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Effects of Drought Stress on Soluble Proteins in two Maize Varieties

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Abstract: Drought stress was generated by PEG 6000. Water potentials were: zero as the control, and –0.15, –0.49, –1.03, and –1.76 MPa as treatments. After 24-h treatment the total soluble protein content of 2 maize (*Zea mays* L.) cultivars (704 and 301) was determined and SDS-PAGE gel electrophoresis in the first dimension was performed. By decreasing water potentials, total soluble protein content first increased, and then decreased in the roots and leaves of both varieties. The decrease in total soluble protein content in the roots of both varieties was equal, but in the leaves of cv. 301 it was greater than in cv. 704. In drought conditions the decrease in root and shoot fresh weight in cv. 704 was greater than in cv. 301. With water potential –1.76 MPa, the accumulation of dehydrin-like 38, 50, 57, and 65 KDa M.W. root proteins and 15, 17, 20, 27, 30, 37, 54, and 59 KDa M.W. leaf proteins increased. However, the expression of 15, 19, and 27 KDa M.W. root proteins, and 22 KDa M.W. leaf protein was induced in both varieties. The accumulation of dehydrin-like proteins in the roots and leaves of cv. 704 was higher than in cv. 301. There was no relationship between protein changes and drought tolerance.

Key Words: Dehydrin-like proteins, Drought stress, Maize, Polyethylene glycol 6000, Protein content, SDS-PAGE

İki Mısır Varyetesinin Çözücü Proteinleri Üzerine Kuraklık Stresinin Etkisi

Özet: Kuraklık stresi PEG 6000 ile oluşturulmuştur. Su potansiyeli kontrole 0 olmak üzere sırasıyla –0.15, –0.49, –1.03, –1.76 MPa şeklinde uygulanmıştır. 604 ve 301 kodlu mısır (*Zea mays* L) varyetelerinin 24 saat muamele sonrası çözünür proteinleri SDS-PAGE jel elektroforezinde ilk boyutta belirlenmiştir. Su potansiyelinin azalması ile toplam çözünür protein muhtevası öncelikle artmış ve daha sonra her iki varyeteninde kök ve yapraklarında azalmıştır. Her iki varyeteninde köklerinde toplam çözünür protein azalması eşit olmasına rağmen 301 nolu varyetenin yapraklarında 704 nolu varyeteye göre daha yüksek bulunmuştur. Kuraklıkta, kök ve gövdenin taze ağırlığı 704 varyetesinde 301 varyetesinden daha fazla bulunmuştur. –1.76 MPa su potansiyelinde dehidrin-benzeri protein birikmesi 38, 50, 57, 65 KDa M.W. kök proteini ve 15, 17, 20, 27, 30, 37, 54, 59 KDa M.W. lif proteini şeklindedir. Fakat her iki varyetede de 15, 19, 27 KDa M.W. kök proteini, ve 22 KDa M.W. lif proteini ifadesi artmıştır. 704 nolu varyetenin kök ve yapraklarında dehidrin-benzeri protein birikmesi 301 nolu varyeteden daha fazla olmuştur. Protein değişiklikleri ve kuraklık toleransı arasında bir ilişki bulunamamıştır

Anahtar Sözcükler: Dehidrin-benzeri proteinler, kuraklık stresi, mısır, Polietilen glikol 6000, Protein muhtevası, SDS-PAGE

Introduction

Drought stress is one of the most important environmental stresses affecting agricultural productivity worldwide and can result in considerable yield reductions (1,2). The physiological mechanisms involved in cellular and whole plant responses to water stress, therefore, generate considerable interest and are frequently reviewed (3-8).

Numerous physiological and biochemical changes occur in response to drought stress in various plant species. Changes in protein expression, accumulation, and synthesis have been observed in many plant species as a result of plant exposure to drought stress during growth (9,10). Both quantitative and qualitative changes to proteins were detected during drought stress (11). In maize (*Zea mays L.*) it has been observed that drought stress increased the expression of 50 proteins, decreased that of 23, and induced the synthesis of 10 proteins, as detected by two-dimensional gel electrophoresis (11). Riccardi et al. (11) examined drought-responsive proteins of 2 maize lines. There was significant quantitive variation in 78 out of 413 leaf proteins, with 38 exhibiting differential expression in 2 genotypes. Proteins

synthesized in response to drought stress are known to be involved in plant response to water stress, such as the RAB17 (response to ABA) protein and enzymes involved in such metabolic pathways as glycolysis, the Krebs cycle, and lignin synthesis (11). Evidence is increasing in favor of a relationship between the accumulation of drought-induced proteins and physiological adaptations to water limitation (11-13).

