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Original Research Paper

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ELECTROPHORETIC SEPARATION OF INDUCIBLE PROTEINS IN MAIZE SEEDLINGS UNDER FUNGICIDE TREATMENT

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ABSTRACT The aim of the experiment was to study the effect of a various concentrations of a systemic fungicide, metalaxyl on the expression of new proteins during early germination of maize. The study was conducted for 7 days. Our investigation showed the appearance of induced proteins ranging from 100-110kDa, 44kDa, 30kDa and 18-25kDa on different days of observation and different concentration of metalaxyl treatment, which is in line with the findings in various abiotic stresses.

KEYWORDS : Germination, metalaxyl, systemic fungicide, abiotic stress

INTRODUCTION

Plants show number of physiological and biochemical changes to the environmental stress in order to overcome or limit the effect of the surrounding stress. Numerous studies and articles have been published on SDS-PAGE exploring their role for different types of protein and isolation from different plants under different environmental conditions.

Studies on the proteomics of major abiotic stresses such as drought, salinity and extreme temperatures (Mohamed et al., 2016), proteomics of low temperature stress (Janmohammadi et al., 2015; Johnová et al., 2016), dehydration stress (Johnová et al., 2016), heavy metal stress (Ahsan et al., 2009; Hossain and Komatsu, 2013), plant biotic stresses (Sergeant and Renaut, 2010) with a special focus on fungal pathogens (Rampitsch and Bykova, 2012a) and *Fusarium* head blight disease (Yang et al., 2013a) are available.

Although many studies have been performed on seed germination for protein changes, present study aimed at investigating the changes in the protein under metalaxyl stress. Various biotic and abiotic factors influence the plants to bring about changes in their system especially protein which have wide functions to perform. It can be either producing new proteins or changes in the protein already present in plants.

MATERIALS AND METHODS

Maize seeds were procured from V.C.Farm, Zonal Agricultural Station, Mandya, Karnataka, India. Seeds were surface sterilized with 0.1% mercuric chloride for 10minutes and repeatedly washed with distilled water for 4-5 times to remove the excess chloride. Seeds of uniform size were selected and soaked for 24 hours in distilled water (control)and with different concentrations (mg/g) of metalaxyl. The germination studies were conducted following the between paper methods recommended by International Seed Testing Association (ISTA 2003). Dose range of fungicide was selected depending on the prescribed concentration that could affect 10-95% of the seedlings with logarithmic intervals. Germination of maize grains was completely inhibited above 8mg/gm of metalaxyl. Hence, maize Seeds were treated with concentration below 8mg/gm i.e. 1.5mg, 3mg, 4.5mg, 6mg and 7mg/gm.

Preparation of protein samples:

For extraction of soluble proteins, seeds were grounded in 50mM phosphate buffer (pH 7.8) and centrifuged in micro-centrifuge machine (Eppendorf) for 10 minutes at 14000 rpm. The supernatant was separated and used for protein profiling (Pek Geok Pang *et al.*, 2012).

Estimation of protein

Protein was estimated as described by Lowry *et al.*, (1951) using BSA as standard.

Sample preparation for SDS-PAGE

The supernatant was mixed with an equal volume of Laemmli solution (1M Tris (pH = 8.8), 0.4 g of SDS, 0.8 g of glycerol, and 0.9 ml 2-ME (mercaptoethanol) in 10 ml dd H_2O, heated in boiling water for 5 min, and frozen until used (Nayer and Reza, 2008). These samples were loaded on to the SDS gels.

Analysis of seedling proteins by using varied gel percentage

SDS-PAGE protocol for analysis of maize seedling protein was standardized using varied gel percentage (Hames and Rickwood, 1990). Four different gel percentages of 10%, 12.5%, 15% and 20% were used in the experiment. Different gel percentage was used to analyze various molecular weight proteins present in maize seedlings. SDS-PAGE was performed in a BIORAD mini gel electrophoretic system according to the method of Laemmli (1970) for 45 minutes.

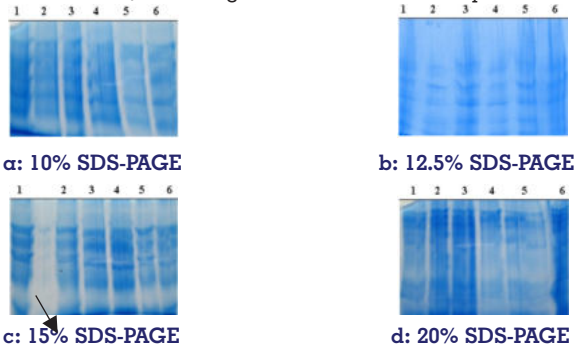
Screening of inducible proteins in maize seedlings

After standardizing the SDS-PAGE protocol, the maize seedlings were analyzed by using 15% SDS-PAGE.

