Pak. J. Bot., 41(2): 731-736, 2009.

WATER STRESS INDUCED VARIATIONS IN PROTEIN PROFILES OF GERMINATING COTYLEDONS FROM SEEDLINGS OF CHICKPEA GENOTYPES

N. BIBI*, A. HAMEED, H. ALI, N. IQBAL, M. A. HAQ, B.M. ATTA, T.M. SHAH AND S.S. ALAM

Nuclear Institute of Agriculture and Biology, NIAB, Jhang road Faisalabad, Pakistan

Abstract

Studies were planned to analyze the response of chickpea genotypes to water stress (10% - PEG) at germination stage. Four genotypes viz., CM-2000, CM-94/99, CM-98 and 6153 were used and data for root length and protein mobilization from cotyledons to seedlings were collected on fifth and seventh day after germination. Analysis of variance revealed highly significant differences among varieties under control and stressed conditions. CM-94/99 showed significant increase ($p<0.05$) in root length while CM-2000 showed significant decrease ($p<0.05$) under the applied stress. SDS-PAGE was utilized to detect variations in protein profiles of these genotypes from 1st till 7th day. Quantitative decrease in some high molecular weight proteins was observed at 5th day while some new germination related proteins appeared on 7th day in controlled samples of all genotypes. However genotype specific variations were observed under the applied stress. In CM-2000 delayed expression (on 7th day) of 100 kDa and 60.8 kDa proteins was observed under water stress while these proteins were expressed earlier (on 5th day) in CM-94/99. Moreover expression of 39.6 kDa and 42 kDa proteins was also delayed (7th day) in CM-2000 but no change in the expression of these proteins was observed in other genotypes.

Introduction

Chickpea provides a protein-rich supplement to cereal-based diets. It is generally grown in arid areas of Pakistan, planted in the post-rainy season, taking advantage of conserved soil moisture. Efforts to identify genes underlying drought tolerance are mostly focused on model species and major cereal crops such as rice and maize (Bruce *et al*., 2002) with lesser attention being given to legumes even though they are known to possess high levels of drought tolerance (Turner *et al*., 2001).

Chickpea has been the focus of research since the inception of systematic research work on pulses in Pakistan. Major importance to chickpea improvement was attributed because it contributes 70-80% to the total pulses area and production. The Thal desert that cannot sustain major cash crops due to low fertility and lack of artificial irrigation is well known as home to chickpea. Because of large variations in seasonal rainfall, the crop suffers occasionally from drought, a severe abiotic stress of chickpea, causing a 40–50% reduction in yield globally (Millan *et al*., 2006).

The root system is considered to be a primary sensor of drought stress and may play an important role in drought avoidance (Davies & Zhang, 1991) by making deep penetration into soil in search of water. Thus drought tolerance in this crop is extremely desirable attribute for moisture deficit areas of the country. However drought is always unpredictable, regular selection of cultivars at a particular site under natural conditions is extremely difficult (Malhotra & Saxena, 1993).

*E-mail: noreen_niab07@yahoo.com

The importance of protein profiling has long been acknowledged in plant abiotic stress studies and previous studies (Kiegle, 2000) have provided useful information on individual enzymes or transporters, measuring their stress-dependent changes in quantity, activity, as well as modifications of structural protein, protein-protein interactions, stress-dependent protein movements, *de novo* synthesis and controlled degradation. Karimzadeh *et al.*, (2006) observed changes in the electrophoretic pattern of the water-soluble proteins from spring and winter wheat cultivars and pointed out accumulation of stress proteins in leaves on exposure to freezing temperature. The present study was designed to document (1) water stress induced changes in protein profiles of germinating cotyledons, (2) root growth and (3) to find morphological and biochemical markers for evaluation of drought tolerance potential of selected genotypes at seedling stage.

Material and Methods

Seeds of 4 chickpea genotypes including two kabuli (CM-2000, CM-94/99) and two desi (CM-98 and 6153) types were obtained from Pulses group at Nuclear Institute for Agriculture and Biology (NIAB). Uniform sized seeds were germinated in growth chamber (FISONS, Loughborough, England) on sterilized water saturated filter papers in large size Petri dishes at 25^{o}C for 24 h (16L/8D). Half of equally germinated seeds were then transferred to Petri dishes lined with filter papers soaked in a 10% solution of polyethylene glycol (PEG - 4000) while other half were placed on water soaked filter papers as control. Experiment was continued under controlled conditions for another eight days. Data for root length were recorded at 7th day of stress application. Cotyledon samples (0.2 g) from germinating chickpea seedlings were collected at fifth and seventh day of stress application for protein profiling.

