Proteomic and transcriptomic analysis reveals evidence for the basis of salt sensitivity in Thai jasmine rice (Oryza sativa L. cv. KDML 105)

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The fragrant Thai jasmine rice cultivar, Khao Dawk Mali 105 (KDML 105), is an economically important cultivar with valuable flavour characteristics, however, it is very sensitive to salinity. To investigate whether genetic characters for salt-tolerance are present, proteomes from the leaf lamina of KDML 105 and a contrasting salt tolerant cultivar (Pokkali) were compared under saline conditions. Ten differential proteins were identified, mainly representing gene products involved in photosynthesis, carbon assimilation and the oxidative stress response. The mRNA transcripts for these proteins were then monitored in both cultivars using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). For Pokkali, the up-regulation of nine identified salt-induced proteins was related to the increase in abundance of the respective mRNA transcripts. In contrast, although mRNA transcripts encoding all ten identified proteins could be detected in KDML 105, only three differential proteins spots were detected in the proteomic analysis. This indicates that although KDML 105 contains elevated transcript level of genes needed for salt tolerance, the posttranscriptional mechanisms controlling protein expression levels were not as efficient as in Pokkali, indicating targets for future genetic improvement.

Key words: Salt stress, fragrant rice, Oryza sativa L., Khao Dawk Mali 105 (KDML 105), proteomics, semi-quantitative RT-PCR.

INTRODUCTION

Salt-affected soils in arid and semi-arid regions are a major factor adversely affecting rice growth and productivity worldwide. This area is likely to increase as a result of increasing irrigation, land clearance, shortage of rainfall and rising temperatures due to global warming (Yeo, 1999). Excess soil solution Na+ imposes root osmotic stress, and this in turn limits the root’s ability to extract water from the soil. Several complementary biochemical and physiological adaptations are generally necessary to establish salt tolerance. These include salt exclusion at the root level, compartmentalization of toxic ions from the intracellular to whole-plant levels, responsive stomata, synthesis of compatible solutes, adjustment in photosynthetic apparatus, alteration of membrane integrity and efficient detoxification of reactive oxygen species (Parida and Das, 2005). Rice plants are relatively sensitive to soil salinity, but salinity tolerance varies
tremendously among varieties providing opportunities to improve crop salt-stress tolerance through genetic means (Flowers and Yeo, 1981; Mohammadi-Nejad et al., 2008; Cha-um et al., 2010). Salt-tolerant rice varieties such as Pokkali have been found to be superior in agronomic characters such as yield, survival, plant height, and physiological traits such as ion exclusion (Heenan et al., 1988; Noble and Rogers, 1992), anti-oxidative systems (Vaidyanathan et al., 2003) and membrane stability (Singh et al., 2007).

Proteomics has been used to identify proteins affected by salinity in several cultivars of rice. Previously identified salt stress-responsive proteins include ones involved in major metabolic processes including photosynthetic carbon dioxide assimilation and photorespiration, photosynthetic oxygen evolution and stress-responsive proteins (Kim et al., 2005; Parker et al., 2006). DNA microarrays have also been applied to monitor changes in the steady-state abundance of salt-stress regulated transcripts (Kawasaki et al., 2001; Rabbani et al., 2003). These studies have identified large numbers of differentially expressed genes. A recent comparative transcriptomic analysis of two contrasting rice cultivars (salt-tolerant Pokkali and salt-sensitive IR64) found a set of genes representing the signal, relay and response classes of salt-regulated genes proposed to confer higher salt tolerance to Pokkali (Kumari et al., 2009).

Several salt sensitive varieties of rice are economically important because of its characteristics other than yield. These include the culinary qualities associated with grain flavour and texture. Thai jasmine rice (also known as Thai fragrant or Hom Mali rice) is sold at a premium price because of its superior cooking and sensory qualities, including fragrance. Thai national authenticity regulations confine jasmine rice to two cultivars. One of these is Khao Dawk Mali 105 (KDM 105) that was introduced in 1958 and continues to be grown extensively despite its low yields and stress-sensitivity (Fitzgerald et al., 2009). The highest quality KDM 105 is produced in the Northeast region of Thailand where high productivity is obstructed by infertile, saline soil and unstable rainfall (Yoshihashi et al., 2002). Compared to the coastal, salt-tolerant Indian cultivar Pokkali, KDM 105 seedlings are sensitive to salt stress as indicated by a greater reduction in plant dry weight, higher Na+/K+ and higher electrolyte leakage (Theerakulpisut et al., 2005). Improvements to the growth performance of KDML 105 in saline laboratory media have been demonstrated after providing the protective metabolite glycinebetaine (Cha-um et al., 2006) but this is not practical in the field.