RESULTS

Analysis of maize seedling proteins by using varied gel percentage

The control and metalaxyl treated seedlings during the early germination period (0-7days) was tested for total soluble protein composition. The result is presented in the Figure 1a to 1d. Effective separation was achieved using 15% SDS-PAGE gel. Our observation reveals that 10% and 12.5% gel are suitable for analyzing high molecular weight proteins (>100 kDa), 15% gel is effective for separation of medium range molecular weight proteins (in the range of 15 kDa to 100 kDa) and 20% gel is effective for low molecular weight protein separation. Thus, the analysis of maize proteins was standardized, and 15% gel was used for further experiments.



a: 10% SDS-PAGE b: 12.5% SDS-PAGE

c: 15% SDS-PAGE d: 20% SDS-PAGE

Figure 45: Analysis of maize seedling proteins by using varied gel percentage

(1: Control, **2:**1.5mg/g metalaxyl, **3:** 3mg/g metalaxyl, **4:** 4.5mg /g metalaxyl, **5:** 6mg/g metalaxyl, **6:** 7mg/g metalaxyl**)**

Inducible proteins in maize seedlings:

Soluble protein profile from the germinating seeds of control and metalaxyl treated seeds were analyzed by SDS-PAGE (15%). The Figure 2 shows the protein profile from 0 - 7 days of germination on 15% SDS-PAGE. During the process of germination there was an increase in the expression of several proteins till the 3rd day. However, there was under expression and disappearance of many proteins on 4th and 5th day of germination. But again on 6th and 7th day the intensity of the protein expressed was more. The proteins observed range from 100-110kDa, 44kDa, 30kDa and 18-25kDa.

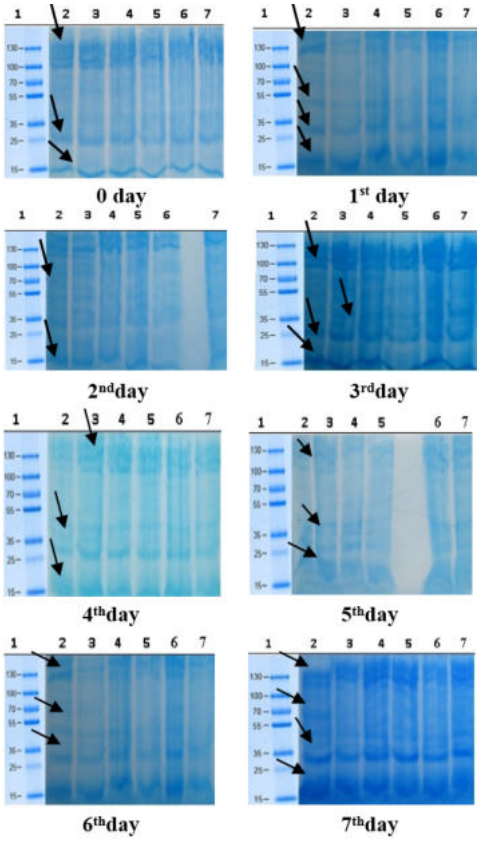


Figure 2: SDS-PAGE (15%) of maize seedling proteins on different days (0- 7day) of germination

(1: Marker protein, **2:** Control, **3: 1.5**mg/g metalaxyl, **4:** 3.0mg/g metalaxyl,**5:** 4.5mg /g metalaxyl, **6:** 6mg/g metalaxyl, **7:** 7mg/g metalaxyl**)**

On 0 day of germination a protein with MW101kDa expressed in control gradually declined with increase in the concentration of metalaxyl. But on the 1st day the same protein could not be seen with 1.5mg, 4.5mg and 7 mg concentrations of metalaxyl. The amount of this protein gradually declined even in control from 2-7 days of germination.

A 25 kDa protein could be seen in both control and treated seeds on all the days. A protein with MW 35kDa appeared on the 1st day of germination in control, 3mg and 6 mg treated seedlings. Not much variation was observed on the 5th day.

However, on 6th day an increased level of 35kDa protein was observed with 3mg and 6mg treated seedlings. With regard to 15kDa protein variation was not observed till 4th day whereas on 6th and 7th day larger amount of expression of this protein in both control and treated seedlings were observed. On 7th day of germination, there was varied expression of protein with metalaxyl treatment. At 3mg, 4.5mg and 7.0mg concentration of metalaxyl, maize seedling expressed some protein bands with molecular weight

The electrophoretic pattern showed proteins with multiple bands ranging from molecular weight from 130kDa- 15kDa with 3mg and 6mg treated seedlings but with 1.5mg, 4.5mg and 7mg metalaxyl treatment enormous decreases in the expression of different proteins were observed.

