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# Drought stress causes alteration of gene expressions in Rice (*Oryza sativa* L.)

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**Abstract :** Water loss from plant tissues under drought conditions results in growth inhibition and in a number of other metabolic and physiological changes. These include abscisic acid accumulation, stomatal closure, changes in leaf water potential , the decreased photosynthesis and solute accumulation. Rice (*Oryza sativa* L.) as a paddy field crop is particularly susceptible to water stress. It is estimated that 50% of the world rice production is affected more or less by drought. To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. We have constructed proteome maps of drought-sensitive IR 20 and drought tolerant Birsa Dhan 101 rice types corresponding to several time points during their response to drought stress. Several up- and down-regulated proteins have been identified by 1D protein gel electrophoresis followed by silver staining.

**Key words: .*Oryza sativa* L., Drought, Rice.**

## INTRODUCTION

Numerous physiological and biochemical changes occur in response to drought stress in various plant species. The alteration of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought tolerance (**Chandler** and **Robertson, 1994; Ouvrard *et al*., 1996**). Both quantitative and qualitative changes of proteins were detected during water stress (**Riccardi *et al*., 1998**). Evidence is increasing in favor of a relationship between the accumulation of drought-induced proteins and physiological adaptations to water limitation (**Bray, 1993; Han** and **Kermode, 1996; Riccardi *et al*., 1998**). Research on rice molecular biology and biotechnology has made significant progress during past years. Genetic transformation of rice has become a routine through *Agrobacterium tumefaciens* based- method (**Tyagi *et al*. 1999, Grover** and **Minhas 2000**). Remarkable success has been achieved in production of transgenic rice plants for increased yield, improved nutritional quality and improved resistance to insects, bacterial, viral and fungal pathogens and against water stress, low temperature stress and salt stress (**Grover** and **Minhas 2000**).

Many studies report changes in the expression of individual genes when rice is challenged by drought stress, and they frequently respond to other abiotic and biotic stresses as well. These include such diverse genes as MAP kinase (Agrawal et al. 2003), DREB genes (Dubouzet et al. 2003), calcium-dependent protein kinase (Saijo et al. 2001), an endo-1,3-glucanase (Akiyama and Pillai 2001), a translation elongation factor (Li Zi and Chen Shou 1999), and glutathione reductase (Kaminaka et al. 1998). Transformation studies have demonstrated that altering the expression of a number of different genes from different pathways can affect the response of rice to water deficit or dehydration . These include genes associated with diverse functions, such as water uptake (aquaporins) (Martre et al. 2002), signaling (kinases) (Saijo et al. 2001) (Liu et al. 2003) and membrane integrity (LEA protein) (Xu et al. 1996) (Rohila et al. 2002) (Babu et al. 2004). The effect of transformation on grain production under stress has not been well documented.

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Plant biology visualizes the proteomic approach as the answer to many of the unlocked / undiscovered functional / structural aspects related to the proteins. It would not be an exaggeration to suggest that discovery of new genes as a result of the efforts done in the various genome sequencing projects will be critically supplemented by the analysis carried out in the field of proteomics. It is envisaged that understanding the functions of the novel genes is a more challenging and difficult task than obtaining just the raw sequence information. The information obtained in this way is sure to help in gene pyramiding effort and cataloging the genes in order to successfully benefit the science in its future endeavors.

The present study was aimed at a better understanding of the molecular adaptation mechanisms of drought stress**.** It is implicated with identifying the proteins that are specifically altered in rice (Oryza sativa L.) during drought stress and during recovery from the stress.

## MATERIALS AND METHODS

Seedlings of Birsa Dhan 101 and IR20 rice type's were raised, subjected to drought stress and transferred to control condition for recovery. For this, MS (Murashige and Skoog) Stock solutions were prepared. For drought stress treatment, 4-day-old germinated seedlings were transferred to plastic trays. The drought stress treatments were carried out at $25^0C \pm 2^0C$ under 1500 lux light intensity with 16 hr. photoperiod, i.e., under controlled condition. Stress was given for different duration of time (54, 60, 66 and 72 hours) and after that, the seedlings were recovered for the same period in water. Samples were harvested after each stress treatment and recovery. Samples for 1D analysis were prepared following the procedure of **Dubey *et al.* (2003).** Shoots of rice seedlings were homogenized in liquid Nitrogen up to the formation of fine powder. Polyvinyl poly pyrrolidone (PVPP) was added to the samples (50 mg/ g fresh weight of the tissue) during homogenization. The powder was then dissolved in **Zivy *et al.* (1983)** buffer containing 30 mM Tris-Hcl (pH 8.8), 1 mM L-ascorbic acid, 1 mM EDTA-Na (Ethylenediaminetetraacetic acid), 5 mM $MgCl_2$, 1 mM dithiothreitol (DTT) and 1 mM phenyl methyl sulphonyl fluoride (PMSF). This extract was centrifuged twice at 10,000 rpm for 15 min at 4°C and the supernatant was transferred to SS34 centrifuge tubes. Then the soluble protein extract was precipitated in pre-chilled acetone and â-mercaptoethanol (0.07% v/v) solution overnight at –20°C. It was again centrifuged and the pellet, which contained the total protein, was dissolved in **Laemmli (1970)** buffer containing 62.5 mM Tris-HCl (pH 6.8), 10% (v/v) glycerol, 5% (v/v) â-mercaptoethanol and 2% (w/v) sodium dodecyl sulphate (SDS). This solution was then heated in boiling water for 5 min. After centrifugation, required amount of supernatant was taken to estimate the protein concentration by **Bradford (1976)** method. Buffer soluble proteins were extracted and analyzed by 1 mm thick, vertical gels. Silver staining of 1D gels were performed according to **Dubey *et al.*, 2003**.