Proteins synthesized in response to drought stress are called dehydrins (dehydration induced) and belong to the group II late embryogenesis abundant (LEA) proteins (14). The dehydrin family of proteins accumulates in a wide range of plant species under dehydration stress, which range in size from 9 to 200 kDa (15). Drought regulation of dehydrin gene expression was observed in both drought-tolerant and drought-susceptible cultivars (16,17).

Dehydrin proteins are also produced in response to various other environmental stresses, such as salt and cold stress (15), and have been characterized as hydrophilic, heat-stable, free of cysteine and tryptophan, responsive to ABA, and rich in lysine (15, 18,19). Dehydrin proteins accumulate along with other LEA proteins in response to a particular stress and have been proposed to play an important role in membrane protein stability and osmotic adjustment (15,20,21). These observations suggest that dehydrins, as well as other LEA proteins, might play a role in the acquisition of desiccation tolerance in seeds (18,21,22). Dehydrins have been most extensively studied in relation to drought stress (15,23).

A proposed role of dehydrin-like proteins in drought stress has been the protection cells from dehydration stress (14,21). Dehydrin-like proteins may also have a role similar to compatible solutes (such as proline, sucrose, and glycine betaine) in osmotic adjustment. Another possible role of stress proteins is to bind with the ions accumulated (ion sequestering) under drought stress and to control solute concentration in the cytoplasm (24). Dehydrin may have a cryoprotective role in macromolecular stabilization by binding water molecules to their hydrophilic surfaces, which reverses or prevents further denaturation of cellular proteins (15). Maturation proteins, which are induced in response to ABA or dehydration, might protect plants under stress by stabilizing cell membranes (21). Dehydrins are indeed a group of proteins whose members differ in their responsiveness to different stimuli and might have a role in response to drought stress.

The aim of present study was to comparatively analyze the effects of drought stress induced by PEG 6000 on total soluble proteins, growth, and the expression of dehydrin-like proteins in the roots and leaves of 2 maize varieties. These specific questions were addressed:

What are the changes in total soluble proteins and growth in the roots and leaves of the 2 maize varieties under drought stress?

What are the changes in the expression of dehydrin-like proteins in the roots and leaves of the 2 maize varieties under severe drought stress?

Is there a relationship between protein changes and drought tolerance?

Materials and Methods

Plant materials and growth conditions

Two maize (*Zea mays L.*) genotypes (cv. 704 and cv. 301) were used. The seeds of both cultivars were germinated in petri dishes on 2 layers of filter paper in an incubator maintained at 25 ºC. After 3 days the seedlings were transferred to plastic pots (15 cm diameter, 20 cm depth) filled with sand and irrigated with half strength Hoagland nutrient solution. Six-day seedlings were transferred to hydroponic cultures in aerated test tubes containing a polyethylene glycol (PEG) 6000 solution of 10%, 20%, 30%, and 40% strengths to achieve water deficit levels of –0.15, –0.49, –1.03, and –1.76 MPa, respectively (25-27), as treatments, and to aerated test tubes containing half strength Hoagland nutrient solution, which served as controls. Stress was applied for 24 h.

Total soluble protein content and fresh weight measurement

Total soluble protein content was determined according to the method of Lowry et al. (28), using bovine serum albumin as the standard. The root and shoot fresh weights obtained with the different treatments were measured with a digital balance (Tecator model 6110).

Protein extraction

Harvested root and leaf samples were immediately frozen in liquid nitrogen and stored at –80 ºC until used. From each sample, 250 mg were extracted in 0.8 ml of Tris-boric buffer (0.09 M Tris, 0.08 M boric acid, 0.93 g/l of Na_2EDTA) and 0.8 ml of 40% w/v sucrose, then

extraction centrifuged at 10000 g for 10 min. 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.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE gel electrophoresis in the first dimension was performed using 15% running gel and 3% stacking gel (29). Running gel: 35.4% w/v acrylamide, 0.62% w/v Bis-acrylamide, 10% w/v SDS, 1M Tris (pH = 8.8), 3 ml of deionized H_2O, 10% w/v APS (ammonium persulfate solution), and 6 µl of TEMED (tetra methylene diamine). Stacking gel: 35.4% w/v acrylamide, 0.62% w/v Bis-acrylamide, 10% w/v SDS, 1M Tris (pH = 6.8), 3.71 ml of deionized H_2O, 10% w/v APS, and 5 µl of TEMED. Running buffer (Tris-glycine running buffer) for the tank contained 0.025M Tris (pH = 8.5), 0.192 M glycine, and 0.1% w/v SDS.

