Indian Journal of Biochemistry & Biophysics
Vol. 41, August 2004, pp. 191-194

Protein profiles of two rice varieties by 2-D gel electrophoresis under moisture stress

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Received 12 November 2003; revised 16 June 2004

Two rice varieties N22 (drought-tolerant) and Panidhan (drought-susceptible) were studied for the protein profiles under control, moisture stress and abscisic acid (ABA) treatments. Both qualitative and quantitative differences were observed. In control and ABA treatment, the number of polypeptides was more in N22, compared to Panidhan. However, a significant number of polypeptides were induced in Panidhan, compared to N22 under mild and severe stress.

Keywords: 2-D Protein profile, moisture stress, rice

Water stress can result from various environmental conditions, such as salinity, dehydration and freezing. To survive and develop normally, plants adapt to stress with various strategies. Protein synthesis responds to environmental stresses such as heat shock[1], water stress[2,3] and osmotic shock[4], etc. There is net synthesis of some proteins and decrease in the others, with or without induction of unique stress proteins. LEA (late embryogenesis abundant) proteins play an important role in the protection of plants under water or salt stress conditions[5]. A set of genes is transcriptionally activated, leading to the accumulation of new proteins in vegetative tissue under osmotic stress[6,7]. Changes in gene expression are involved in physiological responses to water stress. The involvement of a number of transcription factors in stress signal transduction in causing induced gene expression at different levels in different organs and/or at different developmental stages has been reported[8]. Gene expression pattern may vary from tissue to tissue, thus proteins in different tissues differ both qualitatively and quantitatively. Drought stress increases the level of abscisic acid (ABA) and the hormone is involved in the signal transduction of gene expression conferring the adaptation[9]. Some drought responsive and low temperature responsive genes are not induced by ABA treatment[10] suggesting that both ABA-dependent and ABA-independent pathways are involved in signal transduction.

Changes in protein profile of rice cultivars differing in their tolerance to water stress have also been observed[11]. These stress-induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with stress[12]. Thus, understanding the biochemical and molecular basis of drought tolerance shall be helpful in developing strategies for improving drought tolerance in rice. The present study was undertaken to see the effect of water stress on protein profiles of two rice cultivars N22 (drought-tolerant) and Panidhan (drought-susceptible), using two dimentional (2-D) gel electrophoresis.

Materials and Methods

Rice cultivars, N22 (drought-tolerant) and Panidhan (drought-susceptible) were procured from Genetics Division, I.A.R.I., New Delhi and Central Rainfed Upland Rice Research Station, Hazaribagh, respectively. The seedlings (15 days old) of cultivars were planted in pots grown under controlled condition (25$\pm$5°C) and 14 hr D/N photo period and were watered uniformly once a day. Water stress treatment was imposed at vegetative stage (40 days after transplanting) by withholding water. Relative water content (RWC) was computed, using 1 cm long leaf pieces excised from third most recent fully expanded leaf. Approximately 80% and 65% RWC treatments were considered as mild- and severe stress, respectively and leaf tissue was collected and frozen in liquid nitrogen. For abscisic acid (ABA) treatment, 10^{-4} *M* (molar) ABA was sprayed on seedlings on alternate days.

Protein extraction

Leaf tissue (1 g) from both the cultivars was homogenized in liquid nitrogen, resuspended in cold solution of 10% trichloroacetic acid (TCA) in acetone with 0.1% β-mercaptoethanol (β-ME), kept at -20°C overnight, centrifuged the next day and the pellet was resuspended in cold acetone containing 0.1% β-ME for 1 hr. Pellet was dried in lyophilizer for 30 min and stored at -20°C in aliquots. Quantitative estimation of protein was done by Bradford[13] method.

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Protein samples (100 μg) were analysed on 2-D polyacrylamide gel electrophoresis (PAGE) based on O'Farrell's[14] method and on 10% SDS-PAGE as per Laemmlli[15] method.

Results and Discussion

Tolerance is characterized by a delay of both wilting and gradual decrease of leaf water potential, leading to maintenance of cellular turgor. N22 took longer time than Panidhan to attain the desired RWC. The protein profile pattern of both varieties on SDS-PAGE is shown in Fig. 1. Both qualitative and quantitative differences were observed. In N22, intensity of bands of molecular mass 100 kDa decreased both under mild- and severe stress, however, in Panidhan, it remained the same. In N22, bands of 16, 23 and 24 kDa increased only under mild stress, whereas, intensity of bands of 21.5, 32, 36, 39 and 41.7 kDa increased both under mild- and severe stress. However, in Panidhan only the intensity of 21.5 kDa increased under mild stress, while polypeptides of 16, 32 and 39 kDa increased under both mild and severe stress. Three additional polypeptides in ABA-treated, four in mild stress and five in severe stress appeared in 16-40 kDa region in N22, whereas in Panidhan, three additional polypeptides were observed in mild stress only. These differences, however, could not be studied visually, as it was difficult to resolve a large number of bands.