A key challenge is to identify genes that control traits associated with physiological and agronomical parameters leading to higher salt tolerance while retaining grain quality, with the goal to accelerate both conventional and molecular breeding of cultivars with desirable culinary qualities that will grow in saline soil. One question is whether salt sensitive varieties already contain some of these genes but do not regulate them appropriately with respect to salinity. We have identified that although genes for a series of transcripts and proteins that increase in Pokkali seedling leaves when grown in saline conditions are also present in KDML 105, the levels of most proteins do not increase. This suggests that in KDML 105, salt sensitivity may lie in signaling and regulation of the response.

**MATERIALS AND METHODS**

**Plant materials**

The rice seeds of cultivars KDM 105 and Pokkali were provided by Pathumthani Rice Research Institute, Thailand. Seeds were surface-sterilized by soaking in 1.5% (w/v) calcium hypochlorite for 30 min, thoroughly washed and germinated in distilled water. The uniformly germinated seeds were transferred to a plastic grid placed over a 6-L container filled with distilled water. After five days, when the seedlings were well-established, distilled water was replaced by nutrient solution (Yoshida et al., 1976) that was renewed every week. When the seedlings were 21-days-old, the nutrient solution was changed to Yoshida solution supplemented with 120 mM NaCl and after a further seven days, the third leaves were harvested, frozen in liquid nitrogen and stored at -80°C until further use. The experiments were performed in triplicate to obtain three independent samples of rice seedlings for protein and RNA analysis.

**Protein extraction and two-dimensional electrophoresis (2DE)**

The leaves were ground in liquid nitrogen and suspended in 50 mM sodium acetate of pH 5.0. The homogenate was centrifuged at 14,400 g for 10 min. The supernatant was transferred to a new tube and 50% trichloroacetic acid was added to a final concentration of 10% and the mixture was allowed to precipitate on ice for 30 min. Each sample was then centrifuged at 14,400 g for 10 min to obtain a protein pellet. After the pellet was washed with 100 µl ice-cold ethanol and resuspended in 100 µl lysis solution (8 M urea, 4% CHAPS, 40 mM Tris-base), the protein concentration was determined (Bradford, 1976). The protein samples were then diluted into the rehydration buffer (7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2 mM DTT, 0.8% (w/v) IPG buffer and 0.2% bromophenol blue), and allowed to rehydrate for at least 1 h on ice. Samples (100 µg) were then loaded onto immobilized pH gradient (IPG) strips (7 cm, pH 4 to 7, pH 3 to 10, GE Healthcare, Sweden), and isoelectric focusing (IEF) was conducted (IPGphorTM Isoelectric Focusing System, GE Healthcare, Sweden). IEF was performed at 400, 1000 and 2000 V for 1, 12 and 1 h, respectively. After the IEF run, the IPG strip was then equilibrated in equilibration buffer [62.5 mM Tris-HCl Ph 6.8, 2.5% sodium dodecyl sulfate (SDS), 10% (w/v) glycerol and 5% (v/v) 2-mercaptoethanol] for two periods of 15 min each. The second dimension SDS electrophoresis was performed in 12.5% polyacrylamide gels (Hoefer SE600, GE Healthcare, USA). The gels were stained with 0.1% colloidal Coomassie Brilliant Blue (CBB) G-250. The relative molecular mass of each protein was determined using standard markers (Amersham Bioscience, USA) and the isoelectric point (pI) by the migration of the protein spots on the IPG strip. The positions of individual proteins on the gels were evaluated automatically with 2-Dimension software (SineGene, USA).
Table 1. Primers sequences for RT-PCR analysis and their product size.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
</tr>
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<tr>
<td>23 kDa polypeptide PSII (PSII-23)</td>
<td>CCAGGAAGTTTGTGAGGAGC</td>
<td>GAAACACACAGCACCACA</td>
<td>162</td>
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<td>Rubisco large subunit (Rubisco)</td>
<td>GCTGCGGAATCTTTACTGG</td>
<td>GTAGAGGCGGTAAGGCTTG</td>
<td>229</td>
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<tr>
<td>Rubisco activase (RCA)</td>
<td>TGTGGAGAACATGGCAAGA</td>
<td>CGCAGAACCAGTAGAAGGAAC</td>
<td>223</td>
</tr>
<tr>
<td>Putative oxygen evolving complex protein (OEC18)</td>
<td>GCCAAGCCAGTAAGCAAAG</td>
<td>TGAAGTCGAGCAGCATATTCT</td>
<td>181</td>
</tr>
<tr>
<td>Fructose-bisphosphate aldolase, chloroplast precursor, putative, expressed (FBA)</td>
<td>GGGCCGAGACCTTTTCTAC</td>
<td>GTTCATCGAGTTACGATTCT</td>
<td>236</td>
</tr>
<tr>
<td>Sedoheptulose-1,7-bisphosphatase precursor (SBP)</td>
<td>CTCTTGAGGGGTCCAGCATT</td>
<td>ACATGCTGCACTTTTCTCT</td>
<td>214</td>
</tr>
<tr>
<td>40 kDa thylakoid lumen PPIase (TLP40)</td>
<td>GGCCTGAGTAATGGGAGGA</td>
<td>GCTTTGGAGCTACTGCATCC</td>
<td>152</td>
</tr>
<tr>
<td>Phosphoglycolate phosphatase (PGP)</td>
<td>CGATTTCCTCAAGCAAGA</td>
<td>ACCCTGATTTCAGCAATG</td>
<td>242</td>
</tr>
<tr>
<td>2-Cys peroxiredoxin BAS1, (Prx)</td>
<td>TGAGCATGAGGAGCCTTCT</td>
<td>GATCAAGACAGAGCAGCTGA</td>
<td>244</td>
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<tr>
<td>Thioredoxin Type H (Trx)</td>
<td>TGCCGACCTTCATTACATC</td>
<td>TGCATGATATGGAGACA</td>
<td>240</td>
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<tr>
<td>salT (salT)</td>
<td>GGAATATGCCGGTGGTCTCCT</td>
<td>GTCTTGACAGATTGCTGA</td>
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<tr>
<td>Ubiquitin 5 (UBQ5)</td>
<td>ACACCTTCGACCGCCACTACT</td>
<td>ACGCCTAGCCTGCTGGT</td>
<td>69</td>
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</tbody>
</table>

