論文の内容の要旨

生産・環境生物学専攻
平成 26 年度博士課程入学
氏名　モレ　シャミタ　ラオ
指導教員名　山岸　順子

論文題目　Studies on the roles of vacuolar invertase genes in sugar metabolism of rice and sorghum
（イネおよびソルガムの糖代謝における液胞型インベルターゼ遺伝子の役割に関する研究）

In plants, vacuolar invertases (VINs) are proposed to play a role in sugar storage, osmoregulation and response to cold stress.　In the past, VINs have been attributed to roles in cell elongation of seedling hypocotyls in Arabidopsis, fiber cell elongation in cotton, and rapidly expanding tissues in carrot taproot and sugar beet petioles. In addition to its role in facilitating sink expansion, VINs have also been found to play a major role in early plant development in carrot and Arabidopsis. Through its regulation of composition of sugars, it has been found to play a key role in fruit ripening in tomato, and the regulation of stomatal opening in Arabidopsis. Many studies also report the induction of VINs under cold stress. In potato, the unfavorable cold-induced sweetening was attributed to a VIN gene, suppression of which was found to prevent this characteristic. In Arabidopsis, VIN activity was demonstrated to be key in stabilizing the central energy supply under cold stress and combined cold/high light conditions. Contribution of VIN in sugar accumulation in sugarcane and sweet sorghum stems have also been identified. Thus, the involvement of VINs in key physiological functions governing plant development, sink expansion, cold response and sugar accumulation deems its characterization valuable, in agronomic and physiological perspectives likewise.

The current study focusses on isolating key physiological and functional roles of VINs in two major crops, rice and sorghum. This study was undertaken in view of clarifying the contribution of VINs in sugar metabolism in these crop species, mainly with a broader goal of achieving higher sink

strength, in terms of grain yield and stem-sugar yield in rice and sorghum respectively.

1. <u>Mutant analysis of two vacuolar invertase genes, *OsINV2* and *OsINV3* in rice</u>

   Two rice vacuolar invertases, *OsINV2* and *OsINV3*, were assessed for various physiological and agronomic traits using *Tos-17* retrotransposon mutants (KO). In the seedling stage, *OsINV3* was found to regulate the dry matter production, wherein the KO seedlings possessed lower shoot and root dry weights, with observed recovery in the complementation lines. In the reproductive stage, *OsINV3* KO showed shorter panicles with lighter and smaller grains, owing to a smaller cell size on the outer and inner surfaces of the palea and lemma as observed by scanning electron microscopy. Further, a strong *promoter::GUS* expression was observed in the palea, lemma and the rachis branches in the young elongating panicles, which supported the role of *OsINV3* in cell expansion and thus, in spikelet size and panicle length determination. Assessment of field grown KO not only revealed a drastic reduction in the percentage of ripened grain, 1000-grain weight and final yield, but also significantly lower partitioning of assimilates to the panicles, whereby the total dry weight remained unaffected. Determination of the non-structural carbohydrate contents revealed a lower hexose-to-sucrose ratio in the panicles of the mutants from panicle initiation to 10 days after heading, a stage that identifies as the critical pre-storage phase of grain filling, whereas the starch contents were not found to be affected. In addition, a strong *promoter::GUS* expression was observed in the dorsal end of ovary during the pre-storage phase until 6 days after flowering, highlighting a function for *OsINV3* in monitoring the initial grain filling stage. Thus, *OsINV3* was found to be a sink strength determinant in rice, mainly by its role in cell expansion, thus, regulating the spikelet size, and driving the movement of assimilates for grain filling by modulating the hexose-to-sucrose ratio, contributing in grain weight determination and thus, the grain yield.

   Field grown *OsINV2* KO showed a higher total sugar content in the leaves, stem and culm at harvest, a phenotype that couldn't be confirmed by complementation, further supported by its lack of expression at this stage in the *promoter::GUS* lines. However, studies with heterozygous lines of *OsINV2* could shed more light on its role in regulating the stem sugar levels in the field conditions. *OsINV2* and *OsINV3* were found to have contrasting patterns of *promoter::GUS* expression in the development of panicles, with *OsINV2* being expressed in the early initiation stages, and *OsINV3* being expressed in the critical grain filling stage and the stages before heading.

