Pak. J. Bot., 42(6): 4003-4009, 2010.

CONSTITUTIVE EXPRESSION OF *OSC3H33*, *OSC3H50* AND *OSC3H37* GENES IN RICE UNDER SALT STRESS

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Abstract

Abiotic stress is a major problem around the world causing huge losses to cultivated crops. Salinity, low temperature and drought are the most destructive abiotic stresses worldwide. A study was designed to understand the effect of salt stress (control, 50,100, 150 and 200 mM) on the germination of two local rice varieties (Super Basmati and Basmati 2000) along with the cellular injury that salt inflicts on rice seedlings and the expression of regulatory genes (*OsC3H33, OsC3H50* and *OsC3H37*) from CCCH gene family. In both the varieties, continuous reduction in germination was observed as the salt stress increased while better germination pattern was observed in Super Basmati than Basmati-2000. There was an increase in the cellular injury as the salt concentration increased in both varieties. RT-PCR approach was used to isolate and detect the expression of Zinc finger protein genes (*OsC3H33, OsC3H50* and *OsC3H37*) in rice. The expression pattern showed that *OsC3H33, OsC3H50* and *OsC3H37* were constitutively expressed in plants raised in stressed environment but the expression was different under various salt concentrations. The expression of these genes suggests that they might have some regulatory function in abiotic stress tolerance.

Introduction

Abiotic stress is the prime cause of crop loss worldwide, decreasing average yield for most of the major crops by more than 50% and among them, two major abiotic stresses reducing plant productivity are salinity and drought (Serrano *et al*., 1999; Zhu, 2001). It has been found that abiotic stress can enforce limitations on crop yield and also restrict land availability for agriculture, thus emphasizing a greater need for understanding how plants respond to hostile environments (Kawasaki *et al*., 2001; Grennan, 2006).

Rice is salt-sensitive crop, and soil salinity is the single most prevalent soil toxicity problem faced in rice production (Greenland, 1984). Absence of comprehensive understanding of the mechanism of these traits is one of the main limitations in harnessing the biotechnological methodologies for crop enhancement (Schachtaman *et al*., 1992; Ashraf, 2009). Plants can initiate a number of cellular, molecular and physiological variations to respond and adjust to these stresses (Zhu, 2001; Ashraf, 2010). It is necessary to recognize the physiological, molecular, and biochemical mechanisms involved in the tolerance or the sensitivity of plants to salt (Serrano *et al.*, 1999; Mittler, 2006; Calliste *et al*., 2006, Ashraf & Akram, 2009).

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The CCCH gene family of rice is divided into 8 subfamilies having 67 genes. These genes encode zinc-finger proteins conferring tolerance to salt, drought and cold stresses playing important roles in RNA processing as RNA-binding proteins (Wu *et al*., 2004; Wang *et al*., 2008). Due to the enormity of problem associated with soil salinity on crops, a study was initiated where seeds of Super Basmati and Basmati 2000 were subjected to salt (NaCl) stress, to determine the effect of salt stress on germination, cellular injury and gene expression of zinc-finger proteins at seedling stage.

Materials and Methods

Seed germination: Seeds of two rice cultivars, (Super basmati and Basmati 2000) obtained from the National Agricultural Research Centre (NARC), Islamabad were surface sterilized with 0.5% Sodium hypochlorite solution for 30 minutes and treated with control, 50, 100, 150 and 200 mM salt solutions for three days. After providing the stress, seeds were transferred to sterilized disposable Petri dishes (87 mm diameter, 15mm height) and incubated at 25°C. Seed germination was evaluated after every 12 hours up to three days. Seeds were considered to be germinated with the emergence of radicle. After germination, seeds were transferred to water culture (Styrofoam with holes cut in it and surround by a net cloth placed in a tray with distilled water in it) were placed in a growth chamber, with average temperature of 25°C and photoperiod for the day/night cycle kept at 16/8 h respectively with relative humidity of about 70%, till acquiring uniformity of stand and health in appearance.

Cell membrane stability: Cell membrane stability was determined by using fully expanded young leaves (UTECH conductivity meter in µS (micro Siemens). Young leaf (third leaf) was selected from each treatment. Twenty pieces (1 cm diameter) cut from these leaves were submerged into distilled water contained in test tubes. The test tubes were kept at 10ºC in incubator for 24h, followed by warming at 25ºC and measuring the electrical conductivity (C1) of the contents. The leaf samples were then autoclaved for 15 minutes at 120ºC and electrical conductivity of the medium measured again (C2). Cellular injury was determined by using the formula: C1/C2×100, where C refers to conductivity one and two.

Gene expression profiling of OsC3H33, OsC3H37 and OsC3H50 genes: Plant leaves, 0.2g were taken each from control and stress treated plants of Super Basmati and Basmati 2000. Leaves were ground in liquid nitrogen. RNA was extracted by using Trizol reagent method. First strand cDNA was synthesized from RNA with M-MLV reverse transcriptase (Invitrogen) by using OG2 primer (5´-GAG-AGA-GGA-TCC-TCG-ACT-3`). After cDNA synthesis, the desired genes from CCCH gene family were amplified by gene-specific primers for *OsC3H33*, *OsC3H37* and *OsC3H50* genes. The PCR conditions were a pre-denaturation of 5 min at 94°C; 22 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C; an extension for 10 min at 72°C. The PCR products were separated on a 1% agarose gel containing Ethidium bromide and visualized under UV light.