Gel scanning and computer analysis

The CBB-stained gels were scanned using a Cannon scanner (SineGene, USA) at a resolution of 600 dpi. Image editing, spot detection and protein quantification were carried out with 2-Dimension software (SineGene, USA). The gel scanning protocol was first subjected to background subtraction and smoothed to produce a synthetic gel image. The different gel patterns were compared, some spots were manually edited and matched to each other and then the quantities of matched spots were determined. The amount of a protein spot was expressed as the volume, defined as the sum of the intensities of all the pixels that made up the spot. The spot volumes were normalized as percentage of the total volume in all of the spots present in the gel.

Mass spectrometry and database search

The protein spots were excised from the polyacrylamide gels, digested with trypsin and subjected to MALDI-TOF-MS analysis at the Bio Service Unit at the National Science and Technology Development Agency (Bangkok, Thailand). The generations of peak lists of peptide mass fingerprints from raw MS data were conducted by Mascot software (Matrix Science, London, UK, www.matrixscience.com). The acquired peak lists were analyzed by searching NCBI database with the Mascot software.

RNA extraction and RT-PCR assay of gene transcripts

RNA was isolated from leaf tissue previously frozen and ground in liquid nitrogen using the RNeasy plant mini kit (Qiagen, USA) according to the manufacturer’s instructions. The quality and quantity of the isolated RNA was determined using denaturing formaldehyde-MOPS agarose gels and spectrophotometric analysis of the absorbance at 260 nm. cDNA was synthesized from one microgram of RNA treated total RNA using the QuantiTect Reverse Transcription kit (Qiagen, USA) according to the manufacturer’s protocol. The cDNA products were used as templates for PCR amplification of gene transcripts as described in Boxall et al. (2005). The following conditions were used for the PCR reactions: pre-denaturation at 94°C (1 min) followed by 20 to 35 cycles consisting of 30 s at 95°C (denaturing), 30 s at 55°C (annealing), 1 min at 72°C (extension) and a final extension for 7 min at 72°C. 12 pairs of forward and reverse primers were used (Table 1). The primers were designed from the nucleotide sequences of genes encoding ten proteins identified in this study, one house-keeping gene, ubiquitin5 (UBQ5) (Jain et al., 2006) and a previously reported salt-regulated gene (salT) (Claes et al., 1990) using Primer3 (http://frodo.wi.mit.edu/primer3/). The PCR products from RT-PCR amplifications were separated on 3% (w/v) agarose gels and stained with ethidium bromide. The specificity of the primers was confirmed from the size of the PCR band and through cloning (Topo TA, Invitrogen) and sequencing the PCR products.
RESULTS