Thus, we isolate *OsINV3* as a key functional VIN in rice, with critical physiological roles that leads to a compromise in grain yield upon its failure.

2. <u>Role of *OsINV3* in cold stress response during the seedling stage in rice.</u>

   The *OsINV3* WT, KO and the complement lines were tested for recovery following exposure to 4°C, and the WT seedlings showed a higher survival rate when compared to the KO, with an observed recovery in the complement lines. This isolated a key role for *OsINV3* in cold stress recovery in rice seedlings at the 3-leaf stage. Further, acclimatization studies were carried out to establish contrasting survival rates between the WT and KO post-cold stress at 4°C following acclimatization at 12°C. The WT showed greater signs of recovery in comparison to the KO. Estimation of *OsINV2* and *OsINV3* transcript levels revealed a higher transcript level of *OsINV3* during acclimatization, as were the levels of sucrose and hexoses.

   The up-regulation of *OsINV3* in cold-stressed seedlings outlined a possible regulation by ABA, a stress hormone, and sugars, that were found to be accumulated in the cold stressed seedlings. *OsINV3* was found to be strongly up-regulated by ABA within 1.5 hours after treatment (HAT) showing highest transcript levels at 24 HAT, a time-point where maximum *OsINV3* transcript levels in the 12°C treated seedlings was observed. *OsINV3* was found to be strongly up-regulated by glucose, following initial osmotic potential changes until 3 HAT, following which a sharp down-regulation was observed. It showed a strong initial down-regulation by sucrose at 1.5 HAT following which no differences were observed.

   Thus, a strong response of *OsINV3* to ABA and glucose was attributed to be a key finding in demonstrating a mechanism for *OsINV3* induced cold acclimatization, and thus cold tolerance.

3. <u>Role of vacuolar invertase genes, *SbINV1* and *SbINV2* in sugar accumulation in sorghum stems.</u>

   The sugar accumulating potential of global and local sorghum varieties were tested under local conditions. The basis for this study was the dependency of sugar accumulation on temperature and photoperiod, thus creating a necessity to test the performance of the cultivars under local conditions to assess its sugar accumulating potential, thus its efficacy as a bioenergy source.

A strong correlation of sucrose content with brix was established, enabling the large-scale screening of varieties for high sucrose content. There was no major trend observed in terms of starch content in the varieties with respect to their sugar accumulating potential. The morphological characteristics inherent to sweet sorghum, such as, tall stems, greater number of leaves and a longer vegetative period were found to correlate with the stem sucrose content. Assessment of sugars along the stem revealed, maximum sugar accumulation in the intermediate to upper internodes in most of the varieties tested. The Maximum Theoretical Ethanol Yield (MTEY), which was a function of brix and juice volume was isolated as a better indicator of testing the performance of a variety as a potential source of bioethanol, mainly due to a negative correlation of stem juiciness with sucrose content in the varieties tested. Further, the relative expression of *SbINV1* and *SbINV2* revealed a strong negative correlation of *SbINV2* to stem sucrose content, thus isolating it as a key candidate for molecular breeding studies.

In conclusion, the current study isolated key physiological roles for VINs in rice, in regulation of

- Sink size, due to role of *OsINV3* in cell expansion in the spikelet, mainly driven by its osmotic dependence and regulation by glucose.
- Assimilate partitioning to sinks, by *OsINV3* regulation of hexose-to-sucrose ratio during the critical pre-storage phase.
- Cold acclimatization and recovery, owing to *OsINV3* regulation by ABA and sugars,

and, in determination of the sucrose accumulating potential in sweet sorghum, owing to differential expression of *SbINV2,* demonstrating a strong negative correlation with sucrose content, among various sweet sorghum varieties.