Polypeptides were separated on 2-D gel electrophoresis in order to associate some of the protein bands with stress (different treatments). Protein profiles of two varieties were studied under different conditions i.e., control, stress and ABA treatment. A differential pattern of protein expression was observed under different treatments. Some specific polypeptides got induced only in stress condition, while a few were expressed only in control and ABA treatments.

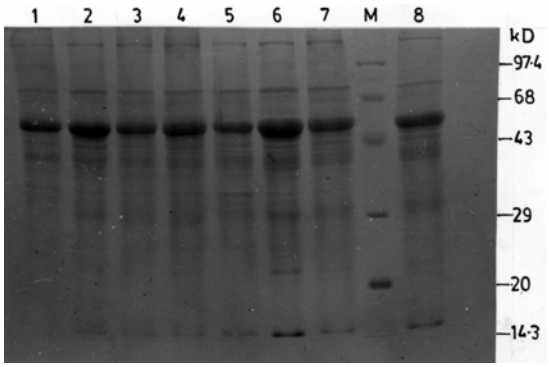

Fig. 1—Protein profile of N22 and Panidhan on 10% SDS-PAGE [Lanes 1, N22 control; 2, N22 ABA; 3, N22 mild stress (RWC 80-85%); 4, N22 severe stress (RWC 60-65%); 5, Panidhan control; 6, Panidhan ABA; 7, Panidhan mild stress (RWC 80-85%); 8, Panidhan severe stress (RWC 60-65%); and marker. 100 μg of total soluble protein was loaded in each lane and the gel was analysed by scanning densitometer]

Study of protein profiles under different treatments

A large number of polypeptides are observed in N22 control, compared to Panidhan control (Fig. 2 A & B). Three polypeptides of 16-18 kDa, pI 7-8 are expressed more in Panidhan control, compared to N22 control, whereas, two low molecular weight (LMW) polypeptides 10 kDa, pI 6-7 are found only in N22 control.

A large number of polypeptides of 20-40 kDa, pI 8-10 are present in Panidhan severe stress, compared to N22, where polypeptides of MW 20-60 kDa, pI 5-7 are observed. Two polypeptides of MW 10-12 kDa, pI 9-10 are highly induced in N22 severe stress, compared to Panidhan (Fig. 2 C & D). In pI 8-10 range, the number of polypeptides were observed to be more in Panidhan mild stress, compared to N22, where polypeptides are more in 7-9 region. Polypeptides of 20-60 kDa are expressed more in Panidhan mild stress, compared to N22 (Fig. 3 A & B). Three LMW polypeptides of 10-12 kDa in pI 7-8

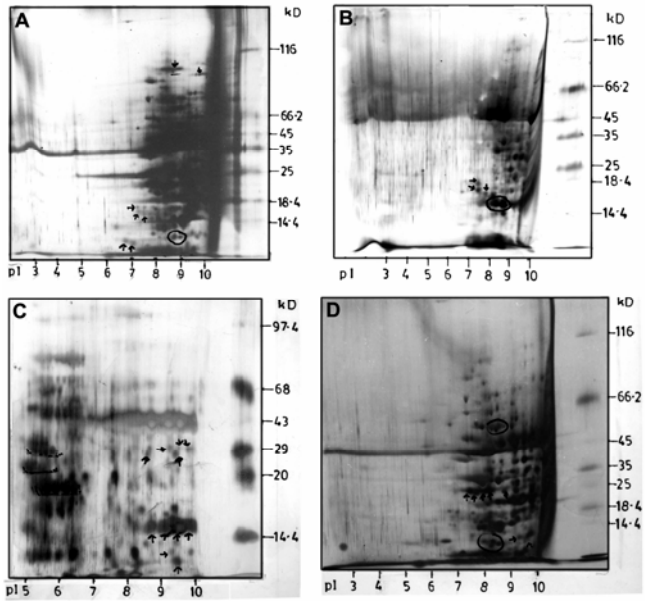

Fig. 2—2-D protein profile (A) N22 control, (B) Panidhan control; (C) N22 severe stress; and (D) Panidhan severe stress [100 μg of protein sample was loaded for both control and severe stress (RWC 60-65%). Isoelectric focussing was done across a pI range (3-10);4.0% polyacrylamide gel was used for isoelectric focussing and 7.5% SDS acrylamide gel for second dimension]

are common in both Panidhan and N22 mild stress. A polypeptide of 25 kDa, pI 8-9 was expressed more in N22 and Panidhan severe stress, compared to N22 and Panidhan mild stress.

