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[1,2]. The extent of damage caused by these stresses varies depending upon, amongst various parameters, the genotypic make-up of the rice plant[3]. Various rice cultivars bred by the plant breeders are shown to be differentially affected by these stress conditions[4]. We are working towards understanding the molecular basis of the stress responses shown in rice plants in response to the above stresses[5–10], with the ultimate aim of isolating new genes which would be important in improving stress tolerance of this crop by plant genetic engineering methods[11–13]. Analysis of protein profiles before and after stress treatments is an important approach for the identification of stress-responsive genes[11,12]. In this communication, we report stress-associated protein alterations in Lal nakanda, a drought-tolerant rice cultivar[14].

Details of seed germination conditions, stress treatments as well as protein extraction and analysis in the present work were similar to those employed for analysis of soluble proteins in Pusa 169 rice cultivar[15].

Levels of a large number of proteins were altered upon exposure of the seedlings of Lal nakanda cultivar to different abiotic stresses. Molecular weights of the prominent protein alterations are shown on the right side of each panel in Figures 1–4. The protein alterations in this cultivar ranged in molecular weights from as low as 10.2 kDa (in response to SS, DS, LS and abscisic acid (ABA)) to as high as 123 kDa (in response to SS and ABA application). To reveal the relationship amongst different stresses with respect to protein changes, protein alterations scored for different stresses are presented in the form of Venn diagrams, separately for the shoot and root tissues (Figures 5 and 6).

Several proteins showed overlapping patterns with respect to their stress-inducibility. These overlapping patterns stretched from two stresses to a whole range of stress conditions for individual proteins. Stress proteins 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 were accumulated in response to various stresses (Figures 5 and 6). A set of five proteins in the molecular weight range of 80 to 100 kDa (i.e. 100, 91, 87, 85 and 78 kDa) showed prominent and parallel accumulation in response to high temperature (HS) as well as other stress conditions (Figures 1 and 3). The co-inducibility of specified genes/proteins has earlier been seen by several other groups in rice[5,16–19]. From such studies, it should be possible to derive information on regulatory elements which enable different genes to respond to stresses in a coordinated/non-coordinated manner. Previously, it has been reported that tolerance to one type of stress often leads to cross-tolerance against the related stress type[20,21]. These common proteins may also provide molecular basis to the cross-adaptability phenomenon. Apart from the proteins which were co-triggered, several proteins were noted to be specific to a given stress type. In shoots, 15 and 13 kDa polypeptides accumulated specifically in response to SS. Equivalent polypeptides for DS were of 60 and 10 kDa. The 104, 93 and 76 kDa polypeptides were specifically accumulated in response to HS. Similar observation have been made for the Pusa 169 cultivar in a related study[15]. It is possible that the proteins unique to a given stress signal have role(s) in governing stress-specific cellular responses.

Multiplicity of ABA action in eliciting stress-specific genes has been shown in rice[5,8,17–19,22,23]. Application of ABA to intact rice seedlings resulted in two kinds of protein alterations. (i) The polypeptides 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,

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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

