Response of Water Saturation Deficit and Some Nitrogenous Compounds in Two Maize Cultivars to Water Stress

Hameda El Sayed* and Mahmoud Aboelyazeed Elhaak**

**Department of Biology, Faculty of Education, Tanta University, Kafr El Sheikh, Egypt and*
***Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt*

(Received 15/2/1993; accepted for publication 5/7/1994)

Abstract. Water saturation deficit (WSD), amino acids, proline and protein content in the leaves were studied in two Egyptian maize (Zea mays, L.) cultivars; Composite 5 and Giza 2 grown under 0, -2, -4, -6, -8, and -10×10^{-5} MPa water potentials prepared from manitol. The study showed that WSD of the two cultivars increased with water stress but at different rates. The two cultivars showed also an equal daily increase in WSD under the same water stress. In both cultivars, water stress lead to the accumulation of protein, amino acids and proline. Higher rates of accumulation of proteins, amino acids and proline occurred at 0 to -2×10^{-5} MPa in both cultivars . Proline increased at rate higher than 15 mg/g D.W. per one MPa decrease in water potential and about 8 mg/g D.W. per day of stress for both cultivars. According to the amount of accumulated proline in response to water stress cv. Composite 5 could be considered more tolerant than cv. Giza 2.

Key words: Maize, (Zea mays, L. cvs. Composite 5 and Giza 2), water saturation deficit, (WSD), amino acids, proline, and protein.

Introduction

Water stress has been reported to induce a pronounced alterations in the metabolism of nitrogenous compounds [*e.g.* 1-3]. Ahmed, *et al.* [4] reported that water stress resulted in some disturbances in nitrogen metabolism. Protein synthesis is one of the biochemical processes that are affected by water stress [*e.g.* 5-7]. Interruption of both protein synthesis as well as proteolysis has been reported in stressed plants [8, pp. 53-88]. However, anabolic activities are usually more severely affected than catabolic activities [9]. Chen, *et al.* [10] and Barnett and Naylor [11] stated that a considerable hydrolysis of proteins may occur in water stressed plants which is always accompanied by varied increments in amino acids. Accumulation of amino acids under drought stress was reviewed by [12, pp. 609-635; 13, pp. 205-259; 14]. This accumulation may be important in plant adaptation by dry environment [15; 16]. Also, adaptation to water stress is closely associated with proline accumulation [14].

Plant sensitive to imposed water stress have been investigated by many workers [*e.g.* 17-19]. The association between susceptibility and water saturation deficit and nitrogen metabolism still needs exploration.

The present work was therefore designed to study the correlations between medium water potential, water saturation deficit and nitrogenous compounds, induced by manntol.

Materials and Methods

Grains of maize (Zea mays, L. Cultivars Composite 5 and Giza 2) were obtained from the Ministry of Agriculture, Cairo, Egypt.

Grains of maize were germinated at 28°C in dark in 9 cm in a diameter glass Petri dishes on discs of filter paper (Whatman No. 1) moistened with mannitol solutions of appropriate water potential as described in [20]. The seedling in pots (25 cm × 35 cm) in the greenhouse. When the seedling were well established after 10 days or seedlings at the first leaf stage were subjected to 0, -2, -4, -6, -8 and -10 × 10^{-5} MPa water potential treatments using mannitol solution as described in [20].

Growth and water saturation deficit

After three days of treatment solutions of mannitol as described before, five leaves were harvested every day for 7 days and washed with distilled water. Leaf samples were weighed as fresh weight (F.W.) then they were immersed in distilled water for 24 hours and then removed to determine the saturated weight (S.W.). After that the saturated leaves were dried in an oven at 60°C for determining the oven constant dry weight (D.W.).

The equations for calculation of water saturation deficit (W.S.D.) and relative turgidity (R.T.) as follow:

$$WSD = [(S.W. - F.W.)/(S.W. - D.W.] \times 100$$
$$R.T. = [(F.W. - D.W.)/(S.W. - D.W.)] \times 100$$

Nitrogenous components

The rest of the leaves were dried to a constant weight at 60°C and ground to powder using grinder. An equal weights of the dry powder were analyzed for their contents of amino acids using the method of Ya and Tunekazu [21], of proline using the method of Bates, *et al.* [22], and of proteins using the method of Lowry, *et al.* [23].