Sample extraction: Samples were macerated in cold pestle mortar by adding 50 m*M* potassium-phosphate buffer (pH 7.4) to fine slurry and then centrifuged at 9,000 g for 15 minutes at 4^{0} C. Supernatant was collected in another set of microcentrifuge tubes.

Protein estimation: Total soluble protein in extracted samples was estimated using Bradford's method (Bradford, 1976) by measuring absorbance at 595 nm using spectrometer (Hitachi U2800) and expressed as µg/g fresh weight.

Protein profiling using SDS PAGE: For electrophoretic separation of proteins, 10 % acrylamide gels with 1mm thickness were used with dissociating discontinuous buffer system as described by Laemmli (1970). Equal amount (70 µg) of protein was loaded in each well. A wide range protein molecular weight marker from Bio Basic UK (DK605) was also run along with protein samples as standard. SDS PAGE was performed at constant voltage (140 V). Gels were stained using coomassie brilliant blue dye and documented by UVIpro platinum gel documentation system (UVI tec UK).

Results and Discussion

Germination induced changes in protein profiles and root traits which probably result from enzymatic action have been reported previously in chickpea by Mansour (1996) while in soybean by Mostafa & Rahma (1987). In the present study differences in root length were observed among all studied genotypes under controlled conditions, with longest root length in CM-94/99 and shorter root length in CM-2000 (Fig. 1). Water stress enhanced root length in all genotypes except CM-2000 whose root length was reduced under applied stress. Significant increase in root length was observed in CM-94/99 (Figs. 1, 2). Smita & Nayyar (2005) also observed reduction in root length of *Cicer arietinum* under water stress and detrimental effects could be due to reduction in root-hair diameter as well as distortion and plasmolysis thus rendering the uptake of available water by roots (Zahran & Sprent, 1986).

Current study deals with protein characteristics and its mobilization during seed germination and have been extensively studied by Ahmad *et al*., (1995), Duranti & Guis (1997) in legumes, Alvarez & Guerra (1985) in lentil, Chang & Harrold (1985) in bean cultivars, Neves & Lourenco (2001) in chickpea and Sathe *et al*., (1983) in *Phaseolus*. They all indicated slow hydrolysis of proteins under stress.

The protein profiling showed genotype specific variations. Greater variations were found in case of kabuli genotypes. A polypeptide of about 39 kDa, 42 kDa and 100 kDa were originally present at 5^{th} day in both CM-2000 and CM-94/99 under controlled conditions. However expression of these polypeptides was altered under stressed conditions. In case of CM-2000 these polypeptides were expressed at seventh day while in CM-94/99 they appeared even at fifth day of applied stress (Fig. 4). The reason might be slow mobilization of these polypeptides in relatively sensitive genotype due to poor enzymatic action under stressed condition (Samarah & Mullen, 2006).

Moreover under controlled conditions a polypeptide of about 60 kDa, which seemed to be a germination related protein, produced at fifth day in CM-2000 and at seventh day in CM-94/99. However water stress delayed (upto 7^{th} day) expression of this particular polypeptide in CM-2000 as compared to CM-94/99 (5^{th} day). No evident differences were observed in protein profiles of desi genotypes (CM-98, 6153).

No stress induced proteins were observed under applied water stress (10% PEG-4000) and thus confirmed the findings of Iqbal *et al*., (2006) about effect of water deficit in cotyledons of cotton seeds germinating under water stress (10% PEG- 4000), while heat shock proteins (HSPs) had been reported in response to desiccation by Burke & Mahony (2001) in cotton seeds.

Delayed mobilization of protein from cotyledon under stress seemed to be responsible for reduced root growth in CM-2000 while adjustment to earlier mobilization in CM-94/99 results an increase in root length under water stress. Combination of these morphological biochemical markers can be a valuable tool in assessment of water tolerance potential of chickpea genotypes at seedling stage.