## RESULTS AND DISCUSSION

We have constructed proteome maps of drought-sensitive IR 20 and drought tolerant Birsa Dhan 101 rice types corresponding to several time points during their response to drought stress. Under control conditions, both the rice types showed comparable percentage germination.

Shoot samples of rice seedlings harvested at variable intervals of drought stress were analyzed for the protein profiles by 1D analysis. Several up- and down-regulated proteins have been identified by 1D protein gel electrophoresis followed by silver staining. A 372 kDa polypeptide was undetected in uninduced control and 72 h stressed sample of IR 20 cultivar and a 205 kDa polypeptide was detected in all samples in equal proportion, pronounced accumulation seen after 66 h and 72 h stressed sample of Birsa Dhan 101 cultivar. The major polypeptide alterations (both up- and down-regulated) noted in the analysis is shown in Figure1and 2 respectively.

The future avenues for further increasing stress tolerance warrant that several stress tolerance-related genes must be pyramided. The realization of this goal can only be achieved if major breakthroughs are made in further identification of the stress related proteins and isolation and cloning of the requisite genes. Genomics and proteomics research will be of great help in constantly expanding the information on newer stress responsive genes and proteins.

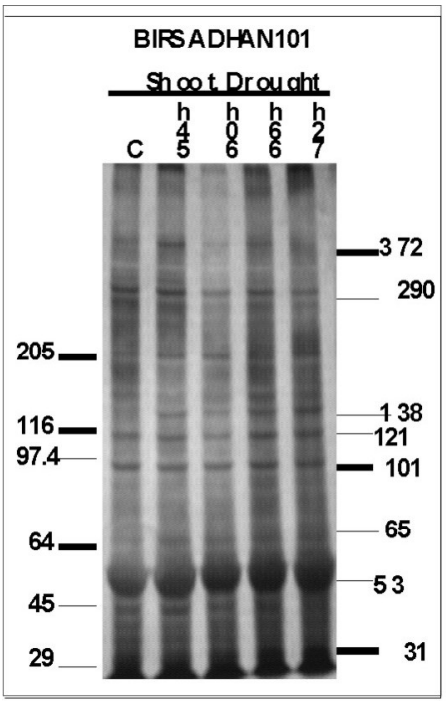


Fig.1. Electrophoretic profiles of proteins of shoot tissue of BIRSA DHAN 101 rice seedlings as revolved on 7.5% uniform SDS-gel in response to 54-72 h drought stress. Equal amount of protein was loaded in each of the lanes. Gel was stained with silver nitrate. Numbers shown on the right side of each panel denote the molecular weights (in kDa) of the matching polypeptides. Duration of stress treatment is shown on the top of each lane. Positions of the standard molecular weight markers in (kDa) are shown on the left side of each panel. C, Control (uninduced)

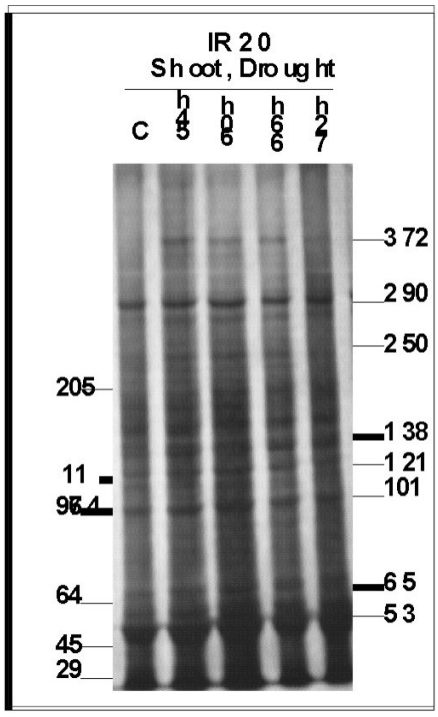


Fig.2. Electrophoretic profiles of proteins of shoot tissue of IR 20 rice seedlings as revolved on 7.5% uniform SDS-gel in response to 54-72 h drought stress. Equal amount of protein was loaded in each of the lanes. Gel was stained with silver nitrate. Numbers shown on the right side of each panel denote the molecular weights (in kDa) of the matching polypeptides. Duration of stress treatment is shown on the top of each lane. Positions of the standard molecular weight markers in (kDa) are shown on the left side of each panel. C, Control (uninduced)

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