Total free amino acids

Free amino acids were determined by the method described by Ya and Tunckazu [21]. An a liquot of 0.1 ml plant extract was heated in a test tube with 1.9 ml of ninhydrine citrate buffer-glycerol mixture in a boiling water bath for 12 min and cooled at room temperature. Then the tube was well shaken and the optical density read at 570 nm. The blank was determined 0.1 ml distilled water and a standard curve obtained with 0.005 to 0.2 mM g glycine.

Free proline

These was estimated using the acid ninhydrine method as described by Bates, *et al.* [22]. Two ml of water extract were mixed with 10 ml of 3% aqueous sulfosalicylic acid. Two ml of this mixture was allowed to react with 2 ml acid ninhydrin-reagent and 2 ml of glacial acetic acid in a test tube for one h at 100°C; the reaction was terminated by cooling the mixture in an ice bath. The reaction mixture was extracted with 4 ml toluene, and mixed vigorously for 15 to 20 s. The chromatophore - containing toluene was aspirated from the aqueous phase, warmed to room temperature, and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve.

Protein

Dry samples collected during the growth study were analyzed for protein content according to [23], after precipitating the protein with 15% TCA at 4°C.

Statistical analysis

Data were statistically analyzed using the least significant difference and regression analysis and correlations coeffectient tests.

Results and Discussion

In both cultivars of *Zee mays,* L. (cv. Composite 5 and cv. Giza 2), water saturation deficit increased in response to the decrease in water potentials of the medium (Fig. 1). This may indicate differential tolerance of the two cultivars to the decrease in water potentials. The relationships between the water saturation deficit (WSD) and water potential of the medium was linear and highly significant in both cultivars of *Zea mays,* L. under different water potentials. The water saturation deficit (WSD) increased greater in cv. Composite 5 than in cv. Giza 2. In both cultivars of maize (cv. Giza 2 and cv. Composite 5)under zero WSD the potentials were 0.04×10^{-5} MPa and 0.4×10^{-5} MPa respectively which indicated that in cv. Giza 2 increased ten times more than in cv. Composite 5. Under zero water potential the results in Fig. 1 indicated a slight lower WSD in cv. Giza 2 (0.273%) than in cv. Composite 5 (3.265%).

Water saturation deficit increased with days of both maize cultivars (cv. Composite 5 and cv. Giza 2) stress (Fig. 2). The increase was greater under 4.04×10^{-5} MPa in both maize cultivars. The relationships between WSD and days of treatment were highly significant. The WSD percentages were; 4.45, 8.78 and 18.85 for cv. Giza 2 and 5.42, 9.06 and 18.35 for cv Composite 5 under 0.0, -2.02, -4.04×10^{-5} MPa respectively. This showed more or less equal increase in water saturation deficit in both cultivars of maize under the same water potential.

The nitrogenous compounds (total free amino acids, free proline and proteins) were

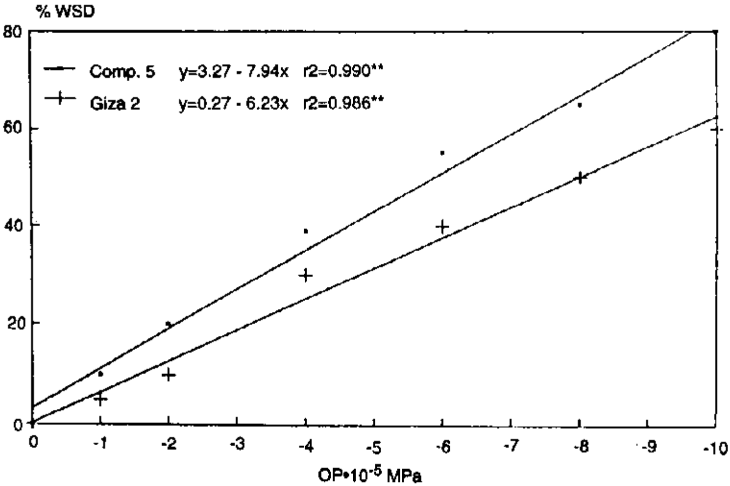

Fig. 1. Relationships between water potential of the medium (OP × 10^{-5} MPa) and water saturation deficit (WSD) of *Zea mays*, L. cultivars (cv. Composite 5 and cv. Giza 2).