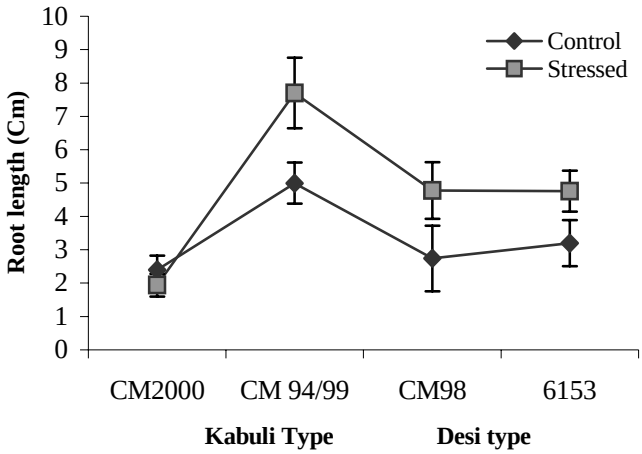

Fig. 1. Comparison of root length of chickpea genotypes growing under control and water stress at 7th day.

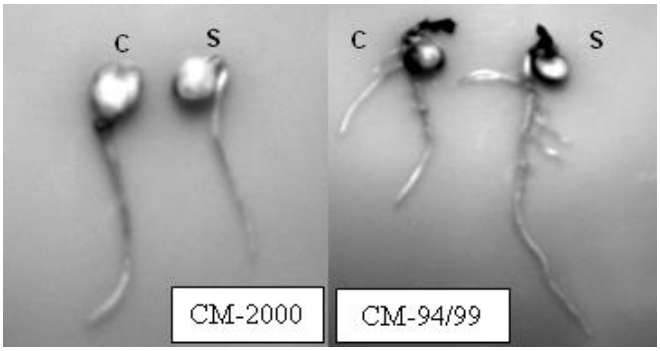

Fig. 2. Effect of water stress on root length of kabuli chickpea genotypes (CM-94/99, CM-2000) grown under control and water stress. C- control seedling and S - water stressed seedling.

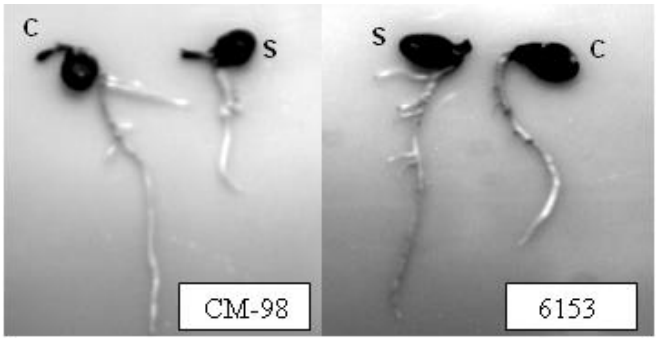

Fig. 3. Effect of water stress on root length of desi chickpea genotypes (CM-98 and 6153) grown under control and water stress. C- control seedling and S - water stressed seedling.

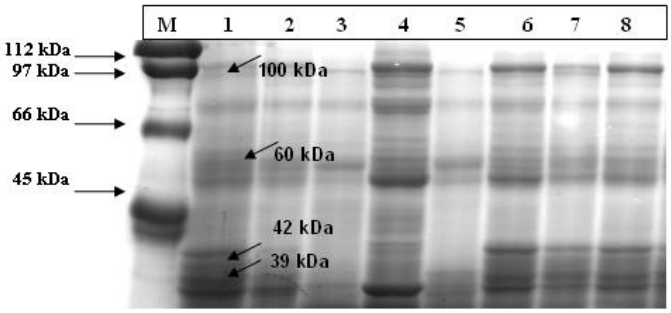

Fig. 4. Proteins profile of cotyledons from chickpea seedling germinating under water stress and control conditions. M. Marker, Lane. 1 & 2. 5th day and 7th day control of CM-2000, Lane. 3 & 4. 5th day and 7th day stressed of CM-2000. Lane. 5 & 6. 5th day and 7th day control of CM - 94/99, Lane. 7 & 8. 5th day and 7th day stressed of CM - 94/99.

References

Ahmed, F.A.R., E.A.M Abdel-Ralim; O.M. Abdel-Fatha, V.A. Erdman and C, Lippman. 1995. The changes of protein patterns during one week of germination of some legume seeds and roots. *Food Chem*., 52: 433-437.

