Although rice supplies over half of the global food requirement, its relatively low micronutrient content presents as a "hidden" malnutrition amongst many people, mainly in developing countries. Biofortification of crops is a way to address this malnutrition-inducing micronutrient deficiency, and rice is an excellent candidate for this approach. However, in cereals like rice, there is often a negative correlation between micronutrient content and yield, making the combination of these two important traits a great challenge for breeders.

Sucrose, produced by plants through photosynthesis, is an important nutrient, which does not only impact yield, but is also involved in a source-sink communication network. A balance between nutrient utilisation in the sink tissues, and nutrient synthesis in source tissues is tightly coordinated; thus, any enhancement of sucrose demand in the sink could stimulate its translocation from the source. Targeted overexpression of sucrose transporters provides a promising method to enhance the uptake capacity and partitioning of sucrose, resulting in increased sink strength. Such a strategy was first reported in wheat by Weichert *et al.* (2010), where the endosperm-specified overexpression of the barley sucrose transporter (*HvSUT1*) led to an increase in seed storage protein synthesis. Of great interest was that higher micronutrient levels were also found in the grain of these transgenic plants (Saalbach *et al.* 2014). A similar strategy used in the Japonica rice cultivar, Nipponbare, found similar results (Huynh 2015), although the formation of insoluble Fe/Zn complexes with phytate resulted in their limited translocation, especially Fe, into the inner endosperm.

Nicotianamine (NA) and 2'-deoxymugineic acid (DMA) are two natural chelators of metal cations, including Fe and Zn. In rice, NA is synthesized by the enzyme, nicotianamine synthase (NAS), which is a product of three *OsNAS* genes (*OsNAS1*, *2* and *3*). Therefore, what is hypothesised here, is that a novel combination of endosperm specific expression of *HvSUT1* and *OsNAS2* will drive more sucrose and micronutrient loading into the grain, and the

availability of an alternative chelator in NA, will increase the translocation of Fe/Zn complexes into the inner endosperm.

To test this hypothesis, research was conducted in three main steps. First, along with single gene constructs of *HvSUT1* and *OsNAS2*, a novel combination of *HvSUT1* and the rice nicotianamine synthase gene (*OsNAS2*) was introduced into Indica rice (cv. IR64) by *Agrobacterium*-mediated transformation. Two promoters, *Glb-1* and *GluA2* cloned from Nipponbare rice, were used to drive the endosperm specific expression of *HvSUT1* and *OsNAS2*, respectively. Multiple homozygous one- or two-insert transgenic lines were identified by quantitative real-time PCR. Also, a *GluA2::uidA* fusion construct was made and transformed into IR64 to confirm the tissue specific expression of the *Glu2A* promoter.

Second, immature grains from T2 plants were used in immunoblot analyses to test tissue specific and temporal expression of the transgenes during grain filling. OsNAS2 was expressed in the rice grain at the highest level 5 days after anthesis (DAA), and then decreased until 15 DAA. HvSUT1 was expressed from 5 DAA to 15 DAA and reached its highest level of expression at approximately 10 DAA. An unexpected vigorous growth phenotype was found in transgenic IR64 rice overexpressing *HvSUT1*, with or without *OsNAS2*, during the vegetative growth phase, but as yet no definitive results have been found for the resulting yield in these transgenic plants. Such a phenotype was not reported by Huynh (2015) in *HvSUT1* transgenic Nipponbare rice.

Finally, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to measure micronutrient concentrations in the grains of the transgenic rice. Fe increased by approximately 100 % and around 30% for Zn, Cu and Mn in the polished grain overexpressing a combination of *HvSUT1* and *OsNAS2*. Histochemical analysis of Fe distribution using Perls/DAB/$CoCl_2$ staining, and Zn distribution using DTZ staining, revealed striking differences in the

distribution and intensity in both dorsal and ventral sides of *HvSUT1+OsNAS2* transgenic grain, not visible in the grains of the non-transgenic and the single construct transgenic lines. Furthermore, based on the molar ratios of phytate to Fe and Zn, micronutrient bioavailability may be improved in these transgenic seed.

In general, these data indicate a positive correlation between HvSUT1 protein levels and the uptake of micronutrients during grain filling in IR64 transgenic rice. The combination of *HvSUT1* and *OsNAS2* expression showed remarkable changes in Fe accumulation and distribution in the grain that may exist in a form more available for uptake in the human gut. A vigorous growth phenotype of the transgenic rice overexpressing *HvSUT1* was an unexpected outcome of this study and is worthy of further investigation. These results are reported in the context of the literature and some speculative hypotheses are raised to stimulate and foster further experimentation.