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Relationships of External Osmotic Potentials with Water Saturation Deficit and Some N Compounds in Two Maize Cultivars

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ABSTRACT. The percentage water saturation deficit (WSD) and the content of nitrogenous compounds free amino acids, proline and soluble proteins were studied in two Egyptian cultivars (Composite 5 and Giza 2) of maize (*Zea mays*, L.) under varying osmotic potentials (0, -1, -2, -4, -6, -8 and -10 MPa) and varying times (from 1 to 7 days of exposure to 0.0, -2.02 and -4.04 MPa osmotic potentials. The obtained relationships were linear and showed significantly different rates of increase in WSD of the two cultivars with the decrease in external water potential or increase in time of stress. Composite 5 cultivar exhibited greater WSD than Giza 2, and the two cultivars showed dissimilar rates of daily increase in WSD under osmotic stress. Free amino acids, proline and soluble proteins accumulated but with greater rates from 0.0 to -2.02 than under from -2.02 to -4.04 MPa in both cultivars. Free proline and amino acids continued to accumulate with time of stress. Proteins accumulation was inhibited after the third day of stress. Proline and amino acids accumulated by greater rates with the increase in WSD in Giza 2. The increase in proline was by about 15 and 8 mg/g D. wt. per MPa decrease in osmotic potential in Composite 5 and Giza 2 cultivar respectively.

Introduction

Osmotic stress was repeatedly recorded to induce pronounced alterations in water saturation deficit and metabolism of nitrogenous compounds in plant species[1]. Proteins synthesis is one of the biochemical processes that still is considered an important effect of osmotic stress[2]. Interruption of protein synthesis as well as proteolysis has been reported in stressed plants[3]. In this respect, Barnett and Naylor[4] stated that a considerable hydrolysis of proteins may occur in water stressed plants which is always accompanied by varied increments in amino acids. But accumulation of amino acids regardless of protein hydrolysis under stress was also reviewed[5]. This accummulation was found to be important in plant adaptation to dry environment[6, 7] and was a function of the level of adaptation[8]. Also, adaptation to water stress is closely associated with proline accumulation *e.g.*[5, 9, 10]. Plants with known sensitivity to imposed stress have been investigated for underlying metabolic changes by many workers *e.g.*[11, 12, 13], but the nature of the relation between susceptibility and water saturation deficit and nitrogen metabolism still needs to be explored. The nature of such studies is important in choosing crops for the newly reclaimed desert area in Egypt. However, the present work was designed to study the relationships between osmotic potential induced by mannitol and the plant water saturation deficit (WSD) in addition to the effect of both osmotic potential and resulted in WSD on the accumulation of amino acids, proline and proteins in two cultivars of Egyptian maize.

Materials and Methods

The present investigation was carried out using Composite 5 and Giza 2 cultivars of the Egyptian maize (*Zea mays*, L.). Seeds of the two cultivars were surface sterilized using 0.1% $HgCl_2$ and cultivated in Petri dishes with distilled water. At the first leaf stage dishes were arranged into two groups, the plants of the first group were subjected to 0, −1, −2, −4, −6, −8 and −10 MPa osmotic treatments using mannitol in distilled water as described earlier by Thakur and Rai[4]. After three days of stress leaf samples from each treatment were weighed as fresh and then saturated for 24 hours in dark at 4 ± 2°C then oven dried and reweighed for determination of water saturation deficit. The other group of dishes was arranged into three subgroups, 14 dishes each, and plants in the subgroups were subjected to total osmotic potentials of 0.0, −2.02, −4.04 MPa by mannitol and nutrient solution. On daily intervals until the seventh day, two dishes from each treatment were harvested and leaf samples were collected for determination of WSD of the plants under each treatment. The rest of plant leaves were dried at 60°C to constant weight and powdered. Then equal weight of the dry matter were analyzed for their contents of amino acids using the method of Ya and Tunekazu[15], proline using the method of Bates *et al.*[16] and of proteins using the method of Lowery *et al.*[17] after precipitating of proteins with 15% TCA (Trichloroacetic acid) at 4°C.

All of the obtained data were statistically analyzed using the least significant difference and regression and correlation coefficient tests according to[18].