greatly higher in stressed than non-stressed (control) plants of both maize cultivars (Fig. 3). Total free amino acids and free proline increased with decreasing water potentials of the medium in both cultivars. The increase in total free amino acids, free proline and proteins appeared to be greater between 0.0 and -3.0×10^{-5} MPa. Protein decreased at lower water potential. The regression equations of the contents of the nitrogenous compounds of the leaves of both maize cultivars subjected to water potentials from 0.0 to -2.02×10^{-5} MPa and from -4.04 to -10×10^{-5} MPa (Table 1) showed a decrease in the rate of total free amino acids and free proline accumulation to the half under lower water potential than at -2.02×10^{-5} MPa. Protein tended to accumulate more than total free amino acids and proline in cv. Composite 5 than in cv. Giza 2 under 0.0 to -2.02×10^{-5} MPa. Henckel [18] found high protein synthesis in stressed plants. Also, the rates of accumulation of amino acids and proline were mostly parallel. The amount of proteins was higher than that amino acids and proline in all treatments in cv. Composites 5 plants, while in cv. Giza 2 was only higher at 0.0 to -2.02×10^{-5} MPa. The amount of amino acids, proline and proteins were higher in cv. Composite 5 than in cv. Giza 2. Protein increased at higher rates in cv. Composite 5 than in cv. Giza 2 at 0.0 and -2.02×10^{-5} MPa. It decreased at equal rates in both cultivars at water potential lower than -2.02×10^{-5} MPa.

In the first two days of stress, protein content increased in cv. Giza 2 under -4.04 × 10^{-5} MPa and under -2.02 × 10^{-5} MPa in cv. Composite 5 (Fig. 4). After that the amount of proteins in both maize cultivars decreased with time of stress. After seven days of

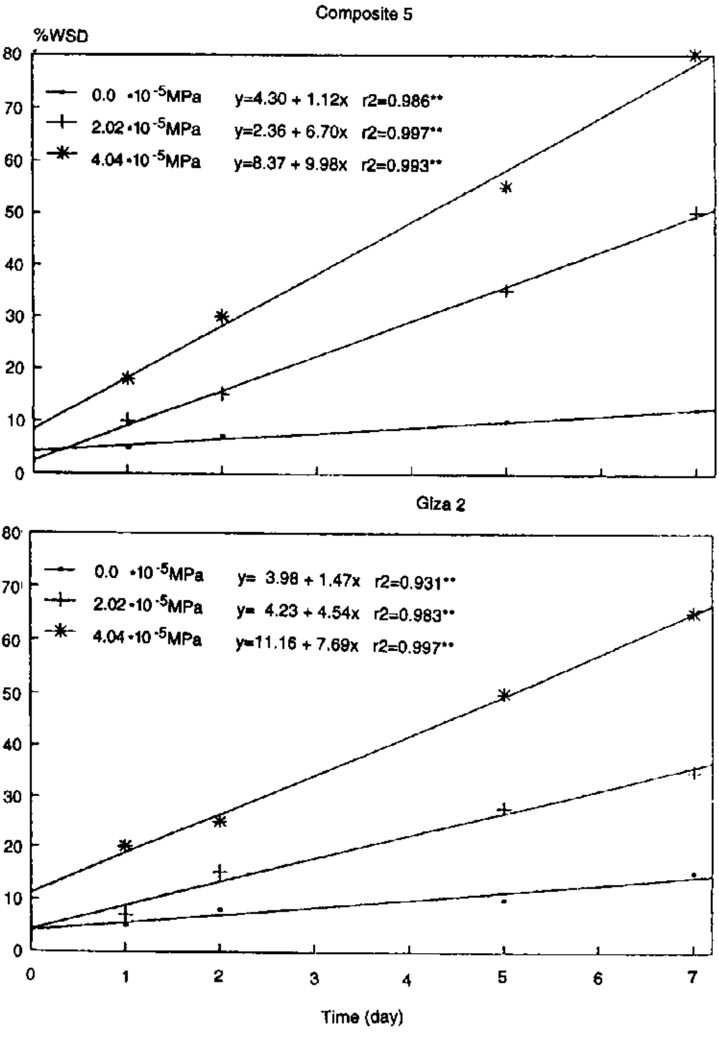

Fig. 2. Relationships between the time of withstand in control, -2.02 and -4.04 $\times 10^{-5}$ MPa water potential and the water saturation deficit (WSD) of both *Zea mays*, L. cultivars cv. Composite 5, Fig. 2(a), and cv. Giza 2, Fig. 2(b).