Alvarez, J. and H. Guerra. 1985. Biochemical and morphological changes in protein bodies during germination of lentil seeds**.** *J. Experimental. Botany*, 36: 1296-303.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein dye-binding. *Anal. Biochem*., 38: 248-252.

Bruce, W.B., O.G. Edmeades and C.T. Barker. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. *Journal of Experimental Botany*, 53: 13-25.

Burke J. and P.J.O' Mahony. 2001. Protective role in acquired thermo tolerance of developmentally regulated heat shock proteins in cotton seeds. *The journal of Cotton Science*, 5: 174-83.

Chang, K.C. and R.L. Harrold. 1988. Changes in selected biochemical components, *In vitro* protein digestibility and amino acids in two bean cultivars during germination. *J. Food Sci.*, 53: 783-804.

Davies, J. William and Z. Jianhua. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42: 55-76.

Duranti, M. and C. Gius. 1997. Legume seeds: protein content and nutritional value**.** *Field Crops Res*., 53: 31-45.

Zahran, H.H. and J.I. Sprent. 1986. Effects of sodium chloride and polyethylene glycol on root-hair infection and nodulation of *Vicia faba* L., plants by *Rhizobium leguminosarum*. *Planta*, 167: 303-309.

Iqbal, N., H. Ali, M.K.R. Khan and M.A. Haq. 2006. Effect of water deficit on protein reserves in cotyledons of germinating cotton seeds. *Science Asia*, 32: 325-327.

Karimzadeh, G., G.R. Sharifi-Sirchi, M. Jalali Javaran, H. Dehghani and Francis. 2006. Soluble Proteins Induced by low temperature treatment in the leaves of spring and winter wheat cultivars. *Pak. J. Bot*., 38: 1015-1026.

Kiegle, E., C.A. Moore, J. Haseloff, M.A. Tester and M.R. Knight. 2000. Cell-type specific calcium responses to drought, salt and cold in the *Arabidopsis* root. *Plant Journal*, 23: 267-278.

Laemmli, U.K. 1970. Cleavage of structure proteins assembly of the head of bacteriophage. T4. *Nature*, 22: 680-5.

Malhotra, R.S. and M.C. Saxena. 1993. The challenge of developing biotic and abiotic stress resistance in cool-season food legumes. In: *Breeding for Stress Tolerance in Cool-Season Food Legumes*, 3: 14-18.

Mansour, E.H. 1996. Biological and chemical evaluation of chickpea seed proteins as affected by germination, extraction and -amylase treatment. *Plant Foods Hum. Nutr*., 49: 271-282.

Millan, T., H. Clarke, K. Siddique, H. Buhariwalla, P. Gaur, J. Kumar, J. Gil, G. Kahl and P. Winter. 2006: Chickpea molecular breeding: New tools and concepts. *Euphytica*., 147(1): 81-103.

Mostafa, M.M. and E.H Rahma. 1987. Chemical and nutritional changes in soybean during germination. *Food Chem*., 23: 257-275.

Neves, V.A. and E.J. Lourenço. 2001. Changes in protein fractions, trypsin inhibitor and proteolytic activity in the cotyledons of germinating chickpea. *Arch. Latinoamer. Nutr*., 51: 269-275.

Samarah, N.H. and R.E. Mullen. 2006. Total soluble and dehydrin like proteins in full rounded and shriveled seeds of soybean in response to drought stress. *Journal of Food, Agriculture & Environmental*, 4: 260-263.

Sathe, S.K., S.S. Deshpande, N.R. Reddy, D.E. Goll and D.K. Salunkhe. 1983. Effect of germination on protein, raffinose oligosaccharides and antinutritional factors in the Great northern bean (*Phaseolus vulgaris* L.). *J. Food Sci*., 48: l796-1800.

Smita and H. Nayyar. 2005. Carbendazim alleviates effects of water stress on chickpea seedlings. *Biologia Plantarum*, 49: 289-291.

Turner, N.C. 1886a. Adaptations to water deficits: a changing perspective. *Aust. J. Plant Physiol*., 13: 175-190.

Turner, N.C. 1986b. Crop water deficits: a decade of progress. *Adv. Agron*., 39: 1-51.

Turner, N.C., C. Wright, Graeme and K.H.M. Siddique. 2001. Adaptation of grain legumes (pulses) to water-limited environments *Advances in Agronomy*, 71: 193-231.

(Received for publication 23 September 2008)