Results and Discussion

Water saturation deficit of the two maize cultivars increased in response to the decrease in osmotic potentials of the growth medium (Fig. 1). The relationships between the percentage of water saturation deficit (WSD) and the external medium osmotic potential were linear and highly significant ($P < 0.01$) for both cultivars. These relationships indicated greater rate of increase in WSD with osmotic potentials in Composite 5 than in Giza 2 cultivar. Also, the same WSD could be exhibited under lower external osmotic potential in Giza 2 cultivar than in Composite 5. However, out of the regression equations of the relationships 50% of WSD was observed under external osmotic potentials of about –6 and –8 MPa for Composite 5 and Giza 2 respectively, which indicates differential tolerance to decrease in external osmotic potential in the two cultivars. Also, zero WSD was attained at positive osmotic potentials of 0.04 and 0.4 MPa respectively in Giza 2 and Composite 5 cultivars, while the zero osmotic potential resulted in lower percentage of WSD in Giza 2 (0.27%) than in Composite 5 (3.3%) which may indicate a normal metabolic activity at higher state of WSD in the latter cultivar. Exposure of the plants of the two cultivars for longer time to osmotic stress increased their percentage of WSD, the increase was greater under –4.04 MPa in both cultivars (Fig. 2). The relationships between the percentages of WSD and days of exposure to osmotic stress treatments were highly significant ($P < 0.01$). The regression equations of these relationships indicate daily increases in percentage of WSD of 1.12, 6.70 and 9.98 in Composite 5 and 1.47, 4.54 and 7.69 in Giza 2 and under 0.0, –2.02 and –4.04 MPa respectively, which showed dissimilar rates of increase in WSD and hence dissimilar control on water loss in the two cultivars under the same osmotic potential. From the regression equations 50% of WSD could be expected to occur at later dates in Giza 2 (at the 10th and 5th day) than in Composite 5 (at the 7th and 4th day) under –2.02 and –4.04 MPa stress treatments. These results disagree with those of Thomas[19], who found no significant changes in the relative water content in *Lolium perenne* until the 6th week of drought. The regression equations indicate also greater tolerance in Giza 2 as indicated by[20] who referred tolerance to water absorption at high osmotic potential.

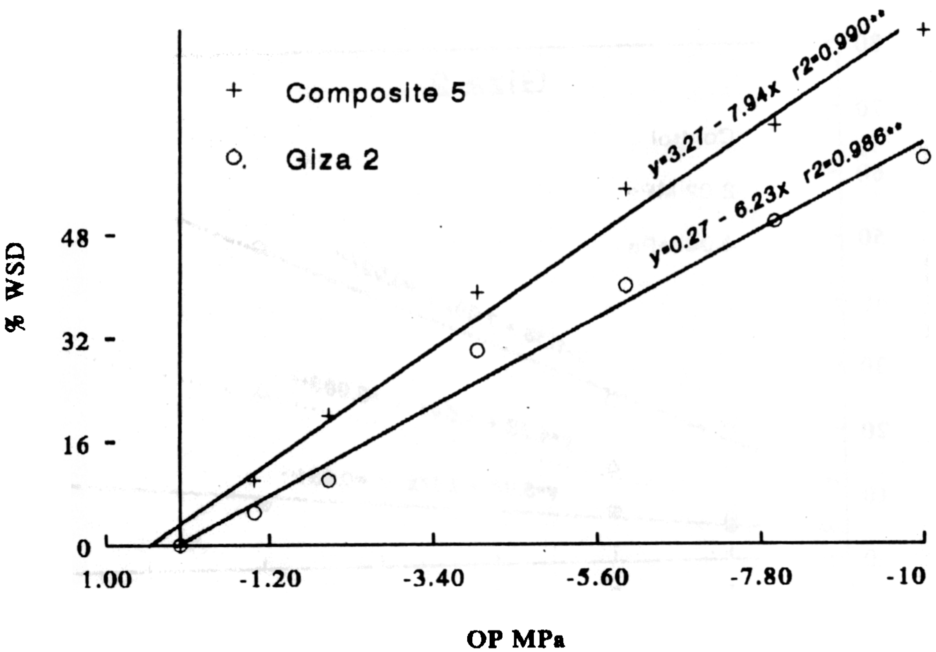


FIG Relationships between osmotic potentials of the growth medium and water saturation deficit (WSD) of Composite 5 and Giza 2 cultivars of *Zea mays*.