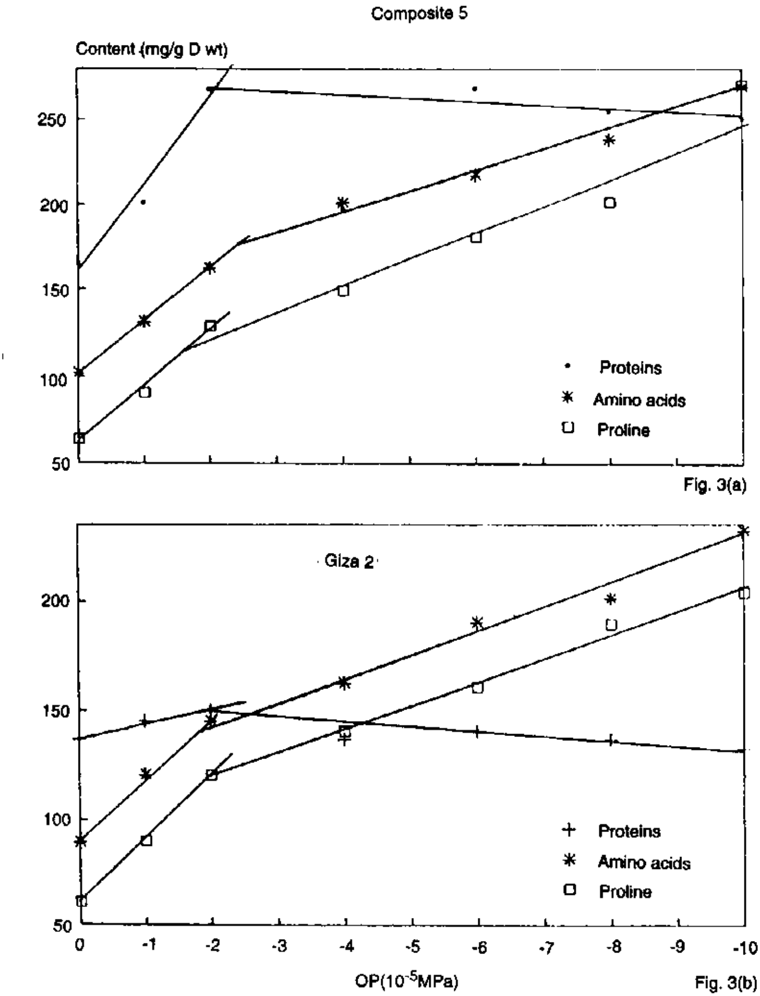

Fig. 3. Relationships between water potential of the medium (OP × 10^{-5} MPa) and the amounts of amino acids, proline and protein (mg/g D.W.) of *Zea mays*, L. cultivars (cv.Composite 5, Fig. 3(a) and cv.Giza 2, Fig. 3(b)).

Table 1. Regression equations of protein, amino acids and proline of *Zea mays*, L. cultivars (cv. Composite 5 and cv. Giza 2) under (A) from 0.0 to -2.02×10^{-5} MPa and (B) from -4.04 to 10.1 $\times 10^{-5}$ MPa water potential

Composite 5			Giza 2		
			Protein		
A	y = 160 - 51.04x	r = -0.987 **	A	y = 137 - 6.68x	r = -0.986 **
B	y = 192 - 7.13x	r = -0.604 ns	B	y = 142 + 0.77x	r = 0.667 ns
			Amino acids		
A	y = 102 - 29.80x	r = -0.999 **	B	y = 90 - 27.70x	r = 0.998 **
B	y = 154 - 11.00x	r = -0.989 **	B	y = 120 - 10.20x	r = 0.986 **
			Proline		
A	y = 62 - 32.20x	r = -0.996 **	B	y = 61 - 29.50x	r = -0.999 **
B	y = 106 - 11.50x	r = -0.994 **	B	y = 97 - 10.90x	r = -0.993 **

** highly significant (p 0.001)

ns nonsignificant

treatment the amount of protein in cv. Composite 5 was higher than the amount of protein in unstressed plant at the beginning of the experiment. An increase in the rate of protein synthesis in stressed maize cultivars over control plants was observed by [24] after the first 48 hours, and decreased as plants dry. It is notable that under stress conditions both maize cultivars attains higher protein contents than control. Stressed and unstressed cv. Composite 5 had higher protein content than cv. Giza 2 plants.