DISCUSSION

In response to environmental stresses, increased gene expression has been noticed which results in the synthesis of proteins, collectively called stress proteins, having putative role in stress protection (Soumen Bhattacharjee, 2008).

Screening of inducible proteins in maize seedlings:

Camila Pegoraro (2011) has reviewed the importance of Heat Shock Proteins in maize and mentions that the cellular homeostasis in all organisms under optimal and adverse growth and development conditions can be ensured by a family of proteins that is highly conserved across species, the heat-shock proteins (HSPs). These proteins were discovered in the salivary glands of fruit flies (*Drosophila*) as a response to heat shock (Ashburner and Bonner, 1979), but currently it is known that these proteins are induced by numerous other stresses (Sule *et al.*, 2004). The cereal grain proteins study is traced back for more than 268 years when wheat gluten was studied for the first time in 1745 (Beccari, 1745). Later on, Osborne (1907) who is considered as father of plant protein chemistry and many others made their efforts in this regard.

The present study shows a variation in banding pattern of proteins extracted from metalaxyl treated seedlings on SDS-PAGE. The protein pattern showed appearance of some new high molecular weight protein bands (140-150kd). The intensity was higher in 3mg of metalaxyl treatment. These variations in the protein bands are due to changes in polypeptides molecular weights, bands intensities, fractionation of some bands, appearance of new bands or unique bands and disappearance of some bands (AL-Huqail and Abdelhaliem, 2015).

In our study, SDS-PAGE analysis of maize seedlings revealed total 3-4 polypeptide bands with molecular weight ranging from 110-18KDa. This is quite in accordance with the findings of AL-Huqail and Abdelhaliem, (2015). They have reported SDS-PAGE analysis with 46 polypeptides bands of different molecular weights ranging from 186.20 to 36 kDa from maize seedlings exposed to electric field.

Several abiotic stress factors can lead to accumulation of HSP genes in plants. HSPs play a very important role in protecting plants against stress and in maintaining cellular homeostasis (Camila Pegoraro *et al.*, 2011). Mitochondrial HSP70 in rice prevents apoptosis induced by heat and oxidation. HSP70 maintains the stability of the membrane and thus inhibits the diffusion of reactive oxygen species (Guiyan Yang *et al.*, 2014).

NaCl stressed but triadimefon treated seedling proteins and only triadimefon treatment but unstressed seedling proteins from Amaranthus showed varied expression of proteins on SDS-PAGE. Triadimefon treated NaCl stressed seedlings showed a significant increase in the band intensity of 110- 120 kDa polypeptides. Triadimefon treatment showed expression of a number of proteins whose molecular weight suggest them to be stress-related proteins, even without subjecting it to salinity (Soumen Bhattacharjee, 2008). Similar to these studies Pinhero *et al.*, (1999) and Kraus *et al.*, (1995) have showed the over expression of many proteins in paclobutrazole treatment under abiotic stresses.

Drought stress induced an accumulation of 20, 22, 27, 30, 54,

and 59 kDa molecular weight proteins in the leaf. The intensity of these proteins increased with progressive increase in the stress (Jiang and Huang, 2002). Drought induced polypeptides have been observed in many studies (Riccardi, 1998; Arora, 1998; Perez-Molphe-Balch, 1996) and are suggested to play a role in water stress tolerance (Nayer Mohammadkhani and Reza Heidari, 2008).

The molecular analysis by SDS-PAGE confirmed the expression of various protein bands ranging from molecular weight 10-60kDa in temperature stress. A study by Geetika Pant (2017) has reported a 57–59kDa heat shock protein band in *C. auriculata* at 42°C. Similar temperature stress induced heat shock proteins between 10 and 50 kDa are reported in maize (Gorinstein *et al.,* 2004), *P. pratensis* (He *et al*., 2005), *B. campestris* and *A. Caudathus* (Kosakivska, 2008), in the seedlings of soyabean and pea (Chen, 1990).

CONCLUSION

In response to the environmental stress plants require changes in the flow of metabolites through suppression of pathways and the induction of various defence genes such as heat shock proteins (HSPs) and reactive oxygen scavenging enzymes. To overcome such environmental stress, generally all plants activate a large set of genes. HSPs are important in protecting the plants against stress. They bring about cellular homeostasis and maintain the normal protein conformation. Hence there is a possibility of expression of new protein which may play a role in the defense mechanism against the stress caused by metalaxyl.

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