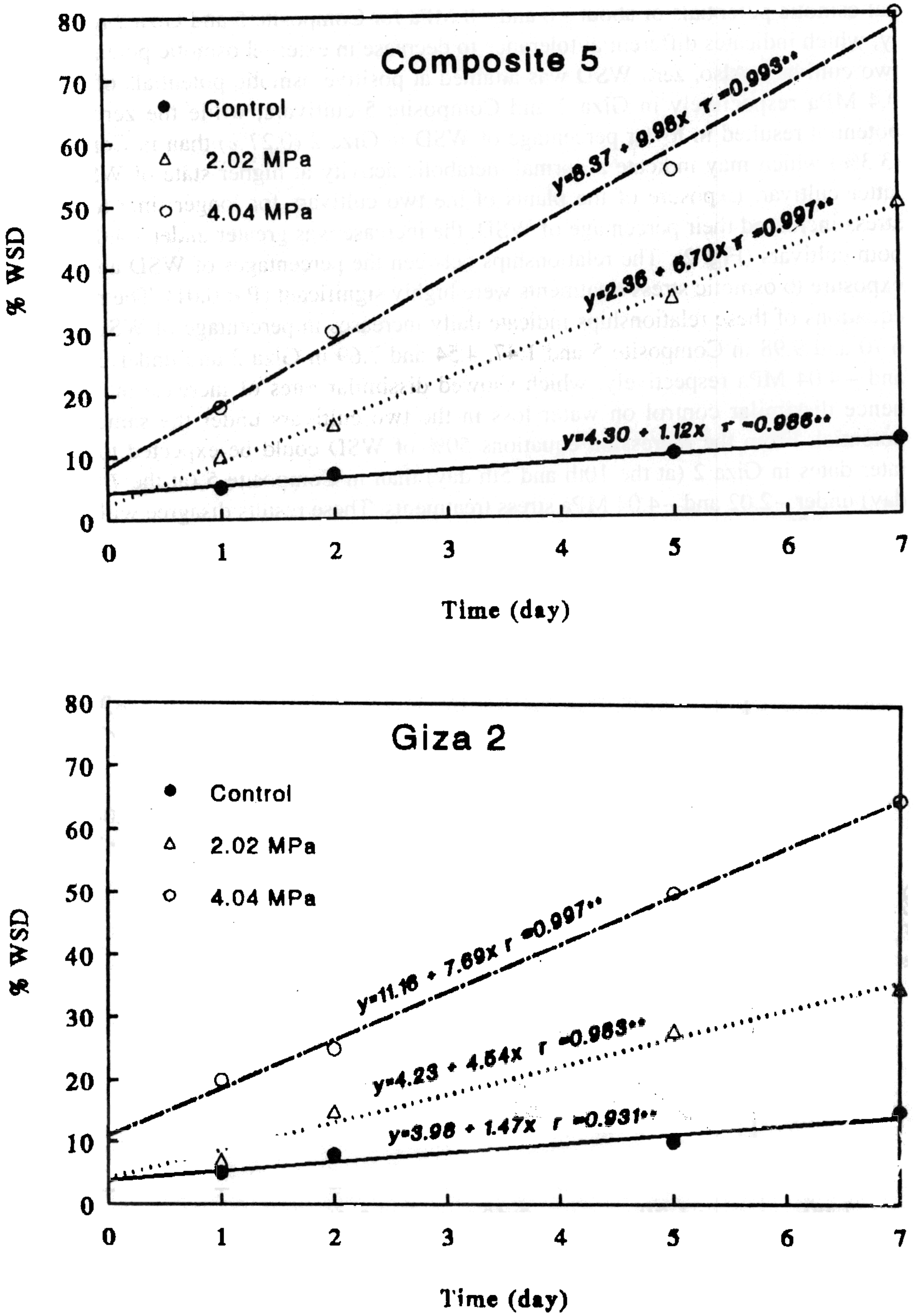


FIG. 2. Relationships between time of exposure to control −2.02 and −4.04 MPa osmotic potentials and water saturation deficit (WSD) of Composite 5 and Giza 2 cultivars of *Zea mays*.

The decrease in external osmotic potential in addition to its effect on the MSD enhanced the metabolism of the nitrogenous compounds towards the accumulation of proteins, amino acids and proline, which showed greater contents in stress than in control plants of the two cultivars (Fig. 3). The best relationships of the contents of proteins, amino acids and proline with the external medium osmotic potentials were the polynomial ones. These relations exhibited regression equations and coefficients of correlation which were highly significant ($P < 0.01$). They showed high rates of accumulation of the proteins, amino acids and proline in the plants of the two cultivars between 0.0 and −3.0 MPa of external osmotic potential but under greater potentials (< −3.0 MPa) there were 50% decline in the rates of accumulation of amino acids and proline. Protein accumulation in the two cultivars otherwise was inhibited and even to a lower value than the control in Giza 2. In this concern, Elhaak and Wegmann[21] found similar trend of variations in proteins, amino acids and proline in *Euphorbia paralias* with 200 and 400M NaCl. In contrary Henckel[11] found progressive increase in protein content with stress. The protein content in Composite 5 was higher than the contents of amino acids and proline under most of treatments but in Giza 2 only under osmotic potentials ranging from 0.0 to −2.02 MPa. This may indicate a variation between the two cultivars in protein response in the adaptation to osmotic stress. It was found also that, the rate of proteins hydrolysis was lower than the rate of increase in amino acids, so proteolysis could not the reason of increase in amino acids especially under low stress treatments where both compounds accumulated. This finding was confirmed by the obtained non significant correlation coefficients between amino acids and proteins in the two cultivars.