The gel was run at 200 V for 4 h, with the voltage gradually increasing to this level during the first 20 min to avoid excessive current flow at the start. After this stage the gels were inserted into fixative solution (45.4% methanol and 9.2% acetic acid) for 2 h, then the gels were inserted into staining solution (0.025% Coomassie Ablue stain, 25% isopropanol, and 10% acetic acid) for 3 h, and finally the gels were inserted into destaining solution (10% methanol and 10% acetic acid) until the bands appeared and image analysis was performed.

Statistical analysis

Mean values of fresh weight and total soluble protein content were taken from the measurements of 4 replicates, and the standard error of the means was calculated. One-way ANOVA was applied to determine the significance of the results between different treatments and then Turkey's multiple range tests ($P < 0.05$) were performed. All the statistical analyses were made using SPSS v.13 for Windows (Statistical Package for Social Sciences, Chicago)

Results and Discussion

The growth of both maize varieties was repressed under drought stress. The fresh weight of roots and shoots were affected by water deficit (Figure 1). With water potentials –0.15 and –0.49 MPa, plants showed higher root and shoot fresh weights, but with –1.03 and –1.76 MPa there was a significant reduction in fresh weight in both varieties. With water potential –1.76 MPa, root fresh weight decreased by 79% in cv. 704 and 66% in cv. 301, and shoot fresh weight decreased by 69% in cv. 704 and 68% in cv. 301. The decreases in root and shoot fresh weights in cv. 704 were greater than in cv. 301.

Kramer (30) reported that the first measurable effect due to water deficit was growth reduction caused by declining cellular expansion. The process of cellular elongation and carbohydrate wall synthesis were very susceptible to water deficit (31), and the growth decrease was a consequence of the turgescence laying down those cells (32).

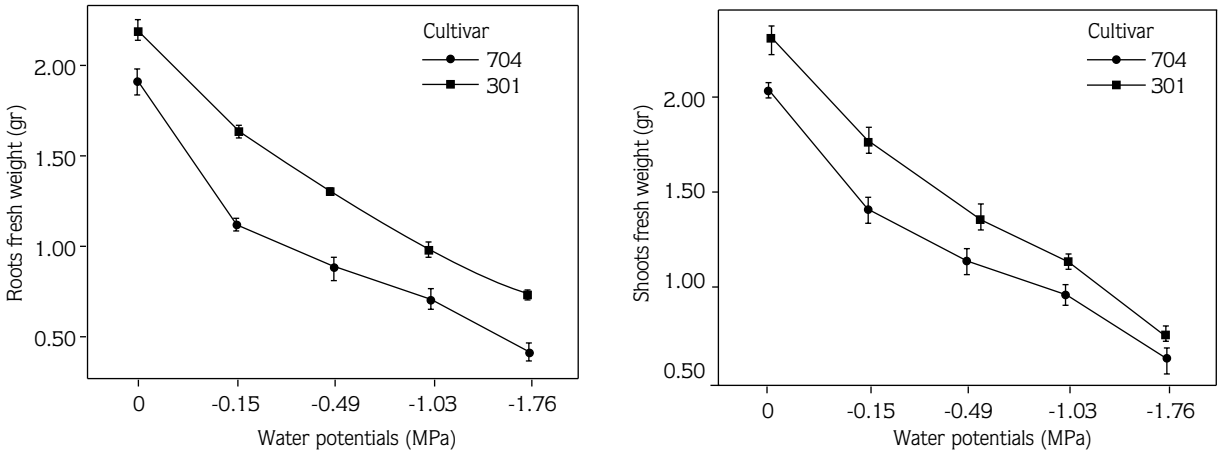

Figure 1. Effects of different water potentials on roots and shoots fresh weights (gr/10 seedlings) in 2 maize cultivars. Results are shown as mean ± standard error ($P < 0.05$), obtained from four replicates.

Under drought stress total soluble protein content in the roots and leaves of both maize varieties first increased and then decreased. With water potential –0.15 MPa, this factor in roots and leaves increased gradually, and total soluble protein content in the roots was maximal, whereas the maximum in the leaves was reached with water potential –0.49 MPa. With water potential –1.03 and –1.76 MPa, total soluble protein content was reduced in roots and leaves of both maize varieties (Figure 2). The amount of reduction was related to drought intensity and drought duration.