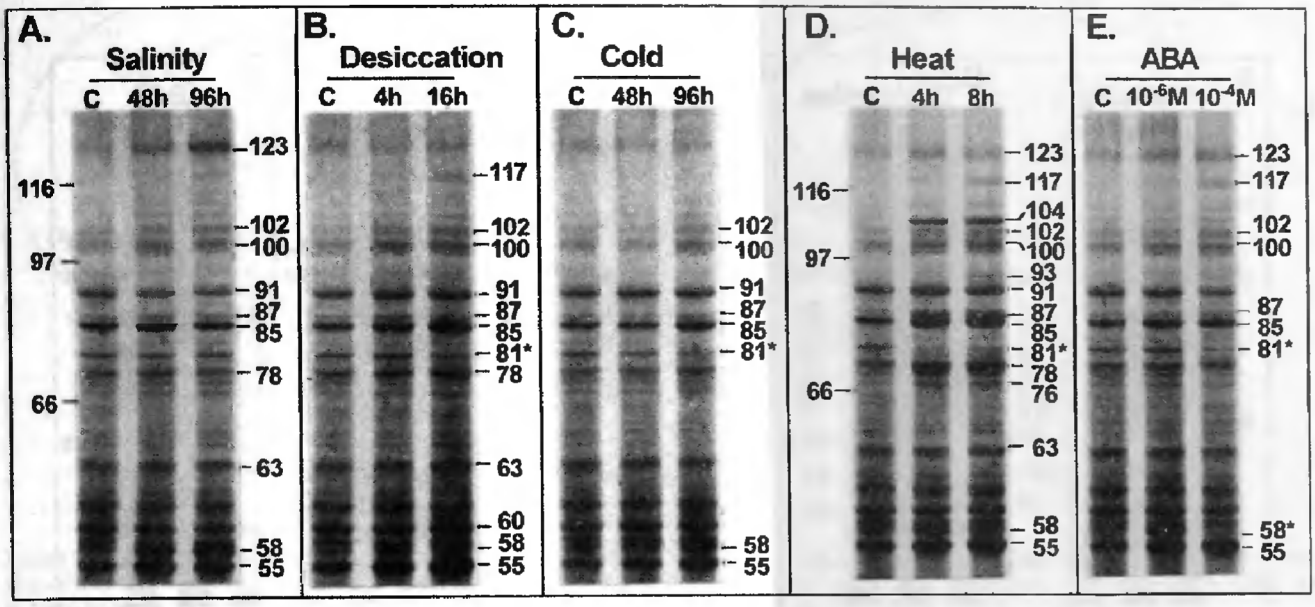

Figure 1. Electrophoretic profiles of the high molecular weight proteins of the shoots of rice seedlings as resolved on 7.5% uniform acrylamide concentration SDS-gel in response to various stress treatments. 20 µg crude protein was loaded in each lane and the gel was stained with silver nitrate. Proteins 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.