Results

Seed germination: In this study it was realized that the stress treatment to the seeds of these plants had an adverse effect on the germination of the seeds as shown in the Fig. 1. It is apparent that the germination of rice seeds reduced drastically with the increase in salt concentration. Highest reduction in germination was recorded in Basmati-2000 as compared to Super-Basmati (Fig. 1).

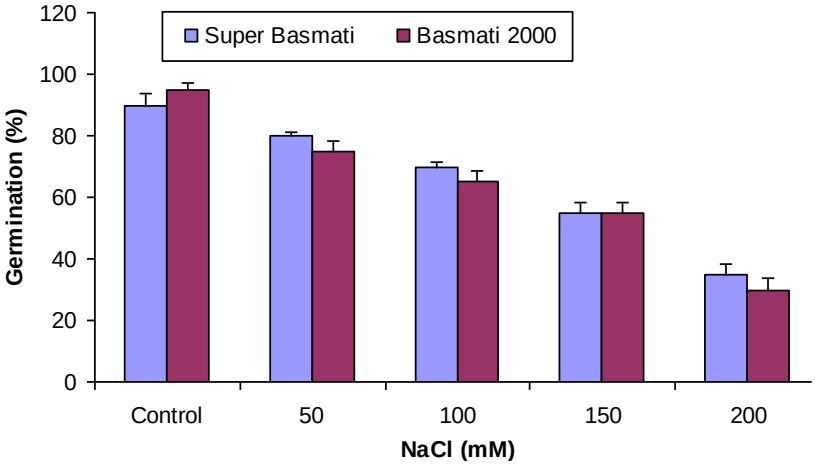

Fig. 1. Effect of various salt concentrations on germination percentage of Super Basmati and Basmati-2000.

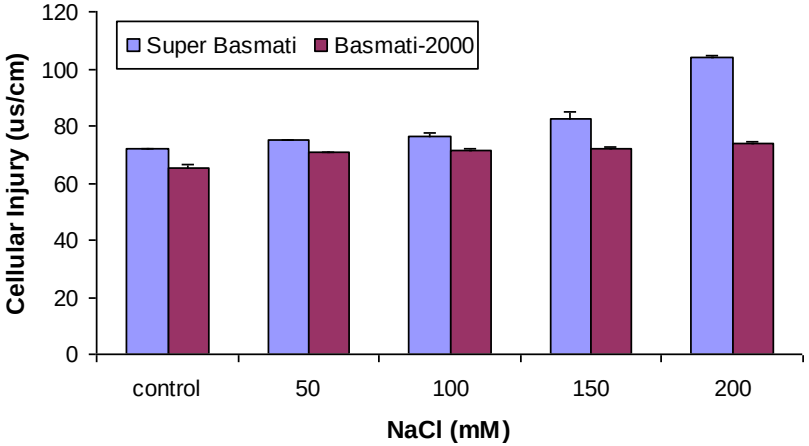

Fig. 2. Effect of various salt concentrations on cell membrane stability of Super Basmati and Basmati-2000.

Cell membrane stability: The data in Figure 2 suggests that salt had a negative effect on the cell membrane stability and as the salt concentration is increased so does the membrane injury. Level of injury was high in case of Super-Basmati than that in the Basmati-2000 (Fig. 2).

Molecular study: After gene specific PCR for *OsC3H33*, *OsC3H37* and *OsC3H50* genes, it was observed that these genes were expressed under stress conditions. For *OsC3H33* gene, expression was high in 100 and 200 mM and low in control and 50 mM salt stressed plants of Super Basmati while in Basmati 2000 low expression was only observed in 50 mM and no expression in 100 mM whereas similar expression pattern was observed in control, 150 and 200 mM salt stressed plants (Figs. 3 and 4). In case of *OsC3H37* gene, expression was high in 100 and 150 mM and low in 200 mM and no expression in control. In Basmati 2000 no expression was observed in control, similar

kind of expression in 50 and 200 mM and low in 150 mM whereas high expression was noticed in 100 mM salt stressed plants (Fig. 5). For *OsC3H50* gene, expression was high in control and 50 mM and low in 100, 150 and 200 mM salt stressed plants of Super Basmati. In Basmati 2000 low expression was only observed in control and no expression in 50 and 200 mM whereas high expression was observed in 100 and 150 mM salt stressed plants (Fig. 6).

