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Review Article

Altering Expression of Regulatory Genes for Enhancing Yields and Stress Tolerance in Rice

[1,2]Tasmia Islam, [1]Sudip Biswas, [1]Sabrina M. Elias, [1]Sarah Sarker, [1]Nurnabi A. Jewel, [1]Farida Yasmin and [1]Zeba I. Seraj*

[1]Plant Biotechnology Laboratory, Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh.
[2]Department of Biochemistry and Molecular Biology, Jagannath University, Dhaka, Bangladesh.

ABSTRACT: Soil salinity is a major abiotic stress for rice production worldwide. It is well established that the salinity zone of south and south-east region of Bangladesh is continuing to expand. In order to utilize the coastal area for rice cultivation in the dry season, production of highly salt tolerant varieties but which also have high yields has become a necessity. This has led to research into introducing salt tolerance into high-yielding commercial rice. The capability of plant cells to retain low cytosolic sodium concentrations is a crucial process associated with the ability of plants to grow in high salt concentrations. Work in our laboratory has shown the role of several transcription factors (*SNAC1* and *HARDY*), promoters (*RD29A*), membrane transporters (*NHX1* and *SOS1*) and other important genes (*G protein*, *PDH45* and *DST*) in conferring salt tolerance. We have also characterized some of the mechanisms that allow plants to tolerate high salt concentrations. This paper provides a summary of this research work, such as the cloning of the above genes in rice to achieve both salt tolerance as well as high yields.

Keywords: Abiotic stress, Stress Tolerance, Rice, Yield, Regulatory genes.

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Corresponding author
Zeba I. Seraj
Dept. of Biochemistry and Molecular Biology, University of Dhaka
Email: zebai@du.ac.bd
Mobile:

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INTRODUCTION

Abiotic stress such as salinity and drought is the major causes of yield penalty in rice [2]. Salinity enforces two stresses on plant tissues: a water-deficit that results from the relatively high solute concentrations in the soil, and ion-specific stresses resulting from altered K^+/Na^+ ratios and Na^+ and Cl^- concentrations that are unfavorable to plants [3]. As salinity stress is ongoing and progressively deleterious hindrance to the growth and yield of rice, due to irrigation practices and increasing demands on fresh water supply, the engineering of salt tolerant rice plants has been a long-held and intensively compulsory objective. Conventional breeding efforts using salt-tolerant relatives of rice has had only limited success [17], although the mapping of quantitative trait loci (QTL) in tomato [6] and rice [7], and methods such as somatic hybridization [14] hold potential as a combination of conventional breeding and a molecular approach. On

the other hand plant adaptation to environmental stresses such as salinity and drought is dependent on the activation of cascades of molecular networks involved in stress perception, signal transduction, and the expression of specific stress-related promoters, genes and metabolites [24]. Therefore, engineering genes and promoters that defend and preserve the function and structure of cellular components can enhance tolerance to stress in rice. In this review we focused on the cloning, transformation and conventional crossing techniques that we had followed to transfer the desire stress tolerant genes and promoters in salt sensitive high yielding as well as farmer popular rice varieties and also the experimentation with transgenic plants that has led to increased salinity and drought tolerance.

Transcription Factors

SNAC1

The NAC transcription factors have been characterized for their pivotal roles in plant growth, development and stress tolerance [24]. These proteins are widely distributed in plants such as Arabidopsis, wheat, soya bean, cotton and rice [31]. The NAC family includes 140 members in rice and 110 members in Arabidopsis [22]. Already some of the NAC proteins have been identified with diverse roles and functions during stress in [11]. Notably, *SNAC1* (a member from NAC family) has already been stated as an enhancer of salt and drought tolerance in rice plants with a concomitant increase in grain yield under field stress conditions compared to control [15]. In our study, a simple and efficient method called in planta method was used for the transformation of *SNAC1* to HY rice variety BRRI dhan-55 [24]. Single *SNAC1* gene insertion was confirmed by Southern hybridization. Several salt tolerance tests were performed in the progenies from the T_1 to the T_3 generation. At seedling stage under salinity stress (120 mM), the transgenic line showed significantly higher chlorophyll content, lower electrolyte leakage, lower leaf damage score and also maintained lower Na^+/K^+ ratio compared to wild-type BR55 [24]. The seedlings of the P4 transgenic line containing the *SNAC1* gene were tested under drought stress by simply withdrawing water. Drought stress-recovered transgenic plants were much healthier and greener compared to the survived wild-type BR55 plants. Transgenic plants had longer shoot, roots and chlorophyll content than wildtype after the drought stress [24].

