



**Identification of Marker for better Iron Acquisition in Arsenic
stressed Rice using Multinutrient Seed Priming Approach**

**Findings
Of the Ph.D. Thesis**

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FINDINGS

Plant immunity holds a central role in enabling crops to withstand environmental stresses. If such immune responses can be primed, plants will be better trained to endure future stresses. Among abiotic stresses, arsenic (As) contamination is particularly alarming due to its high toxicity in both humans and crops. The uptake of As in plants induces inhibition of physiological and metabolic processes, disruption of nutrient uptake, and reduction of yield. Compared to other crops, rice is more susceptible to As toxicity. In anaerobic paddy fields, iron (Fe) undergoes dynamic redox conversions that influence As availability, often creating a complex crosstalk between As-toxicity and Fe-uptake. Such conditions not only alter Fe dynamics but also enhance As solubility, thereby increasing its uptake and toxicity. Rice thus faces the dual challenge of As-contamination and Fe-deficiency, both of which severely limit its growth and productivity. To address this, we employed a multinutrient seed-priming approach aimed at preconditioning rice seedlings. We soaked the seeds in cocktail of nutrients containing Fe, Zn, Si and vitamin-C in pre-screened beneficial concentration. The seed soaking strategy not only imposed a mild osmotic stress but also developed an internal nutrient reservoir, endowing seedlings with a metabolic advantage right from the germination phase onwards, even under As-exposure. In addition, the seedlings were augmented with an optimized Fe-dose to unveil the As–Fe crosstalk under primed conditions. The preparedness of the primed seedlings was analysed through a increased suite of toxicity indicators, modulators ($\text{OH}^\cdot$, $\text{O}_2^\cdot$, H_2O_2), and antioxidant enzyme (SOD, CAT, POD, LOX, APX) activities even without As stress, over control. Beyond stress tolerance, the study further unraveled the energy dynamics of primed seedlings by exploring shifts in the tricarboxylic acid cycle (TCA) enzymes (IDH, SDH, MDH, citrase) and γ -aminobutyric acid (GABA) pathway functioning (GDH, GAD, GABA-T), along with their coordination with nitrogen–phosphorus (NiR, NR, GOGAT, GDH, acid phosphatase, alkaline phosphatase) metabolism and sugar–starch (SPS, SS, β -amylase) fluxes. These acclimatization revealed that priming treatment activated both energy sources *viz.*, TCA and GABA, which assisted seedlings in not only exhibiting enhanced

As resilience but also improving the primary metabolism which supports the elevated seedlings growth. The adjustments were also closely positioned with Fe-metabolism, including Fe-dependent enzyme activities, phytosiderophore release rate, and transcript accumulation of genes in the phytosiderophore biosynthesis route. To attain a holistic understanding, global transcriptomic profiling was executed, highlighting stress memory–associated alterations and signalling molecules. Differentially expressed genes mostly belong to the category of transporters, signalling factors, and defensive molecules. Special emphasis was laid on Fe-related transcriptional regulators, which identified *OsTOM* as a candidate biomarkers for Fe-surplus rice under As stress. Parallely, the whole module of wall–associated kinases (WAKs) and mitogen-activated protein kinases (MPKs) also exhibited differential regulation. Protein–protein interaction analyses delineated a phosphorylation mediated association of OsWAK24 and OsMPK3 that facilitates rapid sensing of As contamination and robust activation of defence cascades. Overall, the results demonstrate that multinutrient priming, coupled with Fe augmentation, effectively boosts rice resilience while maintaining growth and mitigating As toxicity. The integrated transcriptomic and signalling perspectives provide potential molecular targets for engineering a Fe-enriched rice variety with enhanced tolerance to As-contaminated environments.