Total free amino acids and free proline (Fig. 4) increased with time in both maize cultivars. Higher water potential stress treatment resulted in higher accumulation of the two compounds in both cultivars. cv. Composite 5 accumulated higher amounts of total free amino acids and free proline than in cv. Giza 2 during the time of experiment. The increase in proline was more than 15 mg/g dry weight per one MPa decrease in medium water potential and about 8 mg/g dry weight per day of stress in both cultivars. The unstressed plants of both cultivars showed a slight increase in total free amino acids and free proline with time.

Collectively under unstress conditions cv. Giza 2 slightly had greater amounts of free proline and a lower water saturation deficit than cv. Composite 5. On the other hand, proline accumulated at higher rate in cv. Composite 5 than in cv. Giza 2 and the maximum rate of accumulation was between 0.0 and -2.02×10^{-5} MPa. The calculation of the percentages of difference in WSD and the amount of free proline between both cultivars under each water stress treatments indicated about 30% higher WSD and about 38% higher free proline in cv. Composite 5 than cv. Giza 2. This would indicate higher accumulation of proline by about 8%. Aloni and Rosenshtein [25] concluded that the free proline accumulation at the time of dehydration signals drought stress in tomato plants. The possible higher tolerance for cv. Composite 5 than for cv. Giza 2 could also be

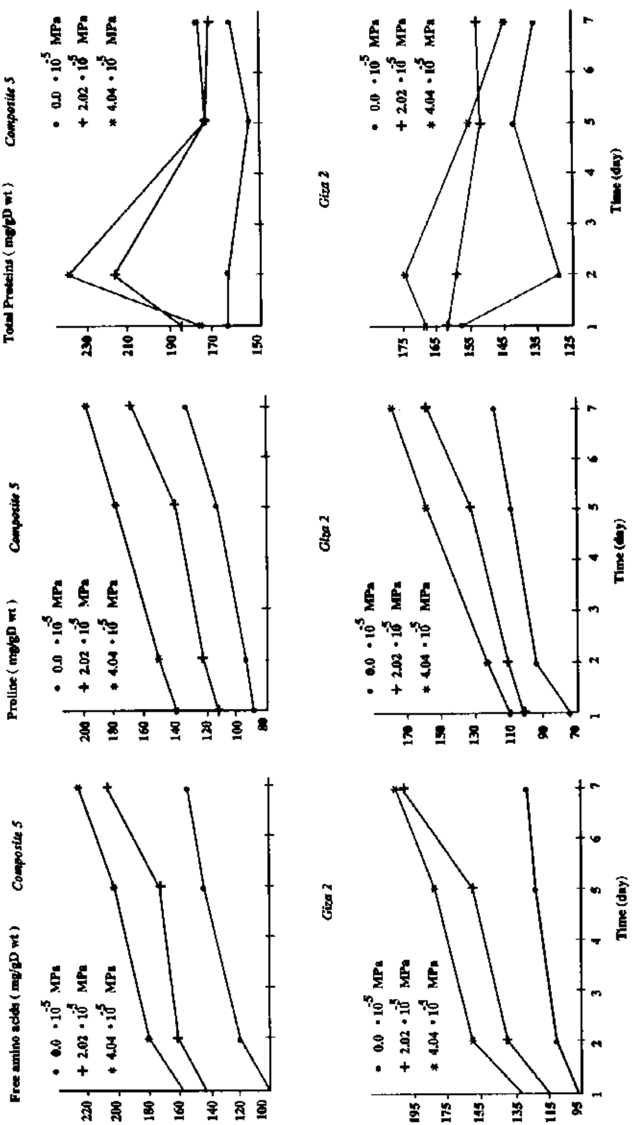

Fig. 4. The variation in the amounts of amino acids, proline and protein (mg/g D.W.) with the time of withstand in control, -2.02 and -4.04 × 10^{-5} MPa water potential of *Zea mays*, L. cultivars (cv Composite 5 and cv. Giza 2).

confirmed by the high amino acids and protein contents after seven days of stress although the plant suffer from higher water saturation deficit.