Drought and Salt tolerance gene (DST)

Drought and salt tolerance (*DST*) transcription factor is a key regulator that plays a vital role in conferring drought and salinity tolerance as well as increase in yield in plants. *DST* enhances grain production through down-regulating *Gn1a/OsCKX2* expression [19]. Moreover loss of *DST* function increases stomatal closure and reduces stomatal density, consequently resulting in enhanced drought and salt tolerance in rice [16]. So, in this study, the expression vector, *pCAMBIA1305.2* containing *DST_amiRNA* was used to down regulate *DST* in two different varieties of rice. Through *in planta* transformation, farmer popular high yielding but salt sensitive variety BR28 and low yielding, aromatic variety kailijira were transformed with the *pCAMBIA1305.2_DST_amiRNA*. Then *DST_amiRNA* transgenic plants were confirmed by artificial microRNA specific PCR. Transformed plants at T_0 generation showed vigorous growth, longer panicle length and higher yield compared to the wild type (WT) BR28. It was confirmed that there was decrease in *DST* expression in the BR28 transgenic plants compared to WT through Semi-quantitative RT PCR. T_1 transgenic plants also showed enhancement in a number of physiological parameters and greater growth compared to WT after 14 days of 120 mM salt (NaCl) stress at seedling stage [10].

Promoters

RD29A

Promoter *RD29A* is a stress inducible gene characterized from Arabidopsis thaliana [18]. No close homologs are identified in relevant crop species, such as maize, rice, sorghum, and soybean Among various stress-inducible promoters, *RD29A* has been widely used to drive transgene expression in a inducible pattern that also minimized the negative effect on plant growth [32]. miR399d gene expression under the regulation of promoter *RD29A* in tomato (*Solanum lycopersicum*) and IPT (isopentyl transferase) gene driven by *RD29A* in tobacco plants showed the potential to improve growth when it was exposed to abiotic stresses such as salinity and drought [13, 26]. Promoter *RD29A* (Responsive to desiccation) comprise of two major cis-acting elements, the ABA-responsive element (ABRE) and the dehydration-responsive element (DRE)/C repeat (CRT), both are involved in stress-inducible gene expression [33]. In our study, *RD29A* promoter was cloned from Arabidopsis and transferred into Bangladeshi rice variety Binnatoa by Agrobacterium mediated tissue culture method [29]. Inducibility of this promoter was analyzed by histochemical *GUS* assay under salinity (100 mM and 200 mM) stress both in seedling and reproductive stage and drought stress only in seedling stage. At both seedling and reproductive stages, very good *GUS* expression was also seen in leaves and rice seeds after 24 hours under salt stress [29]. This timing is vital as it encounters about 24 hours for the toxic effects of salt to reach the leaves. No expression was visible in root samples but a very prominent expression was found in leaves at day 7, 10, 13 and 16 after drought stress [29]. So it can be concluded that the *RD29A* promoter is

most suitable for driving transgenes for conferring stress tolerance.

***Alcohol Dehydrogenase* (*Adh*)**

Alcohol dehydrogenase (*Adh*) gene is induced by abiotic stresses such as hypoxia, drought, and cold stresses [9]. This is mainly expressed in roots [4]. It is reported that GUS gene expression under *Adh* promoter is higher and root specific in Arabidopsis [8]. Therefore, *Adh* promoter can be a promising candidate as a stress inducible promoter to maximize rice production through a transgenic approach. In our study the upstream regions (~1kb) of the *Adh* gene was amplified from the genomic DNA of *Arabidopsis* (*Columbia Ecotype*). Then the amplified product was cloned successively into an entry and promoter-characterization binary destination vector having the reporter gene *β-glucuronidase* (*GUS*) by applying Gateway Technology. A positive clone was confirmed by doing PCR, restriction digestion and sequencing. The construct was then transformed into *Agrobacterium tumefaciens* LBA4404 strain and landrace rice Binnatoa, calli infected with the latter. In both salt and submergence stresses, *Adh* could selectively express *GUS* gene activity up to two-fold compared to control [4].

Membrane Transporters

Vacuolar Na^+/ H^+ antiporter gene (*NHX1*)

Vacuolar NHXs have been shown to be an essential determinants of salt tolerance in plants [12]. Eight NHX homologs have been identified in Arabidopsis and clustered on the basis of function and sequence similarity into three different classes like; vacuolar antiporters (*NHX1*, *NHX2, NHX3*, and *NHX4*), plasma membrane antiporters (*NHX7/SOS1* and *NHX8*) and endosomal/vesicular antiporters (*NHX5* and *NHX6*) [28]. The vacuolar Na^+/H^+ antiporter gene has been transformed for salinity tolerance to many plants like Arabidopsis, rice, tomato, maize, wheat and brassica [2]. Our study reports that the over-expression of vacuolar Na^+/H^+ antiporter from two events, one using the CDS and 5′ UTR (1.9kb) and the other, the complete cDNA (2.3kb) from a salt tolerant rice landrace Pokkali in a tissue culture responsive rice variety [2]. The transgenic plants with these two different constructs were further advanced to the T_3 generation. Then physiological and molecular screening of homozygous plants was conducted at seedling and reproductive stages under salinity (NaCl) stress. At both physiological stages both the transgenic lines were observed to be tolerant compared to WT plants. The transgenic lines containing the CDS with both the 5′ and 3′ UTR were significantly more tolerant compared to the transgenic lines containing *OsNHX1* gene without the 3′ UTR. At the seedling stage, it was found that only the chlorophyll retention was significantly more ($P < 0.05$) in the 2.3 kb event compare to that of the1.9 kb. As well as yield/plant in the best line from the 2.3 kb plants was significantly more ($P<0.01$) compared, respectively, to the best 1.9 kb line and WT plant at stress of 6 dS/m. So it is confirmed that transformation with the complete transcripts rather than the CDS may therefore offer more strong level of tolerance.