With water potential –0.15 MPa, root total soluble protein content increased 1.31 fold in cv. 704 and 1.26 fold in cv. 301, compared to the control plants. With water potential –0.49 MPa, leaf total soluble protein content increased 1.35 fold in cv. 704 and 1.22 fold in cv. 301, compared to the control plants. Then, total soluble protein content decreased in the roots and leaves of both varieties. With water potential –1.76 MPa, root total soluble protein content decreased 39% in both varieties compared to the control plants, and leaf total soluble protein content decreased by 28% in cv. 704 and 30% in cv. 301, compared to the control plants. The increase in total soluble protein content in the roots and leaves of cv. 704 was higher than in cv. 301, whereas the decrease in the roots of both varieties was equal and the decrease in the leaves of cv. 301 was greater than in cv. 704. Total soluble protein content in roots was lower than in leaves.

It seems that the initial increase in total soluble proteins during drought stress was due to the expression of new stress proteins, but the decrease was due to a severe decrease in photosynthesis. Photosynthesis decreased in drought stress (33) and materials for protein synthesis weren't provided; therefore, protein synthesis dramatically reduced or even stopped.

The increase and decrease in total soluble proteins under drought stress was consistent with the findings of Riccardi et al. (11) and Ti-da et al. (34) in maize, and Bensen et al. (35) in soybean. These authors reported that drought stress resulted in an increase of some soluble proteins and a decrease of others.

Several hypotheses may explain the mechanism by which drought stress induces dehydrin-like proteins in a greenhouse experiment. The first hypothesis is that drought may accelerate development, resulting in earlier expression of dehydrin-like proteins. On the basis of this hypothesis, dehydrin-like proteins are expressed because of development rather than experimental stress. Thus, the observed induction of dehydrin-like proteins by drought stress treatments was unlikely to be due to the acceleration of development. The second hypothesis is that the change in water potential under drought stress may result in the expression of dehydrin-like proteins (36). It seems that our results were consistent with the second hypothesis, and the increase in drought stress and decrease of water potential induced the expression of dehydrin-like proteins.

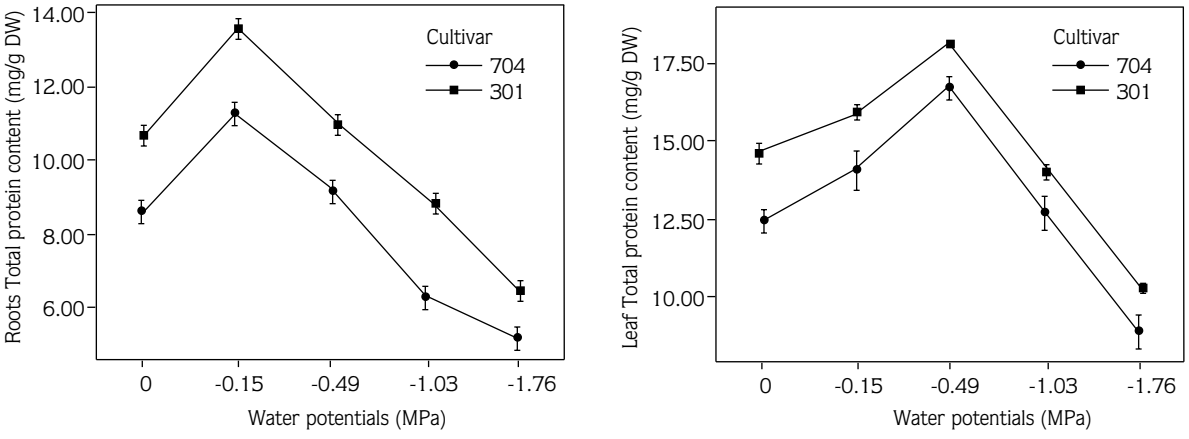

Figure 2. Effects of different water potentials on total soluble protein content (mg/g DW) in the roots and leaves of 2 maize cultivars. Results are shown as mean ± standard error ($P < 0.05$), obtained from four replicates.

The dehydrin-like proteins were detected by SDS-PAGE. It has been reported that dehydrin accumulation is correlated with dehydration when dehydrin accumulation was compared in dried cereal seedlings (37). This is supported by the observation in the present study that dehydrin-like proteins accumulated in the roots and leaves of both maize varieties with decreasing water potentials.

