Analysis of stress proteins at four different developmental stages in field-grown rice, *Oryza sativa* L. (cv. Pusa 169), plants

Ashwani Pareek*, Sneh Lata Singla** and Anil Grover*

Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi 110 021, India

**Present address: Department of Genetics, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi 110 021, India

Grain yield in rice plant is adversely affected due to salinity, water stress and low and high temperature stresses. These stress conditions affect the rice plants differentially at their different developmental stages. We have analysed alterations at cellular level of the various stress-associated proteins at four developmental stages in a high-yielding rice (cv. Pusa 169). These growth stages corresponded to 30-day-old (vegetative stage), 55-day-old (maximum tillering stage), 90-day-old (boat-leaf stage), and 110-day-old (seed-setting stage) plants. The sum total of polypeptide alterations was found to be different at the various growth stages analysed. While the alterations in the levels of some proteins were found to be common at the different stages (such as those with molecular weights of 30, 22.5, 22 kDa in response to salinity; 62, 60, 18.2 kDa in response to desiccation and 87 and 20 kDa in response to high temperature), alterations in levels of some other proteins showed growth stage-dependent difference. Elucidation of the precise identity of these proteins would possibly yield useful information on the response of rice plants to different abiotic stresses.

*For correspondence. (e-mail: pmgb@uscernet.in)

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DS, LS, HS, and ABA treatments, protein extraction and analysis were similar to those employed for analysis of Pusa 169 seedlings.

It has been often observed that the response of a plant to a given stress varies with the different growth stages, i.e. a stress-tolerant plant is not necessarily tolerant at all the growth stages, and likewise, a stress-sensitive plant is not necessarily sensitive at all the growth stages. Such observations are based on certain physiological/biochemical parameters such as reduction in growth rate and perturbations in metabolic activities including specific activities of different enzymes. In this study, we have made attempts to highlight how the stress-induced protein alterations (which represent products of altered gene expression patterns) vary with the growth stages. This analysis was carried out on cv. Pusa 169, a high-yielding rice type, and the four growth stages analysed corresponded to day 30, day 55, day 90 and day 110 after sowing. It is important to note here that while the leaves were harvested from the field-grown uninduced plants, stress conditions were imposed on the excised leaves in the laboratory in this work.

Figures 1–8 show data on alterations in the steady-state protein profiles at the various stages analysed in response to application of different stresses. Figures 1, 3, 5 and 7 represent the prominent protein changes, and the
Figure 3. Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 55-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS: 5 ± 2°C, 96 h; HS: 45°C, 6 h; and ABA: 10⁻⁴ M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase), while those marked with an asterisk show a decline. (b) Analyses of low-molecular weight proteins as revealed by 15-22% linear gradient SDS polyacrylamide-gel. The other details are same as in the panel a.

Figure 4. Venn diagram showing proteins which are altered in response to various stresses in the topmost leaf of field-grown 55-day-old rice plants (cv. Pusa 169). This analysis is based upon the alterations in steady-state proteins as revealed by 7.5% uniform-concentration and 15-22% linear acrylamide gradient SDS gels. Molecular weight (kDa) of individual stress proteins is shown; those marked with asterisk (*) decline in response to stresses while others increase in response to the stress treatments.

molecular weights of the protein alterations are shown on the right side of each panel in the figures. As is clear from these figures, the protein alterations ranged in molecular weight from as low as 15 kDa (in response to DS) to as high as 104 kDa (in response to HS). The results of this analysis have been summarized as Venn diagrams for each growth stage (Figures 2, 4, 6 and 8). In response to SS (200 mM NaCl, 96 h), 13 polypeptides showed alterations at the stage A, 18 at the stage B, 14 at the stage C, and 9 at the stage D. Importantly, alterations in certain specific polypeptides showed similar pattern in response to a given stress signal at all the stages. These include polypeptides with molecular weights of 30, 22.5, 22 kDa in response to SS; 62, 60, 18.2 kDa in response to DS; 87 and 20 kDa in response to HS; and 60 kDa in response to ABA application. However, no LS-responsive polypeptide was found conserved at the different stages analysed in this study. On the other hand, alterations in levels of certain specific polypeptides were found to be unique to a given stage of growth. For instance, accumulation of 64, 44, 24 and 16.2 kDa polypeptides in response to SS was noted only at the stage B. Further, specific polypeptide alterations were detected at either the early or the late stage of plant development but were not observed at all the stages. An example in this category includes a
Figure 5. a, Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 90-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS: 5 ± 2°C, 96 h; HS: 45°C, 6 h; and ABA: 10⁻⁴ M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase, while those marked with □ show a decline). b, Analyses of low-molecular weight proteins as revealed on 15–22% gradient SDS-polyacrylamide-gel. The other details are same as in the panel a.