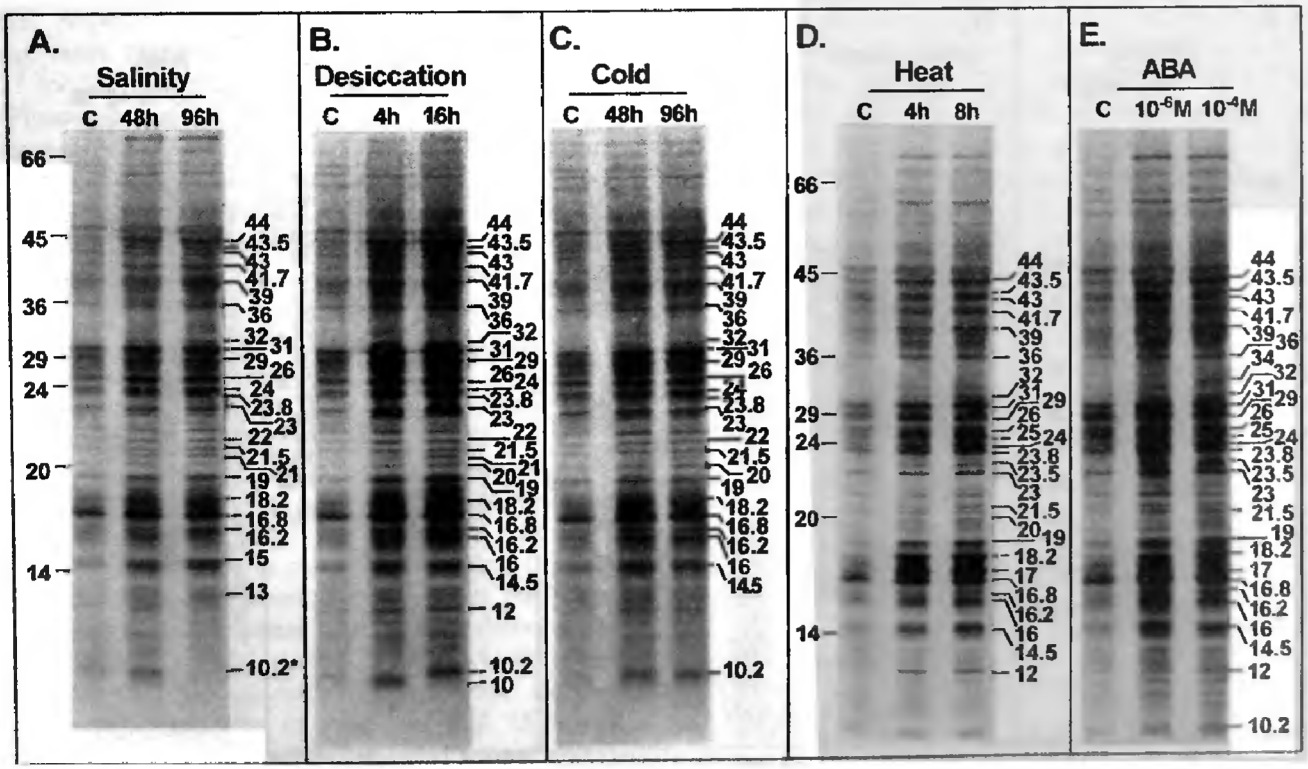

Figure 2. Electrophoretic profiles of the low molecular weight proteins of the shoots of rice seedlings as resolved on 15–22% linear gradient acrylamide SDS-gel in response to various stress treatments. Other details are same as in Figure 1.

in response to ABA as well as HS in shoot tissues. There are several previous reports highlighting such patterns in gene expression[24–27]. 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[28]. 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

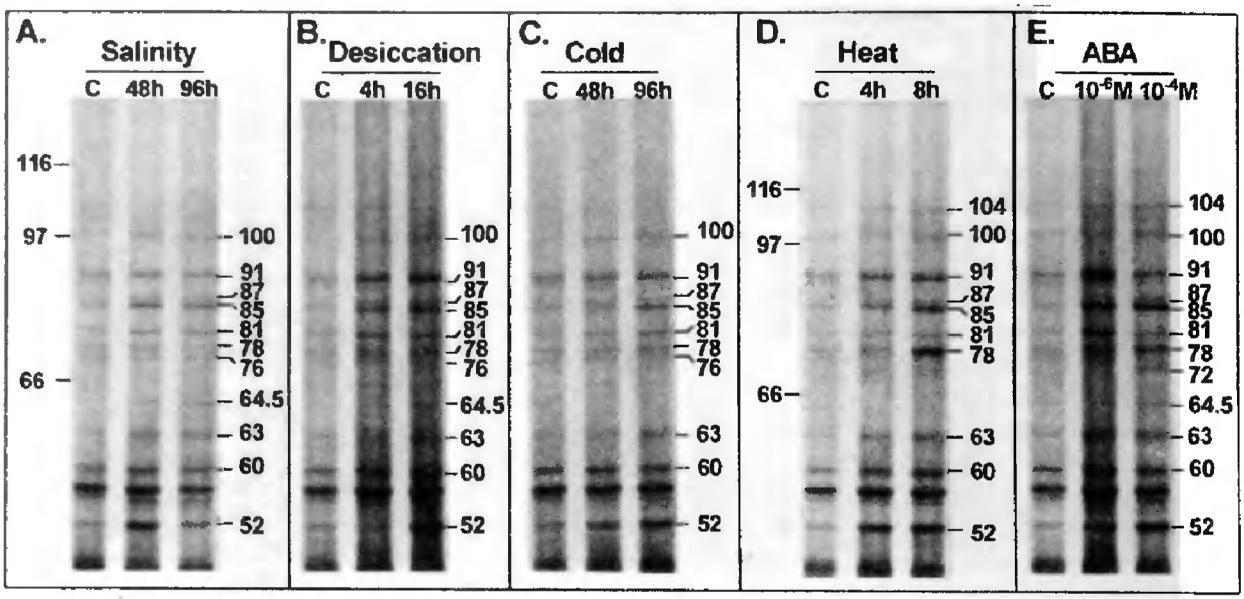

Figure 3. Electrophoretic profiles of the high molecular weight proteins of the roots of rice seedlings as resolved on 7.5% uniform concentration acrylamide SDS-gel in response to various stress treatments. Other details are same as in Figure 1.