Protein expression in leaves

Representative 2DE profiles of leaf proteins from control and salt-stress seedlings of KDML 105 and Pokkali are shown in Figures 1 and 2, respectively. The total number of well-separated and reproducible CBB-stained protein spots was lower in KDML 105 (200) than Pokkali (350), but matched reproducibly among triplicate gels. From the difference map generated by the 2-Dimension software, more than 100 protein spots showed different intensities between the control and salt-treated conditions. Proteins differing by at least 3-fold in average intensity between the control and the salt-treated group were recorded and confirmed by visual inspection of the stained gels. For KDML 105, salinity treatment induced 25 differential protein spots, 16 up-regulated and nine down-regulated while in Pokkali, 32 differential spots were detected of which 25 and seven were up-regulated and down-regulated, respectively. A total of 24 differential protein spots (7 from KDML 105 and 17 from Pokkali, indicated by arrows in Figures 1 and 2) were excised from three replicate gels and analyzed. Of these, 12 spots matched the known proteins with significant (P ≤ 0.05) scores (Table 2). The number of matched peptides ranged from 4 to 13, and percent sequence coverage from 19 to 51%.

All identifications showed a good correlation of theoretical and experimental pl and molecular weight (MW). Three salt-induced proteins (number 1 to 3) (Figure 1) were identified in KDML 105, while nine proteins (number 4 to 12) (Figure 2) were more abundant following NaCl stress in Pokkali. For the remaining 12 spots, no significant matches were found due to either a mixture or a limited amount of protein recovered from the gels.

The majority of the proteins identified as increased in the salt-stressed rice seedlings were associated with a function in photosynthesis, oxygen evolution and the Calvin cycle; others were involved in photorespiration and antioxidant defense systems. In KDML 105, the two that increased in amount were identified as the 23 kDa polypeptide of photosystem II (PSII-23) and Rubisco activase (RCA). The third, which decreased following salt-stress, was identified as ribulose bisphosphate carboxylase/oxygenase large subunit (Rubisco). The nine proteins identified in Pokkali were Rubisco, PSII-23, putative oxygen evolving complex protein (OEC18), fructose-bisphosphate aldolase chloroplast precursor (FBA), sedoheptulose 1,7-bisphosphatase precursor (SBP), 40 kDa thylakoid lumen PPiase (TLP40), phosphoglycolate phosphatase (PGP), 2-cys peroxiredoxin chloroplast precursor protein (Prx) and thioredoxin type H (Trx).

Effects of salt stress on gene expression in rice leaves

The mRNA encoding the ten proteins identified by proteomics, plus two additional control genes (salt-regulated gene, saI T and the house-keeping gene ubiquitin5, UBQ5), was amplified using RT-PCR. Transcripts of all 12 genes were detected in both cultivars (Figure 3). In KDML 105, the relative abundance of FBA, SBP, TLP40, PGP, Trx and RCA mRNA increased in the saline conditions, while the amounts of PSII-23, OEC18, Prx and Rubisco remained the same as in the control (Figure 3a). In Pokkali, the abundance of all transcripts but Rubisco increased (Figure 3b), consistent with the corresponding proteins. Transcripts of the positive control gene saI T were salt-induced in both varieties but more so in KDML 105 while the abundance of transcripts encoding the control house-keeping gene UBQ5 differed little between control and salt-stressed tissues (Figure 3c).

DISCUSSION

Salt-stressed rice leaf proteome

We compared the salt-responsive proteomes from the leaf lamina of two contrasting rice varieties. The majority of proteins identified at increased levels in the saline growth medium in the salt-tolerant cultivar Pokkali were those related to photosynthesis, photorespiration and defense against oxidative stress. Of the three that could be identified in KDML 105, the levels of two (PSII-23, RCA) increased in saline conditions while the level of Rubisco was dramatically decreased (spot number 2) (Figure 1) in contrast to the substantially increased level in Pokkali (spot number 11) (Figure 2). The reduction in Rubisco under salt stress directly affects photosynthetic efficiency and growth of salt-sensitive rice genotypes (Singh et al., 2007). The up-regulation of proteins involved in photosynthesis (PSII-23, OEC18, Rubisco, FBA and SBP; Figure 2) in the tolerant genotype Pokkali points to a higher adaptive ability to adjust the efficiency of its photosynthetic machinery in response to salt stress (Cha-um et al., 2010).