In general, the accumulation of dehydrin-like proteins, as detected by SDS-PAGE, increased in the roots and leaves of both varieties in all treatments, but some of the proteins were not detected in severe drought stress. This means that drought stress resulted in an increase of some proteins and a decrease of others. With water potential –1.76 MPa, the expression of 15, 19, and 27 KDa M.W. root proteins, and 22 KDa M.W. leaf protein was induced in both maize varieties. These changes in protein expression strongly suggest that the induced proteins play a role in plant response to drought stress.

With water potentials –1.03 and –1.76 MPa, 38, 50, 57, and 65 KDa M.W. root proteins increased significantly compared to the control. With water potentials –0.15 MPa and –1.03 MPa, the accumulation of 15, 17, 20, 27, 30, 33, 37, 54, 59, and 115 KDa M.W. leaf proteins increased in cv. 704, and with water potential –1.03 MPa the accumulation of these leaf proteins increased in cv. 301. With water potential –1.76 MPa, the accumulation of 20 KDa M.W. leaf protein (trypsin inhibitor) increased in both varieties. Our results are similar to the results reported by Jiang and Huang (38). They reported that the accumulation of 20, 22, 27, 30, 54, and 59 kDa M.W. leaf proteins was induced by drought stress, and that the amount of these proteins generally increased with progressive water deficit. The 22 and 27 kDa M.W. dehydrin polypeptides significantly accumulated in drought-stressed plants. These results indicated that the accumulation of dehydrin-like proteins was induced by severe drought stress. Wechsberg et al. (39) found that the accumulation of 18, 28, and 31 kDa M.W. dehydrin-like proteins in the seeds of crowfoot (*Ranunculus sceleratus* L.) depended on the stage of drought stress. Accumulation of dehydrin proteins could protect cells from further dehydration during drought stress (13,40). The mechanisms by which drought stress affect drought tolerance are numerous and complex, which may include the induction of some polypeptides and dehydrin-like proteins (41,42).

With water potential –1.76 MPa, 69 KDa M.W. leaf protein decreased in both maize varieties. With this water potential, 83 and 115 KDa M.W. leaf proteins decreased in cv. 704, but were not detected in cv. 301. In severe drought stress (water potential –1.76 MPa), the accumulation of 205 KDa M.W. protein decreased in leaves, but we didn't observe this protein in the roots of either maize variety. The decrease of these proteins in severe drought stress was consistent with the findings of Riccardi et al. (11).

In the roots of both varieties, the accumulation of dehydrin-like proteins increased in drought stress, especially with water potential –1.03 MPa and –1.76 MPa. The accumulation of stress proteins in the roots of both varieties was higher than it was in the leaves. With water potential –1.76 MPa, some of the proteins were not detected in the leaves; therefore, great variation in the level of protein accumulation during drought stress were detected in the roots of both varieties, but we observed only a few variations in the leaves of both varieties (Figure 3). It seems that roots were more sensitive than leaves. Drought probably acted directly on the roots because they immerged in PEG solutions and drought stress in roots was higher than it was in leaves.

In the leaves of cv. 704, the expression of stress proteins with water potential –0.15 MPa was higher than it was with the other water potentials. It seems that in mild drought stress the accumulation of proteins increased, but with water potential –1.76 MPa we didn't observe much protein accumulation. Under drought stress, protein patterns in the roots of cv. 704 were similar to those of cv. 301. In leaves, protein patterns in cv. 704 were similar to those of cv. 301, but with water potential –0.15 MPa the accumulation of proteins increased only in cv. 704 and with water potential –1.03 MPa it increased in both varieties. With water potential –1.76 MPa, the accumulation of proteins decreased in both varieties.

PEG in the medium repressed dehydrins at the protein level. One possible explanation for these results could be that the cell cultures failed to adjust to water stress. As a matter of fact, with callus cultures of poplar Tschaplinski et al. (43) showed that even though PEG in the medium provided osmotic stress, the cultures did not display osmotic adjustment to the drought stress. Another possibility could be that dehydrins in cell cultures do not