References

[1] Farghaly, K.A. "Some Aspects of Resistance to Heat and Drought in Plants under Natural Desert Conditions." *Ph.D. Thesis*, Assuit University, Egypt, 1983.

[2] Imamul-Huq, S.M. and Larher, F. "Osmoregulation in Higher Plants: Effects of Maintaining a Constant Na:Ca Ratio on the Growth, Ion Balance and Organic Solutes of NaCl stressed Cowpea (*Vigna sinesis*, L.)" *Z. Pflanzenphysiol.*, 113 (1984), 163-176.

[3] Migahid, M.M. "A Study of Drought Resistance of Plant Life-forms in the Mediterranean Desert of Egypt." *Ph.D. Thesis*, Alexandria University, Egypt, 1989.

[4] Ahmed, A. M.; Radi, A.F.; Shaddad, M.A. and El Aayed, M.A. "Effect of Phytohormones on Carbohydrate and Nitrogen Metabolism of Some Drought Stressed Crop Plants." *Journal of Islamic Acad. Sci.*, 2 (1989), 93-99.

[5] Nir, I.; Poljakoff-Mayber, A. and Klein, S. "The Effect of Water Stress on the Polysome Population and the Ability to Incorporate Amino Acids in Maize Root Tips." *Isr. J. Bot.*, 19 (1970), 439-462.

[6] Dhindsa, R.S. and Cleland, R.E. "Water Stress and Protein Synthesis. II. Interaction between Water Stress, Hydrostatic Pressure and Abscisic Acid on the Pattern of Protein Synthesis in *Avena coleoptiles*." *J. Plant Physiol.*, 57 (1975), 782-785.

[7] Elhaak, M.A. and Migahid, M.M. Response of Gymnocarpos Decandrum Leaf Callus to Water Stress and ABA Application." *Delta J. Science*, 12, No. 5 (1989) 4184-4197.

[8] Slatyer, R.O. "Physiological Significance Enzymes and Free Proline in Rice Varieties." *Phytochem.*, 16 (1977) 1355-1358 and "Internal Water Relations to Crop Yield." In: J.D. Eastin, F.A. Haskins, C.Y. Sullivan, and C.H.M. Van Bavel (Eds), *Physiological Aspects of Crop Yield*. Madison: Amer. Soc. Agron. Crop Sci., 1969.

[9] Hsiao, T.C. "Rapid Changes in Levels of Polyribosomes in Zea Mays in Response to Water Stress." *J. Plant Physiol.*, 46 (1970), 281-285..

[10] Chen, D.; Kessler, B. and Manselise, S.P. "Studies on Water Regime and Nitrogen Metabolism of Citrus Seedlings Grown under Water Stress." *J. Plant Physiol.*, 39 (1964), 379-386.

[11] Barnett, N.M. and Naylor, A.W. "Amino acid and Protein Metabolism in Bermuda Grass During Water Stress." *J. Plant Physiol.*, 41 (1966), 1222-1230.

[12] Stewart, G.R. and Larher, F. "Accumulation of amino acids and related compounds in relation to environmental stress." In: B.J. Miflin (Ed), *The Biochem. of Plants*. Vol. 5. London and Orlando: Academic Press, Inc. 1980.

[13] Wyn Jones, R.G. and Storey, R. "Proline." In: L.G. Paleg and D. Aspinall (Eds), *The Physiology and Biochemistry of Drought Resistance in Plants*. London and Orlando: Academic Press Inc., 1981.

[14] Rhodes, D. "Metabolic Responses to Stress." In: P.K. Stumpf and E.E. Conn (Eds), *The Biochemistry of Plants, a Comprehensive Treatise*, Vol. 12. London and Orlando: Academic Press Inc., 1987.

[15] Stewart, G.R.; Larher, F.; Ahmad, I. and Lee, J.A. "Nitrogen Metabolism and Salt Tolerance in Higher Plant Halophytes." In: R.L. Jefferies and A.J. Davy (Eds), *Ecological Processes in Coastal Environments*. Oxford: Blackwell Scientific Publications, 1979.