Similarly, the increased phosphoglycolate phosphatase (spot number 9, Figure 2), which is the first enzyme in the photorespiratory pathway in Pokkali may indicate
Figure 1. 2DE-PAGE analysis of proteins extracted from leaves of rice cv. KDML 105. 100 µg of proteins from control plants was loaded on IPG strips with a linear gradient of (a) pH 3 to 10 and (c) pH 4 to 7. 100 µg of proteins from salt-stressed plants was loaded on IPG strips with a linear gradient of (b) pH 3 to 10 and (d) pH 4 to 7. The second dimensional separation was performed using a 12.5% polyacrylamide gel stained with CBB. Proteins showing differential amount between the control and salt-stressed conditions are shown in circles, while those excised for peptide mass fingerprint analysis indicated by arrows and those identified are numbered.

Stimulation of photorespiration as an electron sink to help minimize reactive oxygen species (ROS) production provoked by the increased photosynthetic flux. The low internal CO$_2$ concentration in salt-stressed leaves due to a response of stomata closure, can lead to enhanced rates of photorespiration (Rajmane and Karage, 1986; Fedina et al., 1994). This is an important mechanism for energy dissipation in order to prevent photoinhibition occurring through damage to chloroplast components (Osmond et al., 1997) as well as generating glycine for the synthesis of glutathione, a component of the oxidative stress response system (Noctor et al., 1999).

Salt stress imposed on rice plants is known to induce a secondary oxidative stress (Demiral and Türkan 2005; Kim et al., 2005; Parker et al., 2006). The increased levels of proteins involved in defense against oxidative stress (for example, Prx and Trx) in Pokkali (spot number 10 and 12) (Figure 2) are likely to contribute to more efficient ROS detoxification and thus reduce cellular damage (Vaidyanathan et al., 2003; Dooki et al., 2006).
The increase in TLP40 protein, a cyclophilin with a proposed role in maintenance of the photosynthetic complexes (Peltier et al., 2002) may also contribute to maintenance of metabolic activities. This is consistent with the visual observation of more pronounced leaf-yellowing in the salt-sensitive KDML 105 plants.

Earlier proteomic analyses of the salt-stress response in the rice leaf lamina have reported changes in the abundance of proteins with functions similar to those found in this study. Comparing 2DE profiles of rice leaves (cv. Nipponbare; moderately salt-tolerant), Kim et al. (2005) detected 55 differentially-expressed CBB-stained spots, of which 47 were increased over the control. They were able to identify 33, most of which were involved in major metabolic processes related to photosynthetic carbon assimilation and photorespiration. Our study has also identified an increased level of several of these in Pokkali (Rubisco, FBA, PGP, SBP) as well as proteins of the photosystem II complex (PSII-23, OEC18) while only the PSII-23 protein and RCA were detected at elevated levels under saline conditions in KDML 105. Considering the central role of all these enzymes in carbon metabolism and energy transduction, their increased abundance in Pokkali under salt stress in our study most
Table 2. Identification of salt responsive proteins in the leaves of rice seedlings cv. KDML 105 (spot 1 to 3) and cv. Pokkali (spot 4 to 12) through MALDI - TOF MS.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>Matched protein</th>
<th>Score</th>
<th>Measured MW/pI</th>
<th>Predicted MW/pI</th>
<th>Sequence coverage (%)</th>
<th>Matched peptide</th>
<th>Intensity ratio</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23 kDa polypeptide photosystem II (PSII-23)</td>
<td>81</td>
<td>26.89/6.41</td>
<td>27.09/8.66</td>
<td>45</td>
<td>10</td>
<td>+4</td>
<td>NP - 001058863</td>
</tr>
<tr>
<td>2</td>
<td>Rubisco large subunit (Rubisco)</td>
<td>67</td>
<td>62.30/6.66</td>
<td>53.44/6.04</td>
<td>25</td>
<td>13</td>
<td>-7</td>
<td>ABA96140</td>
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<tr>
<td>3</td>
<td>Rubisco activase (RCA)</td>
<td>90</td>
<td>27.12/4.22</td>
<td>21.73/4.78</td>
<td>39</td>
<td>7</td>
<td>+5</td>
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<tr>
<td>4</td>
<td>23 kDa polypeptide of photosystem II (PSII-23)</td>
<td>67</td>
<td>26.36/7.18</td>
<td>27.17/9.06</td>
<td>32</td>
<td>8</td>
<td>+4</td>
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<td>5</td>
<td>putative oxygen evolving complex protein (OEC18)</td>
<td>104</td>
<td>19.82/8.24</td>
<td>19.77/7.88</td>
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<td>6</td>
<td>+3</td>
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<tr>
<td>6</td>
<td>Putative fructose-bisphosphate aldolase, chloroplast precursor (FBA)</td>
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<td>43.6/5.64</td>
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<td>Phosphoglycolate phosphatase (PGP)</td>
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<td>4</td>
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likely reflects altered patterns of carbon flux that may not be achieved successfully by KDML 105. The fact that we could not detect a similar elevation of most of these proteins in KDML 105, and indeed observed a reduction in Rubisco in saline conditions, may indicate why it is so sensitive to salt stress. We therefore further compared

