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MOLECULAR RESPONSES OF RICE (*ORYZA SATIVA* L.) TO ZINC STRESS

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ABSTRACT:

Seed germination is a complex physiological process in plants that can be affected severely by stresses especially of heavy metals. In order to document the interference during germination by Zinc stress at proteomic and genomic level, an investigation has been undertaken. Seeds were exposed to different concentration of Zinc (0.10 and 0.15 ppm). Protein profiles analyzed by two-dimensional electrophoresis (2-DE) revealed that proteins were differentially expressed in Zinc-treated plants. Restriction digestion banding pattern of EcoRI and Hind III enzymes showed distinct banding pattern in the treated plants compared to the control.

KEY WORDS: Heavy metal stress, *Oryza sativa* L., SDS- PAGE - polypeptides, Restriction enzymes.

INTRODUCTION:

Rice is an important cereal grown in about one-third of the World's total cereal crop area, providing staple food and 35–60% of the calories consumed by more than 2.7 billion people (Khush, 1997). The demand for rice is increasing tremendously as the population in rice-growing areas is increasing at an alarming rate. However, increasing rice production is becoming more difficult because of biotic and abiotic stresses and the continuing decrease of cultivated rice areas due to the expansion of population and industry. It is obvious that increased rice production is necessary to overcome the loss of usable land. The effect of environmental stresses on plants has been one of the main interests in modern biological research. This is connected with radical ecological changes and reduction in biodiversity. Changes driven by ecological stresses can be investigated at molecular and other hierarchical levels. Such stresses as drought, salt, cold, heat, chemical pollutants, heavy metals and others, frequently act together and trigger adaptive and protective mechanisms (Vinocur and Altman, 2005). Stress proteins are critical for maintaining homeostasis under stress (Wang *et al.*, 2004). The up regulation of stress proteins, which occurs against a background of depressive changes in polypeptide formation, relative to normal environmental conditions, is one of the main components of the adaptive response (Lorimer, 2001; Kosakivska, 2008). The genome (DNA) is stable through many stress, the transcriptome (mRNA population) and proteome (protein population) change during development, under biotic and abiotic stress, sometimes rapidly and dramatically (Watson *et al.*, 2003; Rampitsch and Srinivasan, 2006). Heavy metal pollution in soil and water has becoming a serious problem for agriculture and health. Toxic heavy metals share some chemical similarities with the essential metals and in excess can induce the production of reactive oxygen species as well as interact with sulfhydryls to alter protein structure and function (Cox and Hutchinson, 1981). In the present investigation molecular responses of rice was carried out under Zinc stress condition.

MATERIALS & METHODS:

Plant material and stress treatment: The Rice seeds (IR20) were collected from seed testing centre, Tirunelveli and soaked with water for 24hrs. Ten pre-soaked seeds were sown in trays filled with equal amount of garden soil and sandy soil and treated with aqueous solution of different concentrations of Zinc (0.10ppm and 0.15ppm conc.) at regular interval. Distilled water was used in case of control. The Zinc treated germinated plant materials were collected on 10th and 20th day for analysis.

Isolation of protein: The isolation of protein was done as per Lowry *et al.*, (1951) and separated in 10% Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) following the procedure given by Lammelli (1970).

DNA Isolation: Genomic DNA was isolated from the young leaves of the stressed plants as per standard protocol of Doyle and Doyle (1987) and quantified as per Sambrook and Russell (2000).

Restriction Enzyme Digestion : Restriction digestion performed by two restriction enzymes Eco R I and Hind III. The restriction digestion mixture was prepared by sterile double distilled water (13 µl), 10X restriction buffer (2 µl), DNA sample (3 µl) and restriction enzyme (2 µl) in a micro centrifuge tube. The mixture was gently mixed by repeated pipetting or tapping and incubated at 37^0 C. The reaction was stopped by incubating the mixture at 60^0 C for 2 minutes. The reaction product was checked in the Agarose Gel (1%) Electrophoresis.

Determination of Molecular Weight : The molecular weight of the protein and DNA was compared by standard molecular ruler. Standard proteins with molecular weights of 205.0, 97.4, 66.0, 43.0, 29.0, 20.1, 14.8, 6.5, 3.5 and 2.8 kilo Dalton (kDa) were used as markers. 100 base pairs (bp) molecular marker was used as DNA standard ruler.

