

Salinity responsiveness in finger millet analysis



Introduction

Salinity represents a strong limitation for agricultural production worldwide, especially in arid and semi-arid and restricts efficient utilization of available land resources. It is estimated that about 7% of world agricultural land that nearly one half of the total area of irrigated land could be adversely affected by salinization (Kosova 2013). Most of the cereal crops are sensitive to salinity and have limited amount of genetic variation for salinity tolerance in their germplasm. Hence genetic improvement of crops for their tolerance against salinity will be helpful in achieving targeted food production to meet the demands of growing population.

Conventional plant breeding approaches have resulted in limited success in developing salt tolerant crop varieties due to multigenic nature of salt tolerance mechanisms and presence of low genetic variation in major crops. Another problem associated with conventional breeding is that if the gene is present in a wild relative of the crop, there is difficulty in transferring it to the domesticated cultivar, due to reproductive barriers and linkage drag.

Recently, substantial progress in elucidation of salt tolerance mechanisms, especially salt ion signaling and transport, has been achieved due to utilization of modern genetic approaches and high-throughput methods of functional genomics. Genetic engineering has been demonstrated to be successful in developing salt tolerant crop plants (Zhang et al. 2001; Su and Wu 2004; Zhang et al. 2001). Genetic engineering strategies targeting various metabolic pathways viz., accumulation of osmolytes, antioxidant enzymes and up regulation of genes involved in stress responses like ion

transporters, ion channels, transcriptional factors and various signaling pathway components have resulted in production of genetically modified crop plants exhibiting improved level of salinity tolerance (Turan et al. 2012).

Identifying novel genes, analyzing their expression patterns in response to salt stress and determination of their potential functions in salt stress adaptation will provide the basis for effective genetic engineering strategies to enhance tolerance against salt stress (Cushman and Bohnert 2000).

Responses against salinity stress involve many molecular processes such as ion homeostasis (membrane proteins involved in ionic transport), osmotic adjustment and water regime regulation (osmolytes) and scavenging of toxic compounds (Munns and Tester 2008). During recent years, considerable attention has been given towards elucidating the molecular basis of salt tolerance in crop plants. Several important pathways involved in salinity tolerance have been identified in model plants like *Arabidopsis* and rice (Zhu 2003; Walia et al. 2005; Cotsaftis et al. 2011).

It is hypothesized that exploitation of halophytes or distantly related crops or wild progenitors of cereal food crops exhibiting superior levels of salinity tolerance may lead to identification of novel metabolic pathways/mechanisms/genes involved in modulating salinity stress tolerance in crop plants. Several research groups are working on understanding mechanisms of salinity tolerance in *Pennisetum glaucum* (Mishra et al. 2007), *Avecinnia marina* (Mehta et al. 2005), *Porteresia coarctata* (Garg et al. 2014) with a view to identify novel genes for genetic engineering of salinity tolerance in crop plants. But much more concerted efforts are needed to identify and exploit diverse crop species exhibiting superior level

of salinity tolerance which will help in identifying novel genes associated with salinity tolerance.

Finger millet (*Eleusine coracana* L.) is an important minor cereal crop widely grown in Africa and Asia, known for its high degree of tolerance against drought, salinity and blast disease (Shailaja and Thirumeni 2007; Agarwal et al. 2011). Investigating the mechanisms and pathways involved in salt-tolerance of finger millet could facilitate better understanding of the molecular basis of salt tolerance and therefore enable the effective use of genetic and genomic approaches to improve salt tolerance in major cultivated crops. Although a wide range of significant physiological mechanisms and genetic adaptations to salinity stress has been observed, the underlying mechanisms of salt-tolerance in plants are still poorly understood. The best possible approach to explore tolerance mechanisms is to compare the components involved in stress response in tolerant as compared to sensitive plants. The other alternative to overcome this limitation would be to pick up some selected conserved genes which may be used to perform limited transcriptome analysis among the diverse genotypes.

With this background, we planned to understand the physiological and molecular basis of salinity responsiveness in finger millet in comparison to the major cereal food crop, rice. Comparative physiological studies were conducted with a view to prove the superiority of finger millet genotypes over rice in terms of salinity tolerance. Two contrasting finger millet genotypes were used for physiological studies and expression analysis of already identified salinity responsive genes was done. This is the first study

conducted to compare molecular basis of salinity tolerance in finger millet with rice.