Figure 3. Comparison of relative transcript abundance for genes identified via 2DE-PAGE in leaves from (A) KDML 105 and (B) Pokkali under salt-stress for 7 days (shaded) and control (open). The histogram shows mean relative transcript abundance from three biological replicates (+/− standard error) normalized to UBQ5. Representative semi-quantitative RT-PCR results are shown beneath (C) for salt stressed (s) and control (c) samples.
the levels of mRNA transcripts for the proteins to determine whether there were also differences between the cultivars at this level of gene expression.

Relationship between changes in the level of transcripts and proteins

In KDML 105, the levels of several transcripts were unaltered in saline growth conditions (PSII-23, OEC18, Prx, Rubisco) while levels of the remainder increased. This contrasts with the proteins of KDML 105 identified in our study. Levels of RCA strongly increased at both mRNA and protein level in saline conditions, whereas PSII-23 was clearly up-regulated at the protein level but its mRNA abundance was unchanged. The level of Rubisco transcript was unaltered by salinity even though the amount of protein decreased. In salt-stressed KDML 105, an increase in the relative transcript abundance was obtained for several genes (FBA, SBP, Trx) where the protein products were not detected. In Pokkali, the corresponding mRNA transcripts encoding all nine up-regulated proteins were increased. The level of transcripts of RCA, where the protein was not detected, also increased under saline conditions. Similarly, in both KDML 105 and Pokkali, the levels of the control salt-regulated transcript SalT increased. This lack of correlation has been reported by Malakshah et al. (2007) in which they found no concordance between the changes in levels of transcripts and proteins of three salt-responsive genes (remorin, Hir and 14-3-3 protein) in rice roots. The elevated transcript abundance for important photosynthetic and protective genes in even unstressed Pokkali leaves may provide this salt-tolerant rice variety with better innate adaptation to salt stress compared to the sensitive KDML 105. A similar observation was found in a recent comparative transcriptome map of salinity stress response between Pokkali and IR64, a salt-sensitive cultivar (Kumari et al., 2009). These genes included functions in signaling (such as calmodulin binding protein and the zinc finger transcription factor, OSAP1), ion transport (including the vacuolar H⁺-ATPase and a voltage-dependent anion channel), protective proteins (such as late embryogenesis abundant proteins and glutathione-S-transferase II) and proteins with roles in photosynthesis (Rubisco small subunit).

In conclusion, elevated levels of proteins involved in photosynthesis, photorespiration and the oxidative stress detoxification system play important roles in conferring tolerance to salt stress in the rice cultivar Pokkali by providing more efficient metabolic readjustment, thus leading to higher photosynthetic efficiency and hence a better growth performance. In the economically important but salt sensitive variety KDML 105, transcripts of some genes examples are FBA, SBP, TLP40, PGP, Trx and RCA; were up-regulated upon salt stress and increase in only two proteins could be detected (PSII-23, RCA). In addition, levels of the major leaf protein Rubisco decreased in KDML 105, an observation that is frequently observed after tissue damage. This provides evidence that although KDML 105 expresses genes for several proteins needed for salt tolerance, it is unable to induce these stress-responsive processes at both transcriptional and post-transcriptional levels as effectively as Pokkali. With this information, the challenge is how to understand the regulatory basis for these differences. Improve-ments to salinity tolerance in economically important cultivars like KDML 105 will require a comprehensive and integrated knowledge of the transcriptomic, proteomic and metabolomic responses to salt. Such large scale functional genomics studies should allow the key regulators of salt-tolerance to be elucidated and thus facilitate attempts to either breed or engineer these traits into salt-sensitive cultivars like KDML 105.

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REFERENCES