[16] Stewart, G.R. and Lee, J.A. "The Role of Proline Accumulation in Halophytes." *Planta*, 120 (1974), 279-289.

[17] Charles, C.A. and Todd, G.W. "Ribonucleotide Compositional Changes in Wheat Leaves by Water Stress." *Crop Science*, 39 (1968), 319-321.

[18] Henckel, P.A. "Role of Protein in Drought Resistance." *Canadian J. Botany.* 48 (1970), 1235-1241.

[19] Mali, P.C. and Mehta, S.L. "Effect of Drought on Enzymes and Free Proline in Rice Varieties." *Phytochem.*, 16 (1977), 1355-1358.

[20] Thakur, P.S. and Rai, V.K. "Water Stress Effects on Maize: Carbohydrate Metabolism of Resistance and Susceptible Cultivars of *Zea Mays*, L." *Biol. Plant.*, 22 (1980), 50-56.

[21] Ya, P.L. and Tunekazu, T. "An Improved Colorimetric Determination of Amino Acids with the Use of Ninhydrin." *Anal. Biochem.*, 14 (1966), 71-77.

[22] Bates, L.S.; Waldem, R.P. and Teare, I.D. "Rapid Determination of Free Proline for Water-stress Studies." *Plant and Soil,* 39 (1973), 205-207.

[23] Lowry, D.H.; Rosenbrough, N.J.; Farr, A.L. and Randail, R.J. "Protein Measurement with the Folin Phenol Reagent." *J. Biol. Chem.* , 193 (1951), 265-275.

[24] Marnville, J.W. and Paulsen, G.M. "Alterations of Protein Composition of Corn (*Zea mays*) Seedling During Moisture Stress." *Crop Science,* 12 (1972), 660-663.

[25] Aloni, B. and Rosenstein, G. "Proline Accumulation: A Parameter for Evaluation of Sensitivity of Tomato Varieties to Drought Stress." *Physiol. Plant.*, 61 (1984), 231-235.

استجابة العجز من التشبع المائي وبعض المركبات النيتروجينية في صنفين من نبات الذرة تحت تأثير الإجهاد المائي

حميدة السيد* و محمود أبو اليزيد الحق**

** قسم التاريخ الطبيعي، كلية التربية بكفر الشيخ، جامعة طنطا*

*و** قسم النبات، كلية العلوم، جامعة طنطا، طنطا*

(سُلّم في ٢٤ شعبان ١٤١٣هـ؛ وقُبل للنشر في ١٦ محرم ١٤١٥هـ)

ملخص البحث. تمّت دراسة العجز في التشبع المائي (WSD) والمركّبات النيتروجينية (الأحماض الأمينية ، البرولين والبروتينات) تحت تأثير الإجهاد المائي باستخدام المانيتول بتركيزات (صفر، ٢، ٤، ٦، ٨ و ١٠ × ١٠^-٥ MPa) على نوعين من نبات الذرة المصري (تركيبي ٥ وجيزة ٢). اظهرت الدراسة أن العجز في التشبع المائي للنوعين على الرغم من اختلاف معدّلات الزيادة تحت تأثير الإجهاد المائي نفسه. لوحظ أن النوعين من الذرة متساويين في الزيادة اليومية للعجز في التشبع المائي كما أدى عجز التشبع المائي لزيادة تراكم المكوّنات النيتروجينية (البرولين، الأحماض الأمينية والبروتينات) مع صنف تركيبي ٥ عنها مع صنف جيزة ٢. أدت استخدامات تركيزات الإجهاد المائي من صفر إلى ٢ × ١٠^-٥ MPa إلى زيادة معدّلات تراكم البروتين والأحماض الأمينية والبرولين عنها باستخدام الإجهاد المائي المنخفض. كما وجد أيضًا أن كمية البرولين الحر والأحماض الأمينية ازدادت مع الوقت باستخدام الصنفين النباتيين (تركيبي ٥ وجيزة ٢) وأن زيادة البرولين كانت أكثر من ١٥ مجم/جم وزن جاف لكل يوم، كما وجد أن استجابة تأثير الإجهاد المائي على صنف تركيبي ٥ أكثر منها تحملاً مع صنف جيزة ٢ تبعًا لتراكم كمية البرولين.