When the relations were conducted between the contents of nitrogenous compounds and percentage of WSD (Fig. 4), linear relationships with highly significant correlation coefficients ($P < 0.01$) were obtained for amino acids and proline for both cultivars. Proteins showed significant relationships with WSD ($P < 0.05$) in Composite 5 but non significant one in Giza 2. The regression equations of these relationships indicated accumulation of 1.68 and 2.04 mg/g D wt. of amino acids and 1.58 and 2.15 mg/g D wt. of proline by unit increase in WSD in the plants of the Composite 5 and Giza 2 cultivar respectively. However, high contents of amino acids and proline were recorded in Composite 5 but when WSD reached to 50% greater content of both compounds could be calculated in Giza 2 (185 and 207 mg/g D wt.) than in Composite 5 (170 and 203 mg/g D wt.). This denotes to the important role of water status in controlling nitrogenous compounds metabolism which contradicts the reported data of Chu *et al.*[22] who found that accumulation of proline in barley plants was dependent on changes in osmotic potential (by NaCl) rather than on changes in turgor.

Time of stress was found to affect the metabolism of the nitrogenous compounds (Fig. 5). However, the content of proteins increased during the first two of three days in Composite 5 and Giza 2 cultivars under all stress treatments except under −2.02 MPa in Giza 2. During longer stress periods an inhibition of proteins was observed, but the stressed plants still exhibiting greater protein content in comparison with the control especially plants under −4.04 MPa which exhibited the greatest content of proteins. This may indicate an important role of proteins in the plant under stress. The relation-

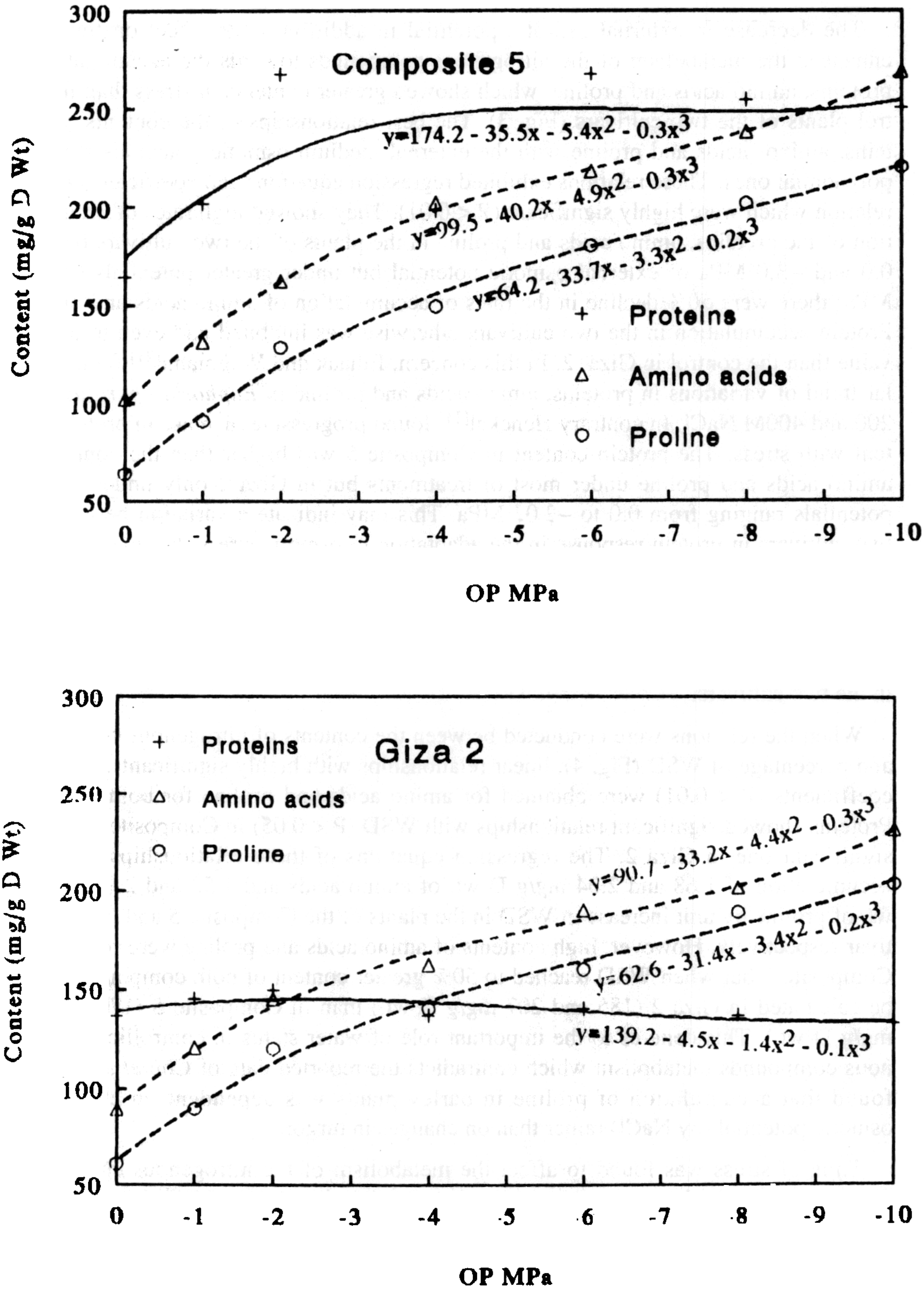
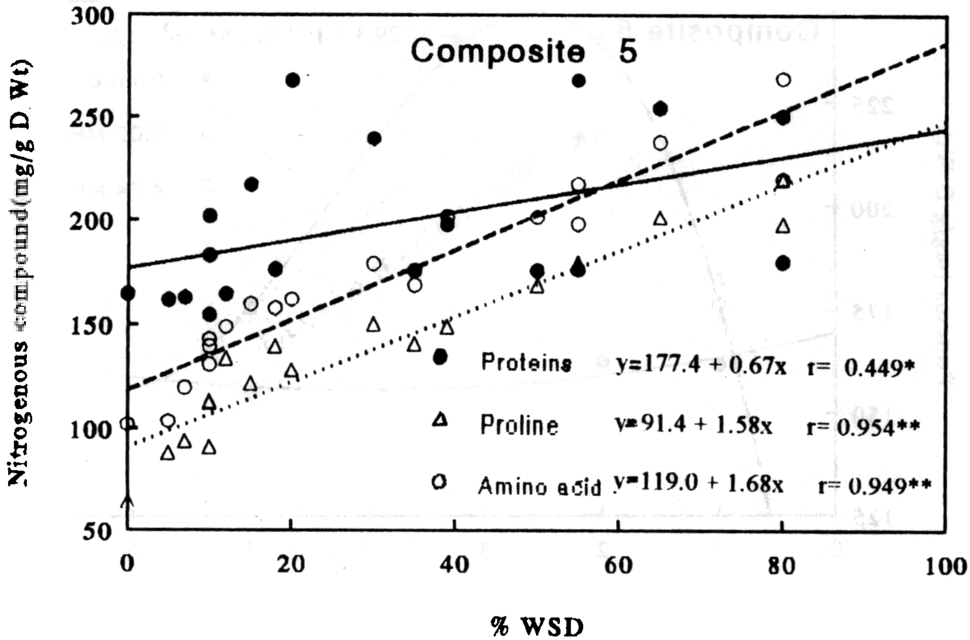