In ABA-treated Panidhan, polypeptides of 14-45 kDa, pI 8-10 are highly expressed, compared to ABA treated N22. In ABA-treated N22 LMW, polypeptides of approx. 12 kDa, pI 6-8 are observed, which otherwise are absent in ABA-treated Panidhan (Fig.3 C & D).

Specific polypeptides in different treatments

A number of specific polypeptides were found under different treatments in both the cultivars. A distinct series of six polypeptides of 97 kDa, pI 8-10 and five polypeptides of 77 kDa, pI 7-8 are specific to N22 mild stress (Fig. 3A). Similarly, four polypeptides of LMW 16-18 kDa, pI 8-10 and a group of four polypeptides of 29 kDa, pI 9-10 were found to be specific to N22 severe stress (Fig. 2C). A fewer polypeptides were observed in ABA-treated N22 in pI 9-10 region. However, three polypeptides of 20-25 kDa, pI 7-8 which were specific to ABA treatment, were not observed in stress and control (Fig. 3C). Similarly, two polypeptides of 12 and 14 kDa, pI 8-9, a polypeptide of 97 kDa, pI 9-10 and a polypeptide of 99 kDa, pI 8-9 were found specific to N22 control

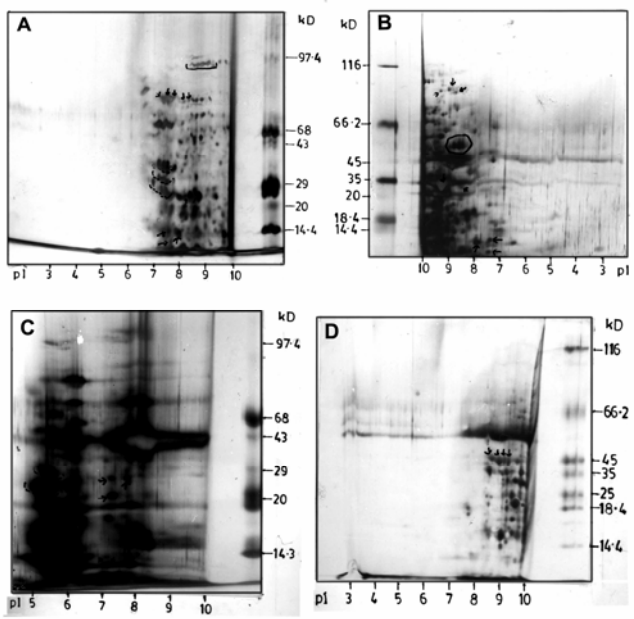

Fig. 3—2-D protein profile (A) N22 mild stress; (B) Panidhan mild stress; (C) N22 ABA; and (D) Panidhan ABA [100 μg of protein sample was loaded for both mild stress (RWC 80- 85%) and ABA treatment. 10^{-4} *M* ABA was sprayed on seedlings on alternate days]

(Fig. 2A). Three groups of four polypeptides were observed both in stress and ABA-treated samples (20-30 kDa, pI 5-7.5), compared to control. Increased number of polypeptides of 14-25 kDa, pI 7-9 were recorded in N22 mild stress, followed by severe stress and control (Fig. 2 A & C, Fig. 3A).

Significantly increased number of polypeptides of 50-100 kDa, pI 8-10 were induced in Panidhan mild and severe stress, compared to ABA-treated Panidhan and Panidhan control. Three polypeptides of 90 kDa, pI 8-10 and a highly intense polypeptide of 35 kDa, pI 9-10 were evident only in Panidhan mild stress (Fig. 3B). Four polypeptides of 13 kDa, pI 7-9 and four polypeptides of 25 kDa, pI 7-8 were specific to Panidhan severe stress (Fig. 2D). Two intense polypeptides of 15-16 kDa, pI 8-9 were control-specific (Fig. 2B) and four polypeptides of 45 kDa, pI 8-9 were specific to ABA-treatment only (Fig. 3D). Three polypeptides of 58 kDa, pI 8-9 were more intense in Panidhan mild stress, compared to severe stress.

A large number of polypeptides show up and down regulation as in one/2-D protein gel analysis[16,17]. Proteins which are up regulated by stress condition (stress proteins) have been observed in response to high and low temperatures, salinity, drought and several other stress factors[16,18,19,20]. Large amount of protein observed in N22 control may be ascribed to inhibition of protein synthesis under stress treatment, since plants under drought are able to maintain water potential gradient by osmotic adjustment[11]. Earlier, aquaporins (proteins that form water selective channels) have been found to play a significant role during the recovery from water deficit[21].

The number of polypeptides are reduced in N22 mild stress, compared to N22 control. The rate of protein biosynthesis shows a general decline during stress condition[22,23,24]. Despite overall reduction in protein synthesis activity, it is interesting to note that cells preferentially synthesize stress proteins. In certain cases, stress proteins play a crucial role in assisting the cells to carry out their metabolic activities during adverse conditions[16,17,23].