25.5 kDa polypeptide which accumulated in response to HS as well as LS at growth stages B, C and D but was not seen at growth stage A. On the other hand, 85 and 81 kDa polypeptides were accumulated at growth stages A, B and C but were not noted at growth stage D.
Figure 7. *a,* Analyses of various high-molecular weight proteins which are altered in the topmost leaf of field-grown 110-day-old rice plants (cv. Pusa 169) in response to various stress treatments (SS: 200 mM NaCl, 96 h; DS: air drying, 16 h; LS: 5 ± 2°C, 96 h; HS: 45°C, 6 h; and ABA: 10⁻⁴M, 24 h) as revealed by 7.5% uniform-concentration SDS-gel following silver staining. The position of standard molecular weight markers is shown on the left side of the panels. The numbers marked on the right side of each panel indicate the molecular weight (kDa) of various proteins altered in response to a given stress treatment (those marked with an arrowhead show an increase, while those marked with * show a decline). *b,* Analyses of low-molecular weight proteins as revealed by 15–22% gradient SDS polyacrylamide-gel. The other details are same as in the panel *a.*

Figure 8. Venn diagram showing proteins which are altered in response to various stresses in the topmost leaf of field-grown 110-day-old rice plants (cv. Pusa 169). This analysis is based upon the alterations in steady-state proteins as revealed by 7.5% uniform-concentration and 15–22% linear acrylamide gradient SDS gels. Molecular weight (kDa) of individual stress proteins is shown in the Figure; those marked with asterisk (*) decline in response to stresses, while others increase in response to the stress treatments.

From the data presented, it is clear that stress-responsive alterations in protein pattern show a clear growth stage-independent as well as growth stage-dependent response. Such studies may provide a lead for understanding the basis of developmental controls on stress-responsive gene expression patterns. Future studies must focus on the precise biochemical identity of various stress proteins. Once this information is available, it should be possible to work out the various components of the signal transduction cascades responsible for the altered patterns with respect to the developmental cues.

Angiotensin converting enzyme inhibitors from ripened and unripened bananas

N. Mallikarjuna Rao, K. V. S. R. G. Prasad* and K. S. R. Pai*

Departments of Biochemistry, and *Pharmacology, Kasturba Medical College, Manipal 576 116, India

Ripened and unripened nendran, rasthali, poovan, robusta, bontha and safed velchi bananas were investigated for inhibition against angiotensin converting enzyme (ACE) using Hip-His-Leu as substrate. The inhibition of ACE by different ripened banana cultivars was much more than that of unripened banana cultivars. The ACE inhibitory activity of ripened and unripened poovan was heat stable and stable to extreme acidic pH and alkaline pH. The ACE inhibitory activity of ripened and unripened banana cultivars was reduced to 25% and 33% respectively on dialysis.

The angiotensin converting enzyme (ACE) is a dipeptidyl carboxypeptidase (EC 3.4.15.1) and plays an important role in the regulation of blood pressure. Several potent inhibitors of this enzyme have been reported to be orally active antihypertensive agents. ACE inhibitors derived from casein, sardines, tuna, bonito and maize protein are shown to be effective in lowering blood pressure. Since these food themselves lacked ACE inhibition and only their derivatives showed ACE inhibitory activity, their antihypertensive role is difficult to assess. Since ripened bananas are consumed raw and unripened bananas are cooked, studies on ACE inhibitors from bananas are helpful in establishing antihypertensive role in food stuffs. Potato, a well known source of serine and cysteine protease inhibitors, contains carboxypeptidase inhibitors. Earlier study from this laboratory indicated the presence of serine and cysteine protease inhibitors in ripened and unripened bananas. In addition, bananas have been reported to be useful in the treatment of hypertension and other cardiac diseases in the indigenous system of medicine in India. A recent report indicated that feeding of ripened banana to rats prevented an increase in blood pressure induced by deoxycorticosterone.

In view of the above-mentioned observations, an attempt was made to identify ACE inhibitors from ripened and unripened bananas consumed all over the world. This communication reports the presence of inhibitors to ACE in six ripened and unripened banana cultivars and some of their properties.

Ripened and unripened bananas nendran-Musa (AB), rasthali-Musa (ABA), poovan-Musa (BAA), robusta-Musa (AAA), bontha-Musa (ABB) and safed velchi-Musa (AB) were procured from local sources. Angiotensin converting enzyme was prepared by using a modification of the method described by Cushman and Cheung. Albino rats of both sexes were used in these experiments. After the animals were decapitated, the lungs were isolated and washed with ice-cold 100 mM borate buffer, pH 8.3, containing 50 mM KCl and frozen until further use. One g of lung tissue was diced and homogenized in 10 ml of the same ice-cold buffer using a motor driven teflon/glass homogenizer at 4°C. The homogenate was centrifuged at 20000 g for 20 min at 4°C. The supernatant was collected and dialysed for 12 h against 20 volumes of the same buffer at 4°C to remove endogenous low molecular weight inhibitors. The dialysed supernatant was used as the enzyme source for angiotensin converting enzyme. Protein content of the supernatant was measured by the method of Lowry et al. using bovine serum albumin as standard. Banana extract was prepared by homogenizing 5 g of ripened or unripened banana without the outer skin in 5 ml of 50 mM borate buffer, pH 8.3. The homogenate was centrifuged at 10000 g for 20 min at 4°C. The supernatant was collected and tested for inhibitory activity.

ACE activity was measured by modification of the method described by Schnaith et al. using hippuryl-L-histidyl-L-leucine (HHL) as substrate. The reaction mixture contained 0.2 ml of 5 mM HHL prepared