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Influence of drought stress on leaf protein pattern of maize genotypes

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Abstract
For evaluation of changes in leaf protein pattern of maize, 12 maize genotypes (PDM 1409, PDM 1415, PDM 1428, PDM 1452, PDM 1465, PDM 1474, PDM 1479, PDM 1485, PDM 1488, PDM 1498, PDM 1430 and PDM 1439) were assayed under both drought stress and non-stress conditions by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) during *rabi*, 2015-16 and 2016-17. Total leaf proteins were extracted and separated on 12% polyacrylamide gels using standard protocols. Protein profiling under moisture stress was also identical to the bands under irrigated condition however there is up regulation of proteins was observed. A protein of 35 kDa, 55 kDa and 155 kDa are expressed in all the genotypes. Among the genotypes these specific proteins were conspicuous in PDM 1409, PDM 1415 and PDM 1465 compared to other genotypes.

Keywords: Leaf protein, maize, moisture stress, sodium dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Introduction
Abiotic stresses are the primary cause of crop loss worldwide reducing average yields in major crop plants, including maize by more than 50 per cent. Among the abiotic stresses, drought is the severe limiting factor for maize production (Ali *et al*., 2011) [2]. Drought is an important environmental constraint that limits the productivity of many crops and affects both quality and quantity of yield. It influences the physiology and metabolism of crops, impairs photosynthetic machinery and other yield-determining processes, and eventually lowers production. Maize (*Zea mays* L.) is the world's most widely grown cereal crop and is the primary staple food in many developing countries. To cope with the stress, numerous morphological, physiological and biochemical changes occur in various plant species. These changes cause the retention of water and the maintenance of photosynthetic activity, while stomatal opening is reduced to counter water deficit. The alternation of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought stress tolerance (Ouvrard *et al*., 1996; Jiang Yand Huang B, 2002) [6, 3]. Both quantitative and qualitative changes of proteins have been detected during the stress (Ahire *et al*., 2005) [1]. Sujin and Ray wu, 2004 [8] reported that soluble proteins in rice leaf with molecular weight of more than 100 kDa were reduced as a result of drought stress, but low molecular weight proteins were increased. The aim of the present study was to evaluate the pattern of leaf proteins in maize under moisture stress and non-stress (control) conditions.

Materials and methods
Plant materials
The Experiment was conducted during *rabi* 2015-16 and *rabi* 2016-17 at dry land farm, S.V. Agricultural College, Tirupati with 12 genotypes (selected through PEG-6000 experiment) which includes ten tolerant (PDM 1409, PDM 1415, PDM 1428, PDM 1452, PDM 1465, PDM 1474, PDM 1479, PDM 1485, PDM 1488, PDM 1498) and two susceptible (PDM 1430, PDM 1439) genotypes. The experiment was laid out in a split plot design with two main treatments, twelve sub treatments and replicated thrice. Main Treatments: 2: i) Irrigated (control) ii) Imposed moisture stress at soft dough stage (60-80 DAS), Sub Treatments:12 Genotypes. Leaf protein from maize genotypes was extracted at 70 DAS in both control and stress treatments.

Protein Extraction

2g of tissue (Maize leaf sample) was ground in 2 ml of 0.1 M Tris buffer pH 6.7 with the help of mortar and pestle. The ground sample was transferred into centrifuge tube of 15ml capacity. The mixture as centrifuged at 5000 rpm for 10 minutes at 4°C. The Supernatant (upper layer) was transferred into another centrifuge tube. To this 0.2 ml, 10 per cent SDS and 20 µl mercapto-ethanol were added. The mixture was boiled the sample on water bath for 2 minutes. After allowing to cool, 0.1 ml of bromophenol blue dye and glycerol 1 ml was added and mixed by stirring. This sample was used for electrophoresis.

Electrophoresis

40-60 µl of sample was loaded into wells with the help of micropipettes. Formation of bubbles was avoided during loading of samples. Then the electrophoresis unit was connected with power supply. The current was turned on allowing 30 mA 220 volts for initial 10 minutes, until the sample travels through the stacking gel. Then current was decreased upto 20 mA till separation of proteins by electrophoresis. After complete separation of protein when tracking dye reached the end of running gel, power supply was turned off. The gel was gently removed from the space between the plates and immersed in staining solution (CBB R-250) contained in a plastic tray. The tray was periodically shaken for uniform staining and this was continued for at least 1 hour. The gel was destained by putting it in 7 per cent acetic acid and methanol solution. The process was continued until background of gel become colourless. The bands were matched with protein markers loaded along with the samples in gel.

Results and Discussion

Water deficit elicits a complex of responses beginning with stress perception, which initiates a signal transduction pathway(s) and is manifested in changes at the cellular, physiological and developmental levels. The set of responses observed depends upon severity and duration of the stress, plant genotype, developmental stage and environmental factors providing the stress. Variability in protein profiles among the maize genotypes under imposed moisture stress and irrigated conditions are presented in Plates 1 and 2.

The banding pattern observed to be similar among genotypes in irrigated condition. Protein profiling under moisture stress was also identical to the bands under irrigated condition however there is up regulation of proteins was observed. A protein of 35 kDa, 55 kDa and 155 kDa are expressed in all the genotypes. Among the genotypes these specific proteins were conspicuous in PDM 1409, PDM 1415 and PDM 1465 compared to other genotypes. However it requires in depth analysis to identify specific proteins expressing in these genotypes under moisture stress condition which can be future line work.

