

Rice is an important food staple and is the main source of nutrients that sustains a large proportion of the world's population. Rice is rich in carbohydrates but otherwise regarded as a relatively nutrient poor cereal staple, especially after processing by polishing to remove the outer grain layers. Polishing removes a range of nutrients that are concentrated in the bran, such as iron (Fe), zinc (Zn), protein and lipids. In addition, nutrient bioavailability can be compromised by the presence of antinutrients, such as phytate, which binds tightly to mineral ions. Nutrient poor diets can contribute to a range of illnesses that are most severe in vulnerable populations in the developing world.

Sucrose, produced by the plant through photosynthesis, is the major assimilate transported in the phloem of rice plants. The phloem is also the long distance transport pathway for other assimilates. At maturity, sucrose transported into the grain is converted into various storage products as future nutrient reserves for the growing embryo. In the rice endosperm, sucrose is mainly converted to starch, however it is also involved in the biosynthesis of other nutrients such as proteins and fatty acids. In addition, minerals such as Fe and Zn, that are taken up from the soil are also transported via similar pathways as sucrose and deposited along with storage compounds in the grain. Therefore, nutrient loading into the seed is a crucial phase in plant growth and the seed becomes a strong sink competing for available nutrients. Sucrose transport in plants is facilitated by sucrose transporter proteins, SUTs, which are highly expressed in the grain aleurone layer and have important roles in assimilate uploading during grain filling.

This study investigated the hypothesis that increasing assimilate uptake into rice grains through overexpression of the barley sucrose transporter *HvSUT1* could change the nutrients in rice. To test this hypothesis, transgenic rice plants (*Oryza sativa* L. cv. Nipponbare) overexpressing *HvSUT1*, driven either by the constitutive rice *Actin-1* ( $Act_{pro}$ ) or endosperm specific rice *Globulin-1* ( $Glb_{pro}$ ) promoter, were generated using *Agrobacterum*-mediated-transformation. These constructs were designated as  $Act_{pro}::HvSUT1$  and  $Glb_{pro}::HvSUT1$  respectively. A number of independent stably transformed plant lines were confirmed by molecular and biochemical assays. Transgene expression was verified by RT-PCR and immunoblotting, which showed that the  $Glb1_{pro}$  drove stronger *HvSUT1* expression in the grain, compared to  $Act_{pro}$ . Transgene function was analysed by [ $^{14}C$ ]-sucrose uptake assays which suggests that transgenic rice grains driven by both promoters had prolonged active sucrose uptake at later stages of grain filling, compared with non-transgenic (NT) grains. Furthermore, grains expressing

HvSUT1 specifically in the endosperm had increased sucrose uptake significantly at 7 days after anthesis (DAA), a crucial time point in grain development.

Grain nutrient composition and distribution was altered in rice grains expressing HvSUT1, compared to the non-transgenic (NT) line, with more significant differences observed in the composition of grains transformed with  $Glb_{pro}::HvSUT1$  compared to  $Act_{pro}::HvSUT1$ . Elemental analysis by Inductively Coupled Plasma Spectrometry (ICP-OES) revealed that transgenic rice grains expressing HvSUT1 had a greater retention of Fe and Zn in the endosperm compared to NT grains. LA-ICP-MS imaging confirmed that elements in the transgenic grains had been redistributed in transgenic rice, with higher counts and greater distribution of micronutrients in the endosperm. Sucrose content increased but uniquely, potassium (K) retention decreased in the endosperm of transgenic grains, which could be explained if the transport processes for these two grain nutrients were linked, possibly as osmotic species involved in cell membrane polarity and/or maintaining cell turgor. A decrease in the soluble starch content, but increases in amino acids and phytate suggested that HvSUT1 overexpression had affected metabolic processes within the grain, leading to changes in the relative proportions of storage products. In addition, the composition of storage proteins and lipids that are also downstream products of sugar metabolism, were modified in rice grains overexpressing HvSUT1.

Overall, the changes in nutrients in the transgenic rice grains support the proposal that increasing sucrose transport can change the nutrient composition of rice and can modify metabolic processes that are responsive to carbon supply. Significantly, in the present study, transgenic grains retained more of the important micronutrients Fe and Zn, in the starchy endosperm. Furthermore, other changes in grain composition suggest that SUT overexpression could be a strategy to improve the nutritional quality of rice. The mechanisms for the changes are yet to be investigated, however, the findings from this study contribute to the understanding of the complex interactions between assimilate partitioning and nutrient deposition in cereal grains. This can aid in future efforts to breed more nutritious staple crops to alleviate nutrient deficiencies in developing countries.