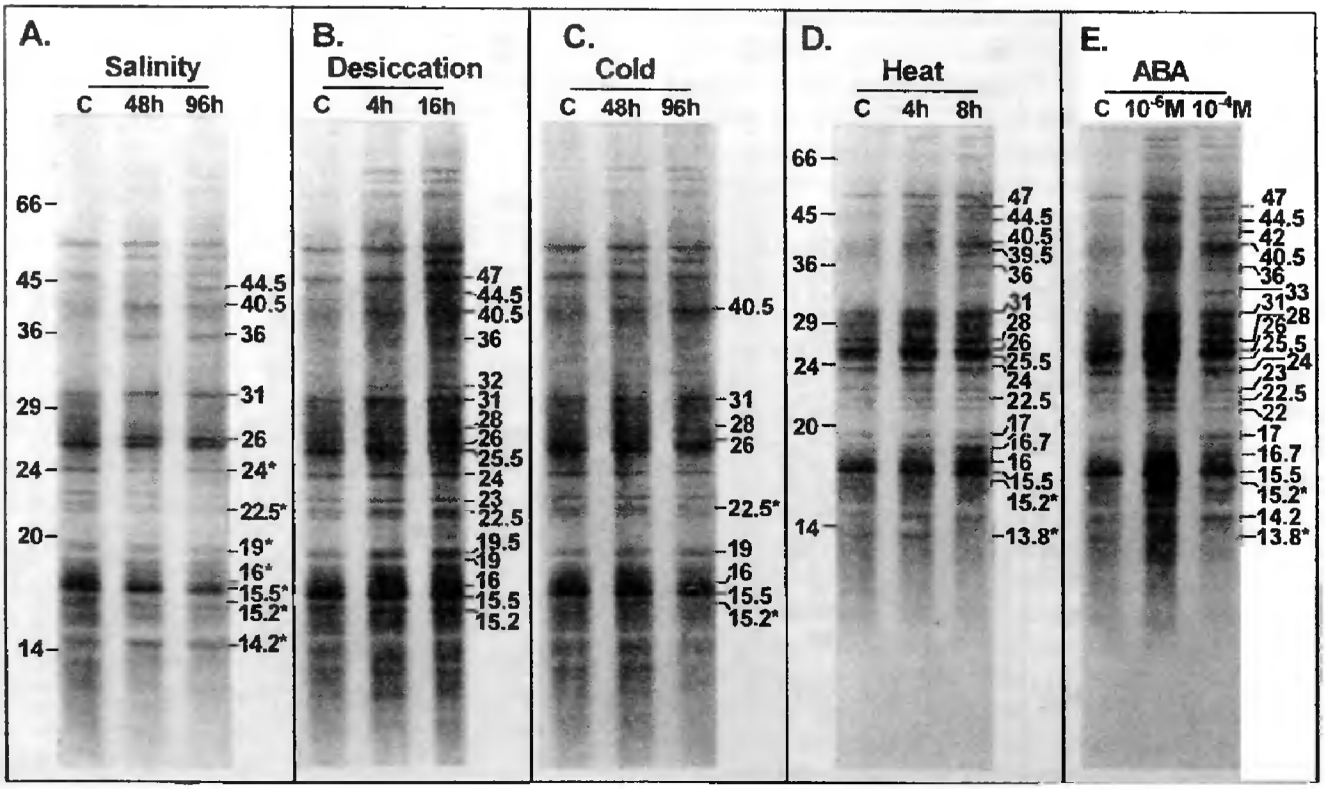

Figure 4. Electrophoretic profiles of the low molecular weight proteins of the roots of rice seedlings as resolved on 15–22% linear gradient acrylamide SDS-gel in response to various stress treatments. Other details are same as in Figure 1.

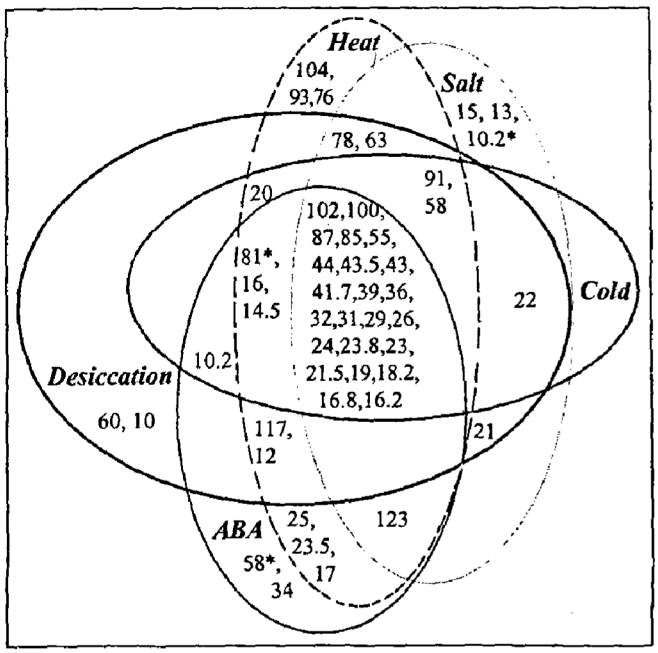

Figure 5. Venn diagram showing proteins which accumulate in response to various stresses in shoots of rice seedlings. This analysis is based upon the alterations in steady state protein profiles as revealed by 7.5% uniform concentration and 15–22% linear acrylamide gradient SDS-gel. Molecular masses (kDa) of individual stress proteins are shown, those marked with asterisk (*) decline in response to stress while others increase in response to stress treatment.