FIG. 3. Relationships between osmotic potentials of the growth medium and the amount of soluble proteins, amino acids, and proline (mg/g d. wt) of Composite 5 and Giza 2 cultivars of *Zea mays*.



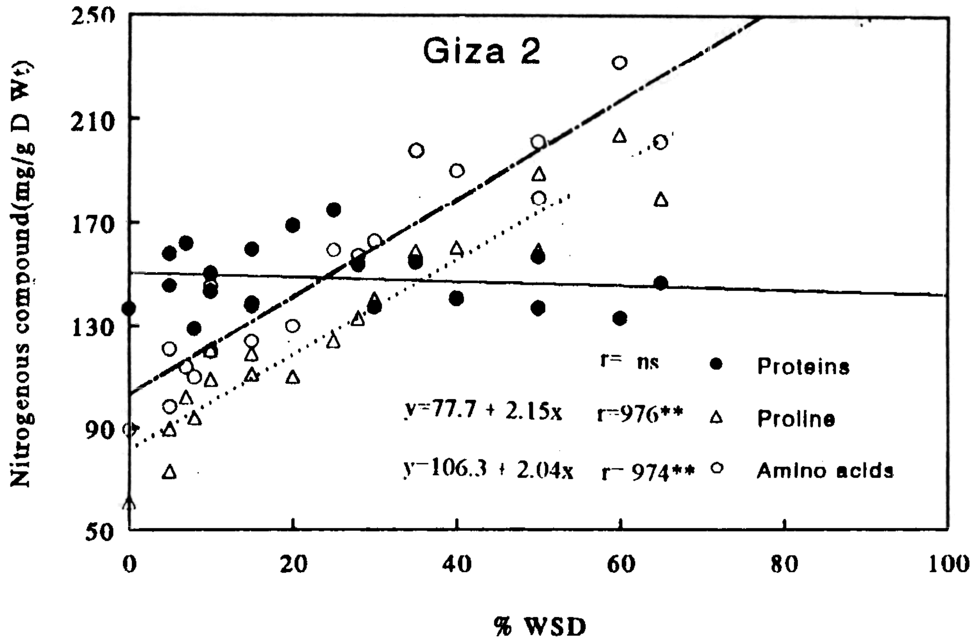
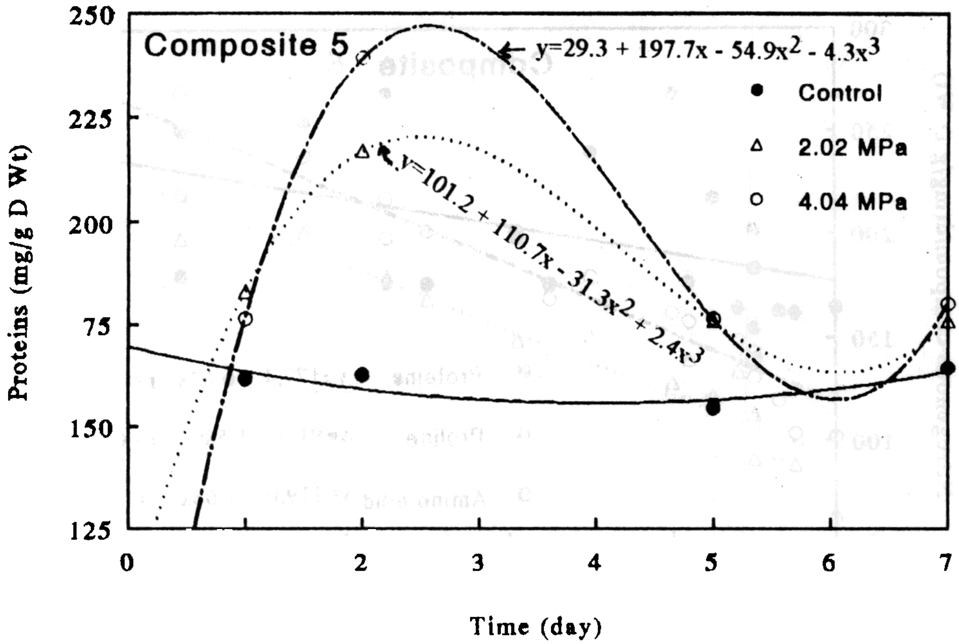