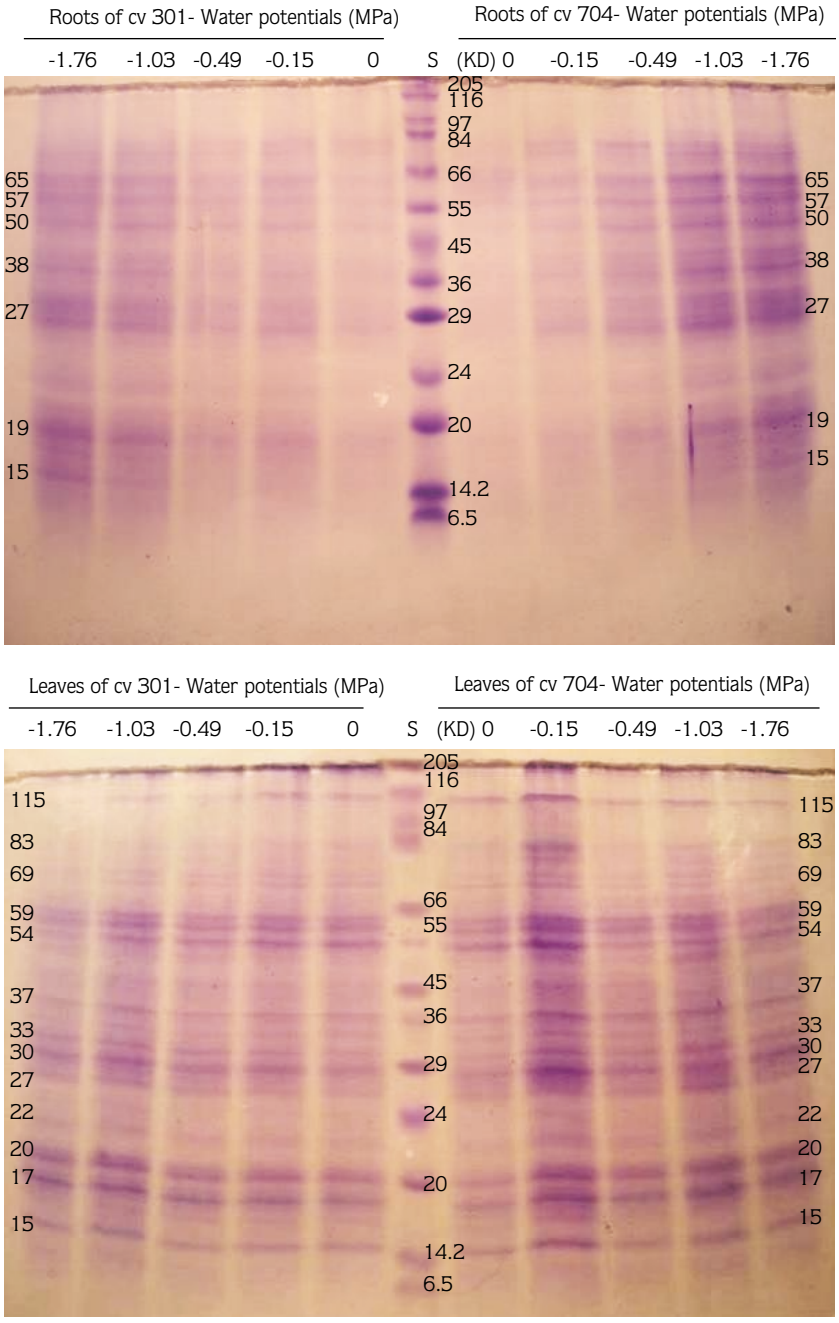

Figure 3. SDS-PAGE of roots and leaves proteins of two maize cultivars in response to different water potentials.

respond to the stress, and other mechanisms and solutes are involved in adjusting the osmotic potential of the cells (44). Our results weren't consistent with Tschaplinski et al.'s findings. We found that PEG 6000 in the medium induced the accumulation of dehydrin-like proteins in the roots and leaves of both maize varieties.

Our original objective was to observe changes in the expression of dehydrin-like proteins and total soluble proteins in the roots and leaves of 2 maize varieties in response to drought stress. We found that by decreasing the water potential, total soluble proteins decreased in the roots and leaves of both maize varieties, but

dehydrin-like proteins increased. The expression of new stress proteins in the roots of both varieties was higher than it was in the leaves, especially with low water potentials. The fresh weight of roots and shoots decreased under drought stress.

With water potential –1.76 MPa, roots and leaves of cv. 301 had higher total soluble protein content and fresh weights than cv. 704. The mean differences in fresh weights and total soluble proteins in both varieties were significant at the 0.05 level between all treatments. Based on these results it seems that cv. 301 has greater tolerance than cv. 704 to severe drought stress; however, the accumulation of dehydrin-like proteins in the roots and leaves of cv. 704 was higher than in cv. 301. It has been reported that the accumulation of dehydrin proteins does not necessarily correlate with the content of the corresponding proteins (40). Drought-induced polypeptides have been observed in many studies (11,45,46) and are assumed to play a role in water stress tolerance. Our results with 2 maize varieties indicated that the accumulation of a 22 kDa M.W. leaf protein, and 15, 19, and 27 root proteins was in response to drought stress, and the other proteins were intensified in drought-stressed plants of both varieties. Therefore, no relationship between protein changes and drought tolerance was apparent in this study, similar to the results reported by Perez-Molphe-Balch et al. (46), and Jiang and Huang (38).