Plasma membrane Na^+/H^+ antiporter (*SOS1*)

Presently, the SOS pathway is one of the most extensively studied mechanisms controlling salt stress response in plants. The SOS pathway is responsible for ion homeostasis and salt tolerance in plants [27]. The SOS proteins also play a role in the dynamics of cytoskeleton under stress [20]. *SOS1* is a plasma membrane Na^+/H^+ antiporter and facilitates Na^+ efflux and control long distance Na^+ transport from roots to shoots, thus protecting individual cells from Na^+ toxicity. *SOS2* is a serine/threonine protein kinase. *SOS3* responds to the Ca^{2+} signal by activating a protein phosphatase or inhibiting a protein kinase that regulates K^+ and Na^+ transport systems. *SOS3* physically interacts with *SOS2* protein kinase and then activates *SOS2*. The *SOS2/SOS3* kinase complex phosphorylates and activates the *SOS1* protein, resulting in an efflux of Na^+ ions [20]. It has been already reported that increased expression of *OsSOS1* results in improved salt tolerance in transgenic Arabidopsis and tomato [23]. In our study *OsSOS1* gene isolated from salt tolerant Pokkali rice and has been expressed under the constitutive promoter, *CaMV35S.* The construct was transformed through a tissue culture-independent *Agrobacterium* mediated *in planta* transformation method into salt sensitive BRRI dhan28. Incorporation of the foreign genes (*OsSOS1*) into the genome of transgenic plants was then confirmed by gene-specific PCR and Southern blot analysis. The level of transgene expression (*SOS1*) was also measured by semi-quantitative RT PCR and real time PCR. The transformants were shown to be salt tolerant compared to wild type in molecular analysis as well as physiological screening [34].

Other Important Gene

Pea DNA Helicase 45 (PDH 45)

PDH45 is a DEAD-box helicase that can regulate the expression of many genes, as it can sense the stress. Helicases belonging to the DEAD box group having the DEAD motif or Asp–Glu–Ala–Asp play a major role in stress management in plants. The effect of DEAD box helicases in plant stress has been validated in mutants [25], in multiple transgenic plants as well as microarray and other transcriptional assays [30]. Earlier work with the Pea DNA helicase (*PDH45*) with over-expression in tobacco, rice, peanut, sugarcane and chili has shown strong salinity tolerance and also providing

drought tolerance in peanut [21]. Furthermore, *PDH45* showed a greater level of salinity tolerance in sugarcane when co-transformed with another gene *DREB2* [5]. In our study *PDH45* gene isolated from pea with a *CaMV35S* promoter was transformed into the Bangladeshi rice variety Binnatoa through Agrobacterium-mediated transformation. Positive integration of the transgene was confirmed by polymerase chain reaction (PCR), semi-quantitative reverse-transcription PCR and Southern blot hybridization. The transgenic seedlings showed significantly higher chlorophyll content, but had decreased root length compared to wild type (WT) under normal physiological conditions. A higher leaf K^+/Na^+ ratio of 0.346 was maintained by the transgenic lines compared to the WT ratio of 0.157, which focused induced ion homeostasis. At the reproductive stage, transgenic rice plants containing *PDH45* showed better fertility and produced higher grain yield by 16% compared to WT plants under continuous stress of 6 dS/m from 30 days till maturity [1].

CONCLUSIONS

Salinity tolerance encompasses a complex of responses at molecular, cellular, metabolic, physiological, and whole-plant levels. From extensive research through cellular, metabolic and physiological level it has been concluded that ion uptake, transport and balance, osmotic regulation, hormone metabolism, antioxidant metabolism, and stress signaling play critical roles in plant adaptation to salinity stress. Taking information of the latest advancements in the field of genomic, transcriptomic, proteomic, and metabolomics techniques, plant biologists are focusing on the development of a complete profile of genes, proteins, and metabolites which are responsible for different mechanisms of salinity tolerance in different plant species. Still, there is lack of the integration of information from genomic, transcriptomic, proteomic, and metabolomics studies, and the combined approach is indispensable for the determination of the key pathways or processes controlling salinity tolerance. In addition, in spite of the significant progress in the understanding of plant stress responses, there is still a large gap in our knowledge of transmembrane ion transport, sensor and receptor in the signaling transduction, molecules in long distance signaling, and metabolites in energy supply. Genetic engineering has been proved to be an efficient approach to the development of salinity-tolerant plants, and this approach will become more powerful as more candidate genes associated with salinity tolerance are identified and widely utilized. The tolerance level achieved due to membrane transporters, promoters, transcription factors and other important genes integration suggests that such kind of study shows great promise for the genetic improvement of stress tolerance in commercial indica varieties of rice.

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