FIG. 4. Relationships between the amount of soluble proteins, amino acids, and proline (mg/d d. wt) and water saturation deficit (WSD) of Composite 5 and Giza 2 cultivars of *Zea mays*.



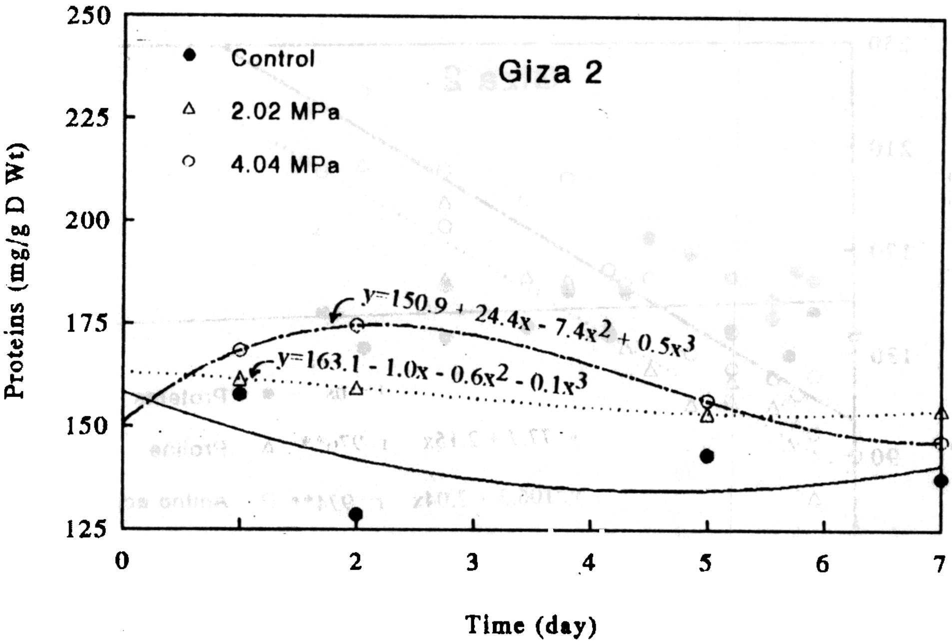
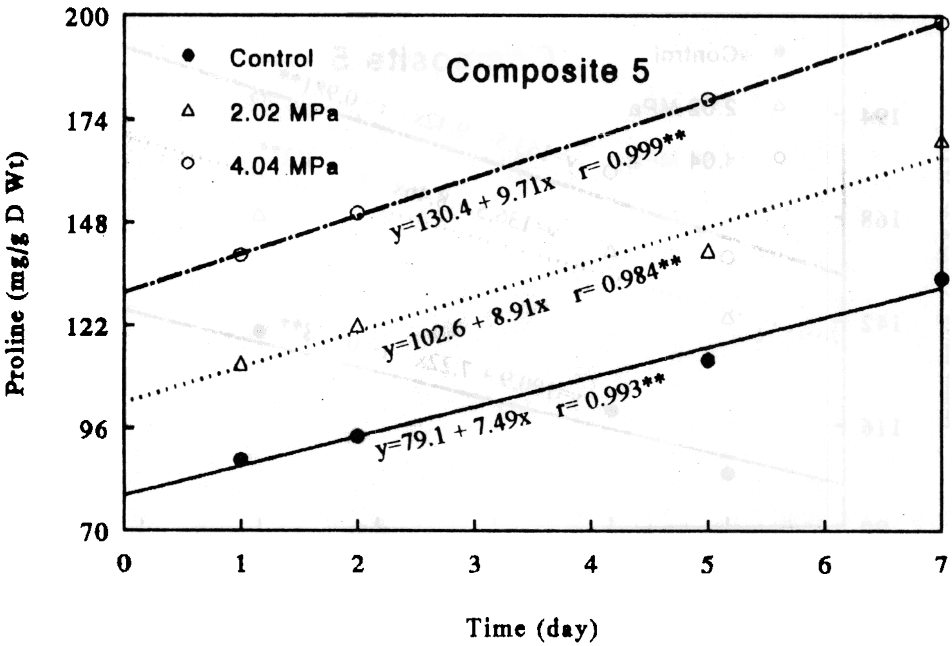