Recently, drought-induced dehydrin proteins have been found in many species (39,45). Drought-induced expressions of dehydrin genes were identified in both drought-tolerant and drought-sensitive cultivars of sorghum (*Sorghum bicolor* L.) (47), and at a higher level in tolerant cultivars of wheat (*Triticum durum* L.) (23) and in sensitive cultivars of cocksfoot (*Dactylis glomerata* L.) (48).

In conclusion, drought stress induced changes in protein synthesis in maize. The accumulation of dehydrin-like proteins was detected in the roots and leaves of drought-stressed plants of both varieties, which could protect plants from further dehydration damage.

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References

1. Boyer JS. Plant productivity and environment. Science 218: 443-448, 1982.
2. Ludlow MM, Muchow RC. A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy 43: 107-153, 1990.
3. Davies WJ, Zhan J. 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, 1991.
4. Kramer PJ, Boyer JS. Water relations of plants and soils. San Diego; USA: Academic Press, 1-495, 1995.
5. Neuman PM. Salinity resistance and plant growth revisited. Plant, Cell and Environment 20: 1193-1198, 1997.
6. Neuman PM. The role of cell wall adjustment in plant resistance to water deficits. Crop Sci 35: 1258-1266, 1995.
7. Smith JAC, Griffiths H. Water deficits: plant responses from cell to community. UK: Bios Scientific Publishers, 1-332, 1993.
8. Turner NC. Further progress in crop water relations. Advances in Agronomy 58: 293-338. 1997.
9. Chen RD, Tabaeizadeh Z. Alteration of gene expression in tomato plants (*Lycopersicon esculentum*) by drought and salt stress. Genome 35: 385-391, 1992.
10. Cheng Y, Weng J, Joshi CP et al. Dehydration stress-induced changes in translatable RNAs in sorghum. Crop Sci 33: 1397-1400, 1993.
11. Riccardi F, Gazeau P, Vienne D et al. Protein changes in response to progressive water deficit in maize, quantitative variation and polypeptide identification. Plant Physiol 117: 1253-1263, 1998.
12. Bray EA. Molecular responses to water deficit. Plant Physiol 103: 1035-1040, 1993.
13. Han B, Kermode AR. Dehydrin-like proteins in castor bean seeds and seedlings are differentially produced in responses to ABA and water-deficit-related stresses. J Exp Bot 47: 933-939, 1996.
14. Close TJ, Chandler PM. Cereal dehydrins: Serology, gene mapping, and potential functional roles. Aust. J. Plant Physiol 17: 333-344, 1990.