RESULT:

Protein Profile: In rice there were about 28 bands (Fig. 1) totally observed on 10th day of Zinc stress. The control had eight bands and their molecular weight ranging from 31.6 to 3.5 kDa. But the 0.10 ppm and 0.15 ppm level concentration showed seven and thirteen banding patterns ranging from 31.6 to 3.5 kDa and 112.0 to 3.2 kDa respectively (Table- I). In control eight bands were observed, but in concentration of 0.10 ppm the number of bands was decreased and one new band of 22.4 kDa was observed. In concentration of 0.15 ppm Zinc, seven new protein bands (112.0, 79.4, 63.9, 22.4, 9.2, 7.0 and 3.2 kDa) were observed. On 10th day at 0.15 ppm concentration protein bands increased and their bands intensity also increased. On the 20th day, the control had twelve bands and their molecular weight varied from 112.0 to 3.5 kDa. But at 0.10 ppm and 0.15 ppm level concentrations nine and twelve banding patterns ranging from 63.0 to 3.1and 63.0 to 3.9 kDa were reported respectively. The total number of bands decreased in 0.10 ppm compared to control. Three new bands (63.0, 7.9 and 3.1 kDa) in 0.10ppm and two new bands (63.0 and 17.5 kDa) in 0.15 ppm were observed respectively.

Restriction Analysis: Usually restriction enzymes recognize only the recognition sites. If the DNA is affected due to stresses. The recognition sequences also are affected or altered and it will be reflected in the banding pattern when such restriction enzymes are used. In our investigation, we used two restriction enzymes EcoRI and HindIII both in the control and the treated DNA and compared the banding pattern (Table- II).

DISCUSSION:

All living organisms respond at the cellular level to unfavourable conditions such as heat shock or other stressful situations of many different origins, by the rapid, vigorous and transient acceleration in the rate of expression of a small number of specific genes (heat shock genes). Consequently, the products of these genes (commonly referred to as Heat Shock Proteins [HSP] or stress proteins), which are also present under normal conditions but in lesser amounts, increase and accumulate in cells to reach, in some instances fairly high concentrations. The mechanisms by which these stressful situations stimulate the stress response appear to converge at the level of transcription and as such it is the activity of heat shock transcription factors which appear to regulate the expression of stress proteins. Several classes of Heat Shock Proteins (HSP) have been described in plants, among them HSP110kDa, HSP90kDa, HSP70kDa HSP60kDa and low molecular weight HSPs (Vierling, 1991). Similar types of proteins were observed in the stressed conditions in our study. The main function of such HSPs is to alter the conformation or assembly of other protein structure. Through genetic analysis and cellular biological research it became clear that the major stress proteins were acting as molecular chaperones in a variety of cellular systems. It now seems that virtually all the major families of stress proteins act as molecular chaperones in at least one system. Molecular chaperons are key components contributing to cellular homeostasis in cells under stress conditions. They are responsible for the protein folding, assembly, translocation and degradation (Knight and Ackerly, 2001). It is well known that members of HSP70kDa family are mainly localised in cytoplasm, where both stress-induced and constitutive polypeptides are expressed (Bukau and Horwich, 1998; Morimoto, 1998). It is very clear that the banding pattern in treated plants are different from the control plants (Fig.2) proving that in treated plants the DNA sequences have been altered by Zn stress. It has been reported that Zn ions inhibit DNA fragmentation and apoptosis induced by various stimuli in different systems (Ciesieslska et al., 2000 and Cai and Cherian, 2003).

CONCLUSION:

Thus, from these experiments, we understand that Rice plants are able to tolerate Zn up to certain concentrations beyond which the proteins and even the DNA are affected. It is evident that the Heat Shock Proteins and chaperones are synthesized in response to Zn stress in Rice plants. It is important that crops like Rice should be protected from such heavy metal stress and measures should be taken to reduce Zn stress.