FIG. 5. Relationships between the amount of soluble proteins, amino acids, and proline (mg/g d. wt) and time of exposure to control −2.02 and −4.04 MPa osmotic potentials in the growth medium of Composite 5 and Giza 2 cultivars of *Zea mays*.


Composite 5
Control
2.02 MPa
4.04 MPa
y=130.4 + 9.71x r= 0.999**
y=102.6 + 8.91x r= 0.984**
y=79.1 + 7.49x r= 0.993**
Proline (mg/g D Wt)
200
174
148
122
96
70
0
1
2
3
4
5
6
7
Time (day)

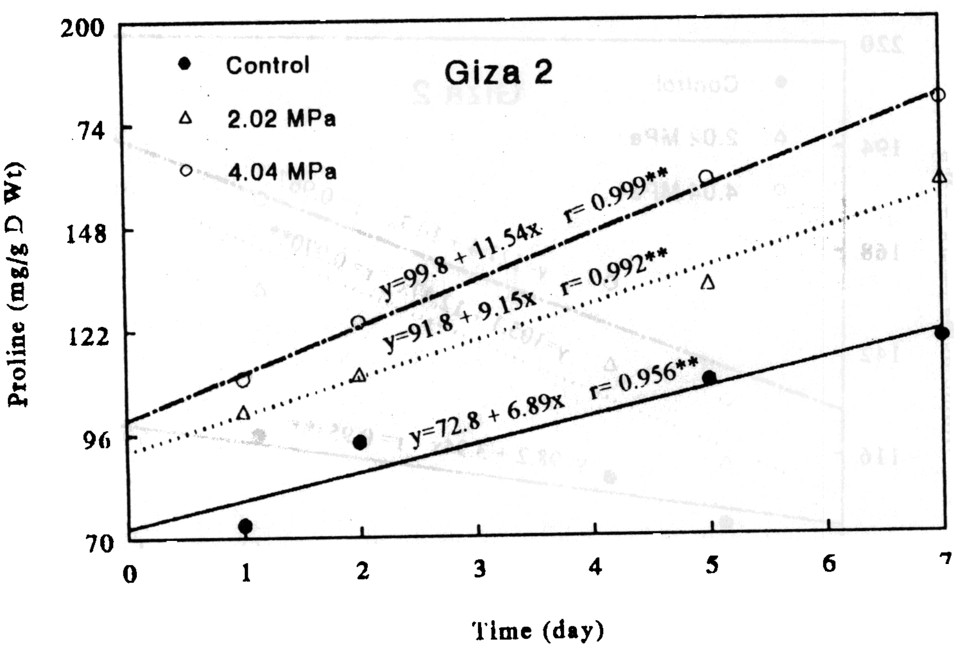
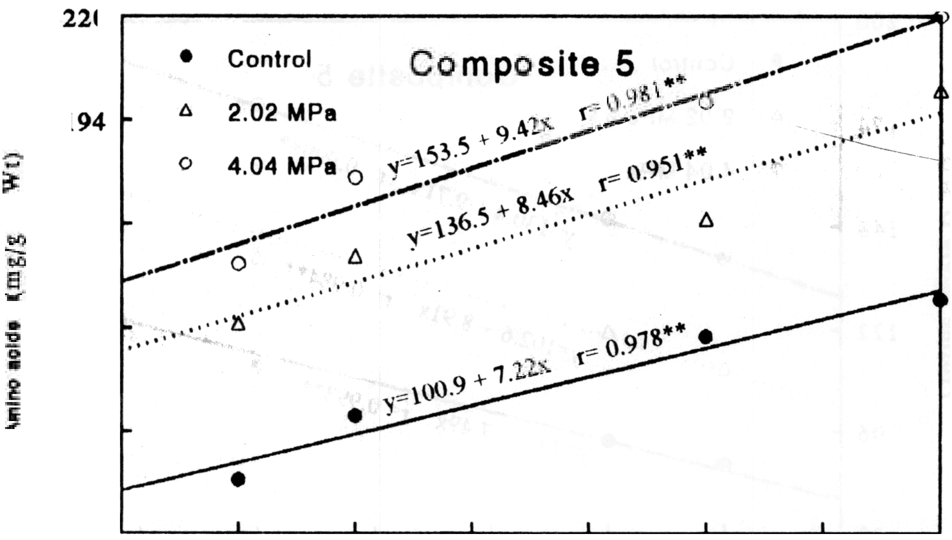
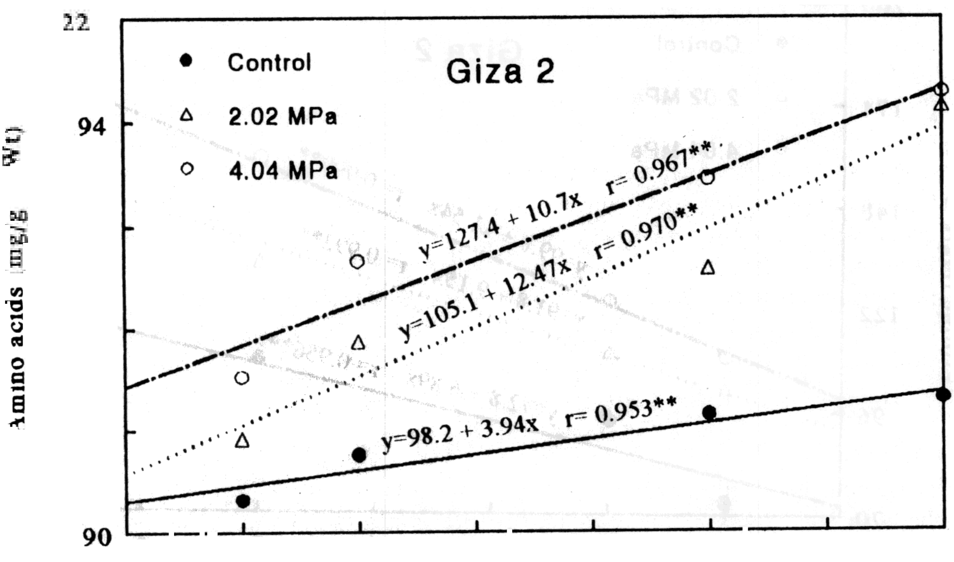