Significantly increased number of polypeptides is observed in Panidhan mild and severe stress, compared to Panidhan control. Proteins play a role in the regulation of plant responses to water stress[25]. They may also have a role in the amelioration of osmotic stress eg. LEA proteins accumulate in roots in response to exogenous ABA[26]. The synthesis and

accumulation of most of the polypeptides in moisture stress, in the present study suggests major mechanisms that underlie adaptation or tolerance to osmotic stress. It is generally assumed that stress-induced proteins may play a role in tolerance, but direct evidence is still lacking and the function of many stress responsive genes are unknown[27]. Stress-associated proteins are either synthesized *de novo* in response to stress or present constitutively at low level and their expression increases in response to stress.

The utility of 2-D protein gel electrophoresis for the study of stress-associated protein is well documented[16,17,28,29]. These proteins can be employed as molecular markers in breeding experiments and can be used to isolate concerned genes[29], as well as regulatory sequences. Availability of full length cDNAs/genes encoding stress-associated proteins may possibly lead to better genetic engineering tools in improving the drought tolerance trait.

References

1 Gupta M, Dhillon S & Nainawatee H S (1991) *Biol Plant* 33, 200-205
2 Bozarth C S, Mullet J E & Boyer J S (1987) *Plant Physiol* 85, 261-267
3 Sachs M M & Ho T H D (1986) *Annu Rev Plant Physiol* 37, 376
4 Fleck J, Durr A, Fritsch C, Vernel T & Hirth L (1982) *Plant Sci Lett* 26, 159-165
5 Xu D Duan X, Wang B, Hong B, Ho, T H D & Wu R (1996) *Plant Physiol* 110, 249-257
6 Chandler P M & Robertson M (1994) *Annu Rev Plant Physiol Plant Mol Biol* 45, 113-141
7 Skriver K & Mundy J (1990) *Plant Cell* 2, 503-512
8 Chen W, Provart N J, Glazebrook J, Katagiri F, Chang H-S, Eulgem T, Mauch, F, Luan S, Zou G, Whitham S A, Budworth P R, Tao Y, Xie Z, Chen X, Lam S, Kreps J A, Harper J.F, Si-Ammour A, Mauch-Mani B, Heinlein M, Kobayashi K, Hohn T, Dangl J L, Wang X & Zhu T (2002) *Plant Cell* 14, 559-574
9 Bray E A (1997) *Trends Plant Sci* 2, 48-54
10 Shinozaki K & Yamaguchi-Shinozaki K (1997) *Plant Physiol* 115, 327-334
11 Tyagi A & Sairam R (2001*) Indian J Agric Biochem* 14 (1&2), 25-28
12 Shinozaki K & Yamaguchi-Shinozaki K (2000) *Curr Opin Plant Biol* 3, 217-223
13 Bradford M (1976) *Anal Biochem* 71, 248-254
14 O'Farrell P H (1975) *J Biol Chem* 250, 4007-4021
15 Laemmli U K (1970) *Nature* (London) 227, 680
16 Pareek A, Singla S L & Grover A (1997) in *Strategies for Improving Salt Tolerance in Higher Plants* (Jaiwal P K, Singh R B & Gulati A, eds), pp 365-391, Oxford and IBH, New Delhi
17 Singla S L, Pareek A & Grover A (1997) in *Plant Ecophysiology* (Prasad M N V, ed), pp 101-127, John Wiley, New York
18 Grover A, Pareek A & Maheshwari S C (1993*) Proc Indian Nat Sci Acad* B59, 113-127
19 Vierling E (1991) *Annu Rev Plant Physiol Plant Mol Biol* 42, 579-620
20 Viswanathan C & Khanna-Chopra R (1996*) Curr Sci* 71, 275-284
21 Martre P, Morillon R, Barrieu F, North G B, Noble P S & Chrispeels M J (2002) *Plant Physiol* 130, 2101-2110
22 Gurley W B & Key J L (1991) *Biochem* 30, 1-11
23 Parsell D A & Lindquist S (1993) *Annu Rev Genet* 27, 437-496
24 Singla S L & Grover A (1994) *Plant Sci* 97, 23-30
25 Ho T H & Mishkind M L (1991) *Plant Cell Environ* 14, 67-75
26 Moons A, Prinsen E , Bauw G, Van Montago M (1997) *Plant Cell* 9, 2243-2259
27 Ingram J & Bartels D (1996) *Annu Rev Plant Physiol* 47,377-403
28 Ramgopal S & Carr J B (1991) *Plant Cell Environ* 14, 47-56
29 Sugiharto B, Ermawati N, Mori H, Aoki K, Yonekura-Sakakibara K, Tomoyuki Y, Sugiyama T & Sakakibara H (2002) *Plant Cell Physiol* 43, 350-354