15. Close TJ. Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant 97: 795-803, 1996.
16. Cellier F, Conejero G, Breitler JC et al. Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflowers. Accumulation of dehydrin transcripts correlates with tolerance. Plant Physiol 116: 319-328, 1998.
17. Wood AJ, Goldsbrough PB. Characterization and expression of dehydrins in water-stressed *Sorghum bicolor*. Physiol Plant 99: 144-152, 1997.
18. Close T, Kortt AA, Chandler PM. A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol Biol 13: 95-108, 1989.
19. Vilardell J, Goday A, Freire MA et al. Gene sequence, developmental expression, and protein phosphorylation of RAB-17 in maize. Plant Mol Biol 14: 423-432, 1990.
20. Carpenter JF, Crowe JH. The mechanism of cryoprotection of proteins by solutes. Cryobiology 25: 244-255, 1988.
21. Dure L, Crouch M, Harada J et al. Common amino acid sequence domains among the LEA proteins of higher plants. Plants Mol Biol 12: 475-486, 1989.
22. Dure L. The Lea proteins of higher plants. p. 325-335. In D.P. Verma ed. Control of plant gene expression. CRC Press, Inc, Boca Raton; 1993a.
23. Labhilili M, Joudrier P, Gautier MF. Characterization of cDNAs encoding *Triticum durum* dehydrins and their expression patterns in cultivars that differ in drought tolerance. Plant Sci 112: 219-230, 1995.
24. Dure L. A repeating 11-mer amino acid sequence domains among the LEA proteins of higher plants. Plant J 3: 363-369, 1993b.
25. Burlyn E, Mirrill R. The osmotic potential of polyethylene glycol 6000. Plant Physiol 51: 914-916, 1973.
26. Nicholas P. Osmotic pressure of aqueous polyethylene glycols. Plant Physiol 91: 766-769, 1989.
27. Steuter A, Mozafar A, Goodin J. Water potential of aqueous polyethylene glycol. Plant Physiol 67: 64-67, 1981.
28. Lowry OH, Rosebrough NJ, Farr AL et al. Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-276, 1951.
29. Hames BD, Rickwood D. Gel electrophoresis of proteins: a practical approach. 2d Ed, IR.L. Press Limited. Oxford, 383, 1990.
30. Kramer PJ. Fifty years of progress in water relations research. Plant Physiology 54: 463-471, 1974.
31. Wenkert W, Lemon ER, Sinclair TR. Leaf elongation and turgor pressure in field; grown soybean. Agronomy Journal 70: 761-764, 1978.
32. Shalhevet J, Huck MG, Schoeder BP. Root and shoot responses to salinity in maize and soybean. Agronomy Journal 87: 512-517, 1995.
33. Havaux M, Canaani O, Malkin S. Inhibition of photosynthetic activities under slow water stress measured in vivo by photoacoustic method. Physiol Plant 70: 503-510, 1987.
34. Ti – da GE, Fang – Gong SUI, Ping BA, et al. Effects of water stress on the protective enzymes and lipid peroxidation in roots and leaves of summer maize. Agricultural Sciences in China 5: 291-298, 2006.
35. Bensen RJ, Boyer JS, Mullet JE. Water deficit-induced changes in abscisic acid, growth, polyamines, translatable RNA in soybean hypocotyls. Plant Physiol 88: 289-294, 1988.
36. Dunbar B, Elthon E, Osterman C et al. Identification of plant mitochondrial proteins: a procedure linking two-dimensional electrophoresis to protein sequencing from PVDF membranes using a fastblot cycle, Plant Molecular Biology Reporter Pages 15: 46-61, 1997.
37. Close TJ, Fenton RD, Yang A et al. Dehydrin: the protein. In: Close TJ, Bray EA, Eds, Plant Responses to Cellular Dehydration during Environmental Stress. American Society of Plant Physiologists, Rockville, 104-118, 1993.
38. Jiang Y, Huang B. Protein Alterations in Tall Fescue in Response to Drought Stress and Abscisic Acid. Crop Science 42: 202-207, 2002.
39. Wechsberg GE, Bray CM, Probert RJ. Expression of dehydrin-like protein in orthodox seeds of *Ranunculus scleratus* during development and water stress. Seed Sci Res 4: 241-246, 1994.
40. Cellier F, Conejero G, Breitler JC et al. Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflowers. Accumulation of dehydrin transcripts correlates with tolerance. Plant Physiol 116: 319-328, 1998.
41. Pruvot G, Massimino J, Peltier G et al. Effects of low temperature, high salinity and exogenous ABA on the synthesis of two chloroplastic drought-induced proteins in *Solanum tuberosum*. Physiol Plant 97: 123-131, 1996.
42. Han B, Berjak P, Pammenter N et al. The recalcitrant plant species, *Castanospermum australe* and Trichilia dregeana, differ in their ability to produce dehydrin-related polypeptides during seed maturation and in response to ABA or water-deficit-related stresses. J Exp Bot 48: 1717-1726, 1997.
43. Tschaplinski TJ, Gebre GM, Dahl JE et al. Growth and solute adjustment of calli of *Populus* clones cultured on nutrient medium containing polyethylene glycol. Can J For Res 25: 1425-1433, 1995.
44. Hanson AD, Hitz WD. Metabolic responses of mesophytes to plant water deficits. Ann. Rev. Plant Phys 33: 163-203, 1982.
45. Arora R, Pitchay DS, Bearce BC. Water-stress-induced heat tolerance in geranium leaf tissues: A possible linkage through stress proteins? Physiol Plant 103: 24-34, 1998.
46. Perez-Molphe-Balch E, Gidekel M, Segura-Nieto M et al. Effects of water stress on plant growth and root proteins in three cultivars of rice *(Oryza sativa*) with different level of drought tolerance. Physiol Plant 96: 284-290, 1996.
47. Wood AJ, Goldsbrough PB. Characterization and expression of dehydrins in water-stressed *Sorghum bicolor*. Physiol Plant 99: 144-152, 1997.
48. Volaire F, Thomas H, Bertagne N et al. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: Water status, solute accumulation, abscisic acid concentration and accumulation of dehydrin transcripts in bases of immature leaves. New Phytol 140: 451-460, 1998.