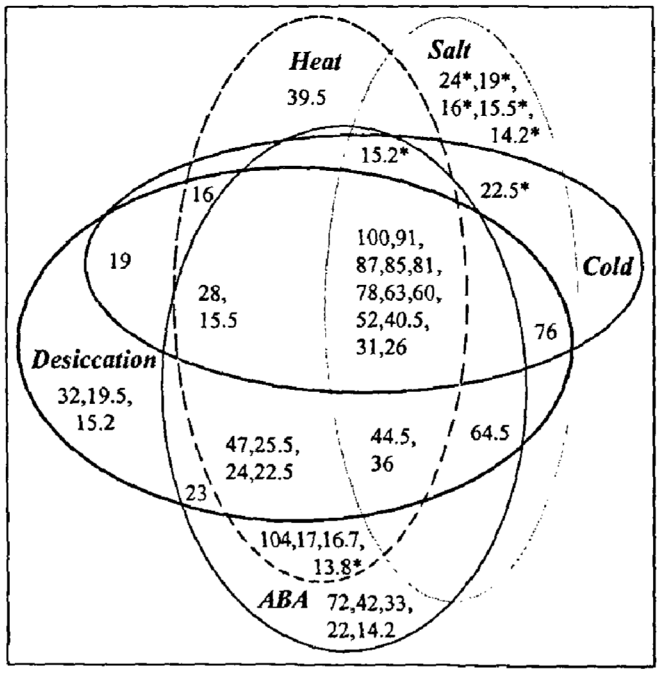

Figure 6. Venn diagram showing proteins which accumulate in response to various stresses in roots of rice seedlings. Other details are same as in Figure 5.

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[15]. Secondly, while some of the stress proteins noted in this study matched in molecular weights to those identified by earlier workers[18,19,23,29], 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[11]. 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.

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Drought-induced enhancement of protease activity during monocarpic senescence in wheat

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The effect of water stress on flag leaf senescence and protease activity during grain development was examined in a wheat variety. Total chlorophyll content and soluble protein content were used as markers for monocarpic senescence. Endopeptidase activities and exopeptidase activities were assayed using ribulose-1,5-bisphosphatecarboxylase (RuBisCo) as a physiological substrate at acidic (pH 4.8), neutral (pH 7.0) and alkaline (pH 8.5) values at three stages during grain-filling period. Water stress enhanced the rate of monocarpic senescence and concomitantly increased the endopeptidase and exopeptidase activities at all pHs tested in the leaves. The above observations showed that different proteolytic enzymes may come into play under water stress which are independent of the reproductive sink effect.

MONOCARPIC senescence is a genetically-programmed decline in physiological functions during which there is a process of protein turnover with the greater effect on protein degradation rather than protein synthesis[1], thereby leading to death of the plant following a single reproductive phase[2]. There have been a large number of studies concerning the role of proteases during monocarpic senescence[3,4]. Leaf senescence, however, is subjected to regulation by many environmental factors such as drought, besides autonomous factors such as reproductive development[5]. There are some reports on the individual effects of drought on proteolytic activities[1,6,7]. However, there have been very few studies on the combined effect of drought and grain development on the proteases activity. In the present study, the effect of drought and monocarpic senescence on proteolytic activities in the flag leaf of wheat has been examined using RuBisCo as the physiological substrate at acidic, neutral and alkaline pH to see if there is any specific increase or a general enhancement of all proteases.

For the present study *Triticum aestivum* var. HD 2329 was field grown in the loamy soils of Water Technology Centre, IARI during rabi season (November–April 1996–97). The crop was sown on 15 November 1996. Cultivation and water stress treatment were carried out as described earlier[8]. Total rainfall was 5.62 cm during the crop season. Total water availability in well watered and stressed region were 43.92 cm and 28.92 cm, respectively. Flag leaf was sampled for water relations and biochemical analyses at anthesis, 15 days after anthesis

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