DIFFERENTIAL EXPRESSION OF CHOLINE MONOOXYGENASE TRANSCRIPT DETERMINES THE PLANT TO BE GLYCINE BETAINE ACCUMULATOR OR NON-ACCUMULATOR

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INTRODUCTION

Globally, salinity is an important environmental problem, because salt affect about 831 million ha and is continually increasing (Martinez-Beltran and Manzur, 2005; Wang et al., 2003). In India, approximately 20% of the cultivated land is salt affected (Saha and Gupta, 1998). Salt stress induces various complex biochemical, molecular, cellular and physiological changes in plants and affects almost all plant processes in all stages of development. In saline soils Na\(^+\) and Cl\(^-\) are dominant ions. High concentration of these ions in soil decreases the osmotic potential of the soil solution, thereby lowering the availability of water to plants (Lloyd et al., 1989; Maeda and Nakazawa, 2008). These also interfere with the assimilation of other essential nutrients resulting in nutrient imbalance (Garcia-Sanchez et al., 2002, Yang et al., 2008) and ion homeostasis (Zhu, 2003; Shabala and Cuin, 2008). It also destabilizes the cellular membranes by displacing the K\(^+\) and Ca\(^{2+}\) (Grattan and Grieve, 1992) and affects their permeability (Dogan et al., 2010). Excess of Na\(^+\) and Cl\(^-\) ions may lead to conformational changes in the protein structure. Salinity can reduce photosynthetic activity by destruction of green pigments, lowering the leaf area or by decreasing the activity of photosynthetic enzymes in calvin cycle (Misra et al., 1997; Abdelkader et al., 2007; Chaves et al., 2009). It is also associated with an increase in reactive oxygen species (ROS) which thereby accelerate toxic reactions like lipid peroxidation, protein degradation and DNA mutation (Jaleel et al., 2007; McCord, 2000). Salinity tolerance is a tightly regulated phenomenon, brought about by adaptations in a range of physiological processes, including ion homeostasis, osmolyte biosynthesis, compartmentation of toxic ions, reactive oxygen species (ROS) scavenging systems and protein turnover for cellular repair (Munns and Termaat 1986; Hasegawa et al., 2000; Mittova et al., 2004; Stepien and Klobus, 2005; Flowers and Colmer, 2008). GB is important because it can confer tolerance to several types of stress by stabilizing protein, maintaining membrane integrity, protecting various components of photosynthetic machinery and scavenges ROS either after application to plants or in transgenics engineered to overproduce GB (Hoekstra et al., 2001, Rontein et al., 2002, Rhodes and Hansen, 1993, Sakamoto and Murata, 2000). To date, there are no reports of GB-accumulating transgenic plants that exhibit undesirable traits when grown under non-stress conditions rather show enhanced yield (Park et al., 2007). Plants have evolved highly integrated sensing and response signaling pathways that regulate developmental processes during salt stress (Tran et al., 2007). The mechanisms of salt tolerance are not yet clear. The elucidation of salt tolerance mechanisms, therefore, is an important issue and has attracted considerable interest in recent years. The aim of this study was to find the hindrance step of GB biosynthesis in plants. Here we study, the expression of transcripts of enzymes that involve in GB biosynthesis such as PEAMT, BADH and CMO in salt tolerant and sensitive plants.

MATERIALS AND METHODS

Plant materials and growth conditions
Two Indica rice *O. sativa*, cv. Lunishree and *O. sativa*, cv. Naveen and two natural halophytes *S. maritima* and *P. coarctata* were used in this study. Seeds of Lunishree and Naveen were obtained from Orissa University of Agriculture and Technology, Bhubaneswar and Central Rice Research Institute, Cuttack. Suaeda seeds were collected from the coastal belt of Odisha and plants of Porteresia from Sundarban mangroves of West Bengal. All plants were grown in growth chamber of maintained temperature at 24 ± 3°C, relative humidity 70–75%, with 14h light (200 μmol m⁻² s⁻¹) and 10h dark periodicity. The surface-sterilized seeds of rice varieties were soaked in de-ionized (Milli-Q) water over night transferred over wet filter paper in a petriplate and kept in room temperature for 36h. Germinated rice seeds were grown in growth chamber hydroponically over nylon nets in 500mL capacity plastic pot containing half-strength Hoagland's solution. The individual pots were watered every day alternately with approximately 50mL of 1/10th Hoagland’s solution or Milli-Q water except on the penultimate day of the stress application. The surface-sterilized seeds of Suaeda were directly shown to pot containing sterilized soil in growth chamber. After four weeks, the seedlings were transferred to soil in pots of 500cc volume and set to acclimatize and grow for eight weeks. During this period, individual pots were watered everyday with Milli-Q water except on the penultimate day of the treatment. The plants of Porteresia were planted in 5000cc pot in growth chamber for six week to adopt in non saline environment. After six weeks a tiller was transferred to another 5000cc pot and allowed to grow for eight week. At this time new tillers were produce. This process is done at least three times before used for experiment. Finally one tiller was allowed to grow in 500cc of plastic pot containing sterile soil.

