclass. The unique top metabolic predictors were mostly involved in specialized metabolism (54%), while 35% represented central metabolism. The most important subclasses were carbohydrates, amino acids, flavonoids, and terpenes. Given pearl millet resilience and its potential as a climate change-resistant cereal, this research holds significant implications for agronomy, nutrition, and health. In perspective, using the predictive biomarkers

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to confirm the most promising accessions, especially those related to grain production and human nutrition.

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