Material and Method

Genetic Materials Used

Seeds of two contrasting genotypes of rice (*Oryza sativa*) {FL478 (tolerant), White Ponni (Susceptible)} and finger millet (*Eleusine coracona*) {Trichy 1 (tolerant), CO12 (Susceptible)} in terms of salinity tolerance were evaluated for their responses against salinity stress under greenhouse conditions.

Nucleus seeds of rice genotypes were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India and finger millet genotypes were obtained from Millet Breeding Station of Tamil Nadu Agricultural University, Coimbatore, India.

Effect of salinity stress during germination

Contrasting genotypes of rice {FL478 (tolerant), White Ponni (Susceptible)} and finger millet {Trichy 1 (tolerant), CO12 (Susceptible)} genotypes were assessed for their ability to germinate under salinity stress. Twenty seeds of both rice and finger millet genotypes were allowed for germination under different concentrations of NaCl solutions (0 mM, 50 mM, 100 mM, 200 mM NaCl solution) in petri-dishes with adequate replications. Germination percentage was calculated based on the number of seeds successfully germinated and vigor index was calculated based on the shoot length and root length on 10th day of germination.

Effect of salinity stress during vegetative stage

<https://assignbuster.com/salinity-responsiveness-in-finger-millet-analysis/>

Imposition of salinity stress

Contrasting genotypes of rice and finger millet genotypes (three seedlings per pot) were grown in perforated pots of 15 cm diameter and 20 cm height (having 3–5 mm holes on the side walls and bottom) filled with 2 kg of field soil mixed with required amount of fertilizer [1. 25 g of $(\text{NH}_4)_2\text{SO}_4$, 0. 08 g Muriate of potash (KCl), and 0. 08 g single superphosphate (SSP)]. Three pots were placed inside a large tray containing irrigation water and grown up to 20 days under greenhouse conditions. Plants were grown during June–August when air temperature ranged from 26 to 34 °C during the day and from 20 to 27 °C during the night and relative humidity ranged from 60 to 80 %. Salinity stress was imposed on 21st day when plant has reached to 5 leaf stage by adding desired concentrations of NaCl viz. 150 mM and 300 mM along with suitable control pots irrigated with normal water. Progression of salinity stress was monitored by periodically measuring the electrical conductivity (EC) of soil (from pot) and water (collected from tray) samples collected from both control and salinity stressed trays.

Physiological and biochemical responses of contrasting rice and finger millet genotypes under salinity stress

Contrasting genotypes of rice viz., FL478 (tolerant) and White Ponni (susceptible) and finger millet viz., CO 12 (susceptible) and Trichy 1 (tolerant) were evaluated for their physiological and biochemical responses viz., osmotic tolerance ability, salt accumulation pattern and sugar accumulation pattern during salinity stress.

Measurement of Osmotic tolerance ability

<https://assignbuster.com/salinity-responsiveness-in-finger-millet-analysis/>

For assessing the osmotic tolerance ability of contrasting rice and finger millet genotypes, freshly emerged leaf (5-6cm) was marked and increase in leaf length was measured at every 24hrs interval during the initial 6 days of salinity stress along with control plants. Terminal leaf elongation rate per day (24 h) was calculated based on the observations recorded.

Salt accumulation pattern

Salt (Na^+ and K^+) uptake, transport and accumulation pattern of contrasting rice and finger millet genotypes was assessed by determining the (Na^+ and K^+) contents in shoots and top 3 leaves collected under normal and salinity stress conditions. Tissue samples collected at 21 DAS (days after stress) were washed with de-ionized water, dried in a hot air oven (70 °C) and then ground into fine powder. Ground samples were digested with triple acid mixture (sulfuric acid, perchloric acid and nitric acid in the ratio 9: 2: 1 v/v). Na^+ and K^+ concentrations in the triple acid digested extract were estimated using Flame Photometer (Elico, CL378).

Determination of total soluble sugar content

Total soluble sugar (TSS) content in the top three leaves of control and salinity stressed plants (21 days after stress) of contrasting rice and finger millet genotypes was determined using anthrone reagent method (Yemm and Willis 1954). Fresh leaf sample (100 mg) was ground in liquid nitrogen and pigments were removed using acetone extraction. TSSs were extracted in 80 % ethanol and were estimated by the anthrone reagent method using glucose as the standard.