Discussion

The percent seed germination is optimum during non-saline conditions where as it decreases with the increase in salt concentration (Dkhil *et al*., 2010; Siddiqi *et al*., 2007). Salt negatively affected the growth of the seeds, whereas the number of seeds that grew was reduced as salt stress was increased (Fig. 1). Salt causes inhibition of seed germination which could be attributed to specific ion toxicity (Huang & Redmann, 1995). Similar kind of results has also been reported by Lima *et al*., (2003) according to him the viability of seeds under salt stress was reduced as the concentration of salt was increased. Germination decreases as salt stress increases, it is reduced to only 25% at 900 mM NaCl stress (Khan *et al*., 2009). Commencement of germination is reduced as salt concentration is increased to 150 mM (Alam *et al*., 2004). It has been reported that seed germination, survival of rice seedlings and overall plant growth is extensively reduced due to high salt concentration (Zeng *et al*., 2000; Narale *et al*., 1969; Huang *et al*., 2008). Salt reduces germination percentage as well as causes an increase in the time for germination (Jamil *et al*., 2007).

Salt stress inflicts injury to cellular membranes as the level of salt increases causing excessive loss of ions and ultimately affecting the survival of the plants (Zeng *et al*., 2001). In this study it was observed that the severity of the injury to cell membrane was increased due to increase in the salt concentration (Fig. 2). Salt causes insufficient uptake of water due to which Na+ accumulates in plant cells causing excessive cellular damage (Atienza *et al*., 2007; Golldack *et al*., 2003; Lee *et al*., 2003; Ren *et al*., 2005). The same was noticed by Jamil *et al*., (2009) while working on *Brassica napus* L., where increased injury to the plants was observed as the concentration of the salt stress was increased. Salt causes changes' to cell membrane integrity (Chen *et al*., 2007). Salinity causes an increase in electrolyte leakage hence increase in cellular injury (Lutts *et al*., 1996). The cell electrolyte leakage increases by increasing salt stress (Hoque & Arima, 2000).

OsC3H33, OsC3H37 and *OsC3H50* genes are responsible for regulatory functions including RNA processing and stress tolerance induced by salt stress (Wang *et al*., 2008; Jin *et al*., 2010). In this study it was observed that salt induced the expression of the desired genes but the expression was different under various salt concentration (Figs. 3, 4, 5 and 6), which might suggest their role in salt stress tolerance and also suggest that these genes play important roles as regulators, as was observed in a study conducted by Malik *et al*., (2002) according to him, CCCH gene family has been observed to play certain roles in regulating abiotic stresses including salt stress, *AtMYB2* transgene was observed to tolerate increased salt concentration. The same was reported by Sun *et al*., (2007) where the genes under consideration were *AtSZF1* and *AtSZF2* from the same CCCH gene family, concluding that the expression of the genes was induced by providing salt stress to the plants. Many regulatory genes in plants are induced due to various abiotic stresses (Seki *et al*., 2001). In another study conducted by Wang *et al*., (2008) the CCCH gene family is known to play regulatory functions. The expression was also observed in control plants which was also reported by Misra *et al*., (1997) according to him zinc finger protein genes often express constitutively in rice tissues.

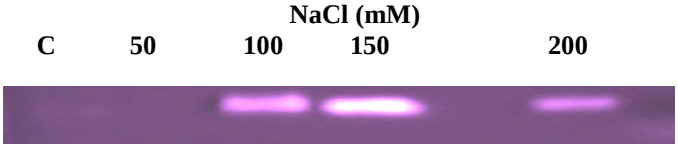

Fig. 3. Expression pattern of OsC3H37 gene in Super Basmati under control and different salt stress conditions.

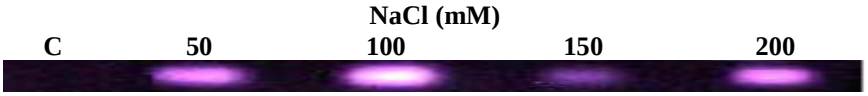

Fig. 4. Expression of OsC3H37 gene in Basmati 2000 under control and different salt stress conditions.

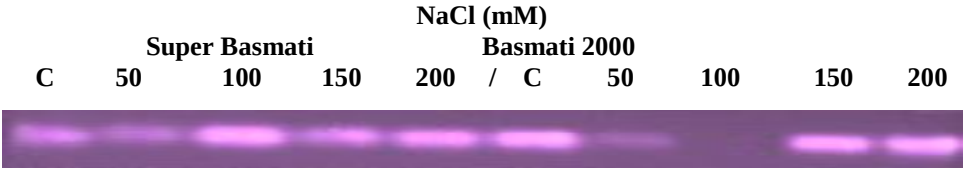

Fig. 5. Expression pattern of OsC3H33 gene in Super Basmati and Basmati 2000 under control and different salt stress conditions.

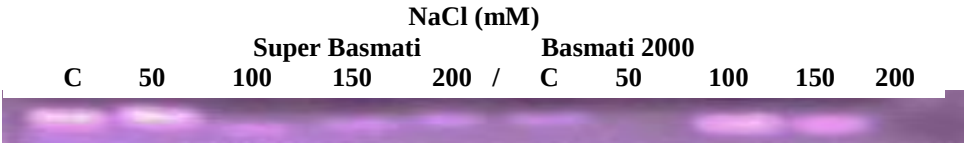

Fig. 6. Expression pattern of OsC3H50 gene in Super Basmati and Basmati 2000 under control and different salt stress conditions.

Acknowledgement

This work was supported by research grants from the Higher Education Commission, Pakistan

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(Received for publication 4 January 2010)