Protein alterations associated with salinity, desiccation, high and low temperature stresses and abscisic acid application in Lal nakanda, a drought-tolerant rice cultivar

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Lal nakanda is a drought-tolerant rice cultivar. We have identified 95 steady-state high and low molecular weight proteins which are up-accumulated (such as those with molecular weights of 102, 100, 87, 85, 55, 44, 43.5, 43, 41.7, 39, 36, 32, 31, 29, 26, 24, 23.8, 23, 21.5, 19, 18.2, 16.8 and 16.2 kDa in shoots and 100, 91, 87, 85, 81, 78, 63, 60, 52, 40.5, 31 and 26 kDa in roots) or down-accumulated (such as those with molecular weights of 81, 58 and 10.2 kDa in shoots and 24, 22.5, 19, 16, 15.5, 15.2, 14.2 and 13.8 kDa in roots) in this rice cultivar when intact seedlings are subjected to salinity (NaCl), air drying and high and low temperatures. Several proteins (such as those with molecular weights of 100, 91, 87, 85 and 78 kDa) were found to be co-regulated in response to the above stresses. On the other hand, proteins specific to a given type of stress (such as 15 and 13 kDa in response to salinity stress; 60 and 10 kDa in response to desiccation stress and 104, 93 and 76 kDa in response to high temperature stress) were also noticed. Exogenous application of abscisic acid mimicked several of the protein perturbations caused by the imposition of stresses.

ABiotic stresses such as salinity (SS), desiccation (DS), low temperature (LS) and high temperature (HS) stresses adversely affect rice cultivation to a significant extent.

The extent of damage caused by these stresses varies depending upon, amongst various parameters, the genotypic make-up of the rice plant

Various rice cultivars bred by the plant breeders are shown to be differentially affected by these stress conditions

We are working towards understanding the molecular basis of the stress responses shown in rice plants in response to the above stresses, with the ultimate aim of isolating new genes which would be important in improving stress tolerance of this crop by plant genetic engineering methods

Analysis of protein profiles before and after stress treatments is an important approach for the identification of stress-responsive genes. In this communication, we report stress-associated protein alterations in Lal nakanda, a drought-tolerant rice cultivar.

Details of seed germination conditions, stress treat-
16.8 and 16.2 kDa in shoots and 100, 91, 87, 85, 81, 78, 63, 60, 52, 40.5, 31 and 26 kDa in roots were accumulated in response to ABA application as well as in response to all stresses tested in this study. These proteins might involve ABA as a signal transduction component. (ii) However, some ABA-associated protein alterations were shared by specific stress conditions. For example, 25, 23.5 and 17 kDa proteins were accumulated

Figure 1. Electrophoretic profiles of the high molecular weight proteins of the shoots of rice seedlings as resolved on 7.5% uniform acrylamide concentration SDS-gel in response to various stress treatments. 20 μg crude protein was loaded in each lane and the gel was stained with silver nitrate. Proteins marked with asterisk (*) are those which decline in response to the stress treatments and those not marked with asterisk increase during the stress treatments. Numbers shown with various marks are the molecular weights (in kDa) of proteins. Duration of each treatment is shown at the top of each lane (ABA was given for 24 h). Positions of the standard molecular weight markers are shown towards the left side of the Figure. C: control.

Figure 2. Electrophoretic profiles of the low molecular weight proteins of the shoots of rice seedlings as resolved on 15-22% linear gradient acrylamide SDS-gel in response to various stress treatments. Other details are same as in Figure 1.
in response to ABA as well as HS in shoot tissues. There are several previous reports highlighting such patterns in gene expression. In rice, leaf-specific WcS 19 gene is shown to be induced (by light) during acclimation to low temperature but is not affected by ABA. Taken together, this analysis may indicate that the role(s) of ABA may be diverse and there might be multiple signal transduction pathways for stress-responsive genes/proteins which may or may not involve ABA.

On the whole, 33, 38, 31 and 40 polypeptides showed

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![Figure 3. Electrophoretic profiles of the high molecular weight proteins of the roots of rice seedlings as resolved on 7.5% uniform concentration acrylamide SDS-gel in response to various stress treatments. Other details are same as in Figure 1.](image)

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![Figure 4. Electrophoretic profiles of the low molecular weight proteins of the roots of rice seedlings as resolved on 15–22% linear gradient acrylamide SDS-gel in response to various stress treatments. Other details are same as in Figure 1.](image)
alteration in their level in response to SS, DS, LS and HS, respectively, in shoots (Figures 1 and 2). The equivalent number of polypeptides were 23, 28, 19 and 27 for SS, DS, LS and HS respectively, in root samples (Figures 3 and 4). From the general picture of stress response emerging from this study, two points are noteworthy. Firstly, numerically speaking, more protein alterations were scored in shoots than roots for all the stresses. Similar observations were made in Pusa 169 rice cultivar. Secondly, while some of the stress proteins noted in this study matched in molecular weights to those identified by earlier workers, it appears that majority of proteins noted here have not been analysed previously. It is possible that the latter class of proteins represent novel stress proteins of rice.

The intra-species variations in salt response have been reported in several crop species. The understanding of the underlying genetical basis of such variants can provide useful clues. It can help in finding (i) whether differential gene expression changes are the basis of variations in salt response and (ii) the relative value of the constitutive and inducible gene expression changes in controlling salt-tolerance. Comparative account of the protein alterations in Pusa 169 (ref. 15) and Lal nakanda (this study) seedlings revealed several important observations. The number of polypeptides which showed altered patterns in response to different stresses were 95 in Lal nakanda while 73 steady-state proteins were found to be either up- or down-regulated in response to various stresses in cultivar Pusa 169. Further, polypeptides of 102, 43.5, 43, 25, 24, 23.8, 19, 17, 16, 12 and 10 kDa in shoots and 26, 25.5, 24, 19.5, 16, 15.2, 14.2 and 13.8 kDa in roots were specific to Lal nakanda cultivar. On the other hand, polypeptides of 112, 48, 37, 33, 30, 22.5 and 18.6 kDa in shoots and 27.5 kDa in case of root tissues were specific to Pusa 169. In both the cultivars, maximum number of protein alterations were noted in the case of HS while minimum number of protein alterations were noted in the case of LS. Importantly, the protein profiles of the uninduced (control) shoot and root tissues of the two cultivars were by and large similar. Detailed characterization of stress proteins in these two cultivars may shed further light on the mechanism(s) of stress tolerance and provide clues on gene/proteins responsible for imparting stress tolerance.