Other physiological responses of contrasting finger millet genotypes to salinity stress

Gas exchange parameters were recorded in the third leaf (from top) of control and salinity stressed plants of rice and finger millet genotypes between 1000 hours and 1200 noon at 11 DAS (days after stress) using LI-COR 6400-XT photosynthesis system (LI-COR Biosciences, Nebraska, USA). The instrument was set with the following conditions: photo-synthetically active radiation 1, 500 $\mu\text{mol of photon m}^{-2} \text{ s}^{-1}$; ambient levels of CO_2 and temperature; leaf area 3 cm^2 and flow rate of 500 $\mu\text{mol s}^{-1}$.

RNA isolation, Northern blotting and hybridization

Expression analysis of already reported salinity responsive candidate genes in response to salinity stress in the leaves of contrasting rice and finger millet genotypes were studied by northern blotting. Top 3 leaves of both rice and finger millet genotypes were collected and frozen immediately in liquid nitrogen from both control and stressed plant (300mM NaCl) when susceptible rice variety viz. White Ponni has shown salinity symptoms i. e., 11 days after salinity stress. Total RNA was isolated from stressed and control leaf samples using One Step RNA Reagent (Biobasic Inc., Canada) as per manufacturer's protocol. The integrity of RNA was assessed by formaldehyde agarose gel electrophoresis. Total RNA was quantified using Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). 20ug of RNA mixed with RNA loading dye (1: 1) was denatured at 75°C for 10mins and separated on denaturing agarose gel as described by Streit et al. (2008). The gel was stained with ethidium bromide

and photographed. Gel was processed and RNAs were transferred to positively charged nylon membrane (Pal Corporation) using 20XSSC buffer. After capillary transfer to the membrane, RNAs were fixed by exposing the membrane to UV cross linker (Hoeffer, Piscataway). DNA fragment of candidate genes to be used as probe were isolated from rice cloned in pTZ57R TA cloning vector and confirmed by sequencing. Double-stranded probes were radioactively labelled with (α - 32 P) dCTP using DecaLabel DNA Labeling kit (Fermentas) and probes were purified using Sephadex G-50 spin column (GE Healthcare). Radiolabelled probes were denatured on boiling water bath snap cooled on ice and used for hybridization as described by Streit et al. (2008). RNA blots were pre-hybridized in ULTRAhyb® at 45°C for 4–8 h. The blots were hybridized with 32 P-labelled denatured probes at 45°C for 20 h in the same but fresh buffer. The blots were initially washed at room temperature with 2XSSC and 0.1% SDS followed by twice wash with 1XSSC and 0.1% SDS at 45°C for 20 min each.

The blots were initially washed at room temperature with 2XSSC and 0.1% SDS for 30 min and then washed with different stringencies for different probes to decrease background. Hybridized membrane were dried on blotting paper and exposed to Kodak XAE-5 film with cassette having Kodak intensifying screen for 1–6 d. The resulting radiograms were scanned in an LKB 2201 densitometric scanner.

Results

Effect of salinity stress on rice and finger millet genotypes during germination stage

Screening of contrasting genotypes of both rice and finger millet against salinity stress at germination stage revealed the superiority of finger millet over rice in terms of salinity tolerance at germination stage. At lower concentration of salinity stress (i. e 50mM NaCl) the susceptible genotypes of both finger millet (CO12) and rice (White ponni) has shown better germination percentage and vigor index as compared to tolerant genotypes. Tolerant rice genotype FL478 was found to possess better germination percentage ($35 \pm 2.9\%$) and vigor index (128.2 ± 10.6) in comparison to susceptible White Ponni where germination percent and vigor index was found to be $16.7 \pm 1.7\%$ and 70.9 ± 7.1 respectively. Both finger millet genotypes i. e. CO12 and Trichy1 has shown almost similar germination percent and vigor index at 100mM of NaCl stress. Both rice genotypes (viz. FL476 and White Ponni) did not show any germination beyond 100 mM NaCl stress (Table 1); whereas both susceptible (CO12) and tolerant (Trichy 1) finger millet genotypes were able to germinate even at 300 mM NaCl stress (Table 1). At 300 mM NaCl stress Trichy 1 has shown better germination percent (40.0 ± 1.6) and vigor index (32.0 ± 1.3) as compared to CO 12 germination percent (24.4 ± 0.9) and vigor index 24.4 ± 0.9 .