A total number of 35 protein bands were detected by electrophoresis of flag leaf proteins in stress and non-stress conditions of wheat. Most of the bands under the stress conditions were similar to those in non-stress environment and specific bands were rare. Under drought stress, some low molecular weight proteins were intensified, while high molecular weight proteins were faint (Najaphy *et al*., 2014) [4]. Water deficit stress increased concentration of soluble proteins in chickpea leaves up to 43% in comparison with normal watering treatment, but didn't significantly affect electrophoretic pattern of protein profiles (Najaphy *et al*., 2010) [5].

Shafina *et al.* (2015) [7] analysed 24 rice genotypes under drought stress for proteins by SDS-PAGE to estimate their genetic diversity for the purpose of genetic improvement under drought conditions and profiling. He concluded that screening of genotypes for protein profile using SDS-PAGE is highly effective to identify drought tolerant donors with good seed proteins.

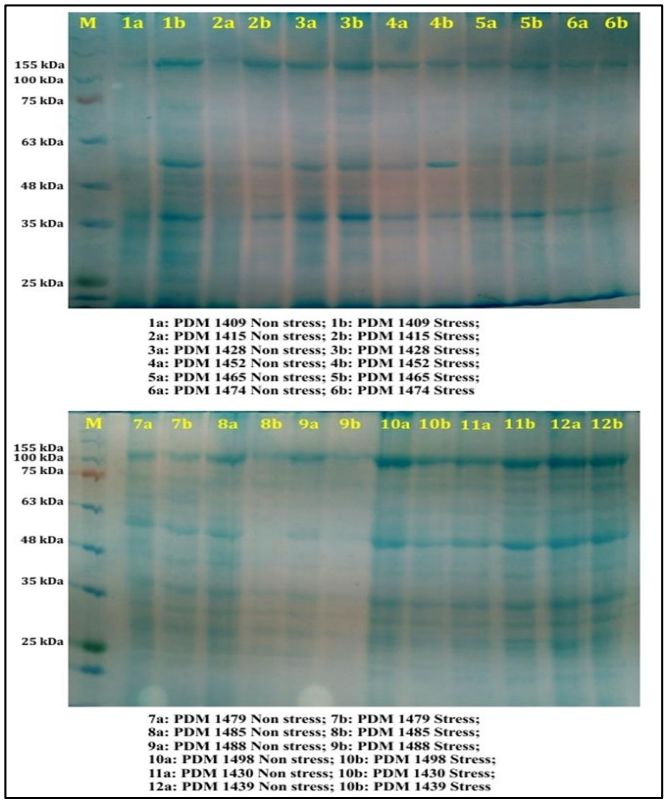

Plate 1: Protein polymorphism using SDS PAGE among maize genotypes during *Rabi*, 2015-16

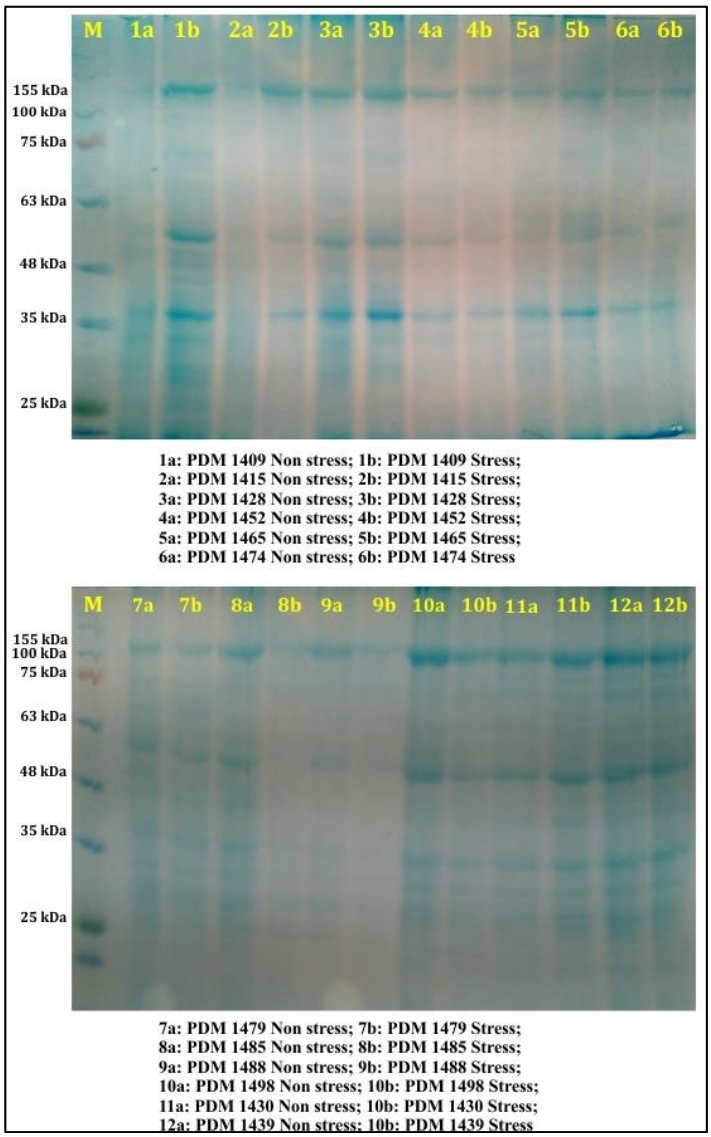

Plate 2: Protein polymorphism using SDS PAGE among maize genotypes during *Rabi*, 2016-17

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