**Stress application**

After 10 days, the rice seedlings in individual beakers were treated with 1.5% (7.5g for 500mL) of NaCl. For this, initially 2.25g NaCl was dissolved in 15mL of 1/10th strength Hoagland’s solution and was poured into individual beakers. After incubation for 1h in dark, another 100 mL of solution containing 3.5g NaCl was poured into the pots, increasing their final NaCl treatment concentration 2.5% (in 150mL treatment volume) to about 2.5%. It was determined earlier that 100mL water was totally absorbed by soil in the pots, while additional 50mL was partly absorbed and rest in under the soil. Controls were applied with the same volume of Milli-Q water.

**RNA isolation and cDNA preparation**

Leaves of the seedlings/plants were harvested after 6h of final treatment for RNA isolation. Total RNA was isolated from the leaves of control and NaCl exposed plants following the LiCl method (Sambrook et al., 1989) and treated with RNase-free DNase (Promega, USA) to remove any DNA contamination. The quality and quantity of the RNA in each preparation was checked spectrophotometrically using Nano Photometer (Implen, Germany). First-strand cDNA of Suaeda was synthesized from 1μg of total RNA using the ImProm- II Reverse Transcription System (Promega, USA). The cDNA sample was diluted five times for PCR use.

**PCR amplification**

PCR amplification of desired genes fragment of *S. maritima* was performed with DNA Engine Dyad (Bio-Rad, USA) by using PCR buffer and GoTaq flexi DNA polymerase (Promega, USA). A total of 30 cycles of amplification was performed with template cDNA denaturation at 94°C for 20s, primer annealing at 55°C for 30s and primer extension at 72°C for 30s. An initial denaturation step of 95°C for 3 min and a final extension step of 72°C for 7min were performed. PCR-amplified DNAs were analyzed by gel electrophoresis on 1% agarose (Sigma, USA) containing ethidium bromide for 40 min at 85 V in TAE buffer. Amplified products were purified from gel by QIAquick Gel Extraction Kit (QIAGEN) as described in manual. The primers for the genes were designed based on the nucleotide sequence information in NCBI data base.

**Northern blot**

10μg of individual total RNA samples were blotted onto a Nylon N+ membrane (GE Health Care) by a vacuum transfer apparatus (Amersham Biosciences, UK). The blots were air-dried and UV cross-linked at 150mL using a UV cross linker (GS Gene linker, Bio-Rad). The RNA blots were hybridized with a 32P-dATP-labelled probes prepared by random primer labeling kit (Prime-a-Gene Labeling System, Promega, USA). The air-dried membrane was exposed to Imaging Screen K (Bio-Rad, USA) for 15 min and scanned by Phosphoimager (Pharos FX plus Molecular Imeger, Bio-Rad, USA).

**RESULTS AND DISCUSSION**

Under salt stress plant tries to produce GB, at least in transcription level (Fig. 1). Our blotting results show PEAMT, CMO and BADH transcripts were over expressed in Suaeda. Although there is over expression in Porteresia, it is less than Suaeda. In case of cultivated variety of rice, the PEAMT and
BADH showed enhance expression, where as there is no significant change in CMO transcript.

The expression of CMO increasing well in Suaeda and Porteresia and both are tolerant to salt. But the differential expression in Naveen was negligible, where as Lunishree showed little over expression and was less sensitive then Naveen. The rate of over expression of genes was higher in Suaeda followed by Porteresia, Lunishree and Naveen. This matches with their tolerance capacity. PEAMT catalyses the methylation of phosphoethanolamine (P-EA) to phosphocholine (P-Cho) or phosphatidylcholine (Ptd-Cho) by multi step reaction, which then metabolise to choline (Cho). The intermediate compounds have many other roles than GB biosynthesis. Ptd-Cho is major component of cell membrane. So PEAMT is present in cell always. Ptd-Cho inhibits PEAMT by feedback mechanisms. BADH which catalyses the last steps in GB bio-synthesis has stress inducible promoter. So, there was enhanced expression in any stress. What about CMO? CMO oxidizes Cho to betaine aldehyde (BA) reducing NAD (P+). BA is toxic to plant. Its immediate consumption is necessary; otherwise plant will dye while trying to live. So plants tightly regulate the expression of CMO and restricted it to chloroplast. Cultivated plant was grown under favorable condition, so by the mean time they either lose the gene or inactivated.

REFERENCES