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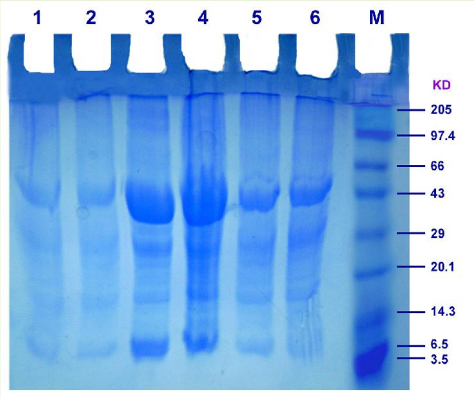
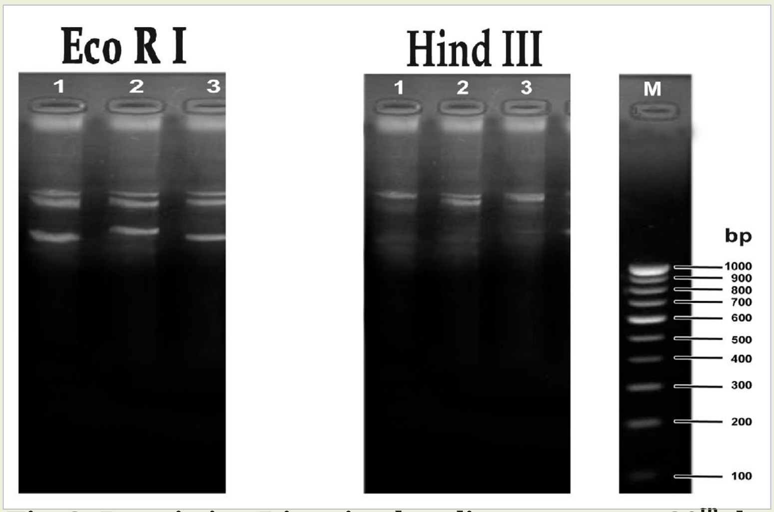


1. 10th day Control
2. 10th day 0.10ppm
3. 10th day 0.15ppm
4. 20th day Control
5. 20th day 0.10ppm
6. 20th day 0.15ppm

M. Molecular Weight

Fig.1. Effect of Zinc on Protein in *Oryza sativa*



1. Control
2. 0.10ppm
3. 0.15ppm

M. Molecular Weight marker.

Fig. 2. Restriction Digestion banding pattern on 20th day zinc treated rice plant

TABLE 1: MOLECULAR WEIGHT OF PROTEINS IN *ORYZA SATIVA* UNDER ZINC STRESS

Band Number	1OTH DAY			2OTH DAY			**Molecular Marker (Lane 7)**
	Control (Lane 1)	0.10ppm (Lane 2)	0.15ppm (Lane 3)	Control (Lane 4)	0.10ppm (Lane 5)	0.15ppm (Lane 6)	
1	-	-	-	-	-	-	205.0
2	-	-	+	+	-	-	-
3	-	-	-	-	-	-	97.4
4	-	-	+	-	-	-	-
5	-	-	-	-	-	-	66.0
6	-	-	+	-	+	+	-
7	-	-	-	+	+	+	
8	-	-	-	+	+	+	43.0
9	+	+	+	+	-	-	
10	+	+	-	+	+	+	29.0
11	-	+	+	+	-	+	-
12	-	-	-	-	-	-	20.1
13	+	-	+	-	-	+	
14		+			+		14.8
15	+		+	+	-	+	-
16	+	+	+	+	-	+	-
17	-	-	+	+	+	+	-
18	+	+	+	-	+	+	-
19	-	-	+	+	+	-	6.5
20	+	-	+	+	-	+	-
21	+	+	-	+	-	+	3.5
22	-	-	+	-	+	-	2.8

TABLE 2: DNA BANDING PATTERN OF *ECORI* AND *HIND III* DIGESTION IN ZINC TREATED 20TH DAY *ORYZA SATIVA*

Band No.	*EcoRI*			*Hind III*		
	Control (Lane-1) bp	0.10ppm (Lane-2) bp	0.15ppm (Lane-3) bp	Control (Lane-1) bp	0.10ppm (Lane-2) bp	**0.15ppm (Lane-3) bp**
1.	-	-	-	-	-	**1798.8**
2.	1778.2	1778.2	1778.2	1778.2	1778.2	**1778.2**
3.	1584.9	1584.9	1584.9	-	-	-
4.	-	-	-	-	1603.6	-
5.	-	-	-	1584.9	-	-
6.	-	-	1288.4	-	-	-
7.	-	1258.4		-	-	-
8.	-	-	-	1208.3	-	**1208.3**
9.	-	-	-		1188.8	
10.	-	-	-	-	1174.3	-
11.	**1098.0**	-	-	-	-	-