Giza 2
Control
2.02 MPa
4.04 MPa
y=99.8 + 11.54x r= 0.999**
y=91.8 + 9.15x r= 0.992**
y=72.8 + 6.89x r= 0.956**
Proline (mg/g D Wt)
200
.74
148
122
96
70
0
1
2
3
4
5
6
7
Time (day)

FIG. 5. (Contd.)


Composite 5
Control
2.02 MPa
4.04 MPa
y=153.5 + 9.42x r= 0.981**
y=136.5 + 8.46x r= 0.951**
y=100.9 + 7.22x r= 0.978**
22(
194
Amino acids (mg/g Wt)


Giza 2
Control
2.02 MPa
4.04 MPa
y=127.4 + 10.7x r= 0.967**
y=105.1 + 12.47x r= 0.970**
y=98.2 + 3.94x r= 0.953**
22
94
90
Amino acids mg/g Wt)

ships between time of stress and the content of proteins were polynomial and their coefficients of correlation were highly significant (r = 1) in both Composite 5 and Giza 2 cultivars under each of –2.02 and –4.04 MPa osmotic stress treatments. The regression equations of the relationships are :

Proteins = 101.2 + 110.7 day – 31.3 day^2 + 2.4 day^3 for Composite 5 at –202 MPa

Proteins = 29.3 + 197.7 day – 54.9 day^2 + 4.3 day^3 for Composite 5 at –4.04 MPa

Proteins = 163.1 – 1.0 day – 0.6 day^2 + 0.1 day^3 for Giza 2 at –2.02 MPa

Proteins = 150.9 + 24.4 day – 7.4 day^2 + 0.5 day^3 for Giza 2 at –4.04 MPa

After the third day of stress the regression lines showed similar rate of decrease in proteins with stress treatments –2.02 and –4.04 MPa in Composite 5, but different rates in Giza 2 which showed greater decrease with –4.04 MPa treatment. This manifests the deleterious effect of osmotic stress on proteins in the latter cultivar. An increased rate of protein synthesis during the first 48 hours, in stressed maize over control, which later decreased as plants were depleted by moisture stress was also observed by Marnville and Paulsen[23]. It is also important to note that Composite 5 attained greater content of protein under all osmotic stress treatments along the time of experiment than Giza 2 cultivar.

Free amino acids and proline (Fig. 5) progressively accumulated with time of stress in both Composite 5 and Giza 2 cultivars. Higher external osmotic stress led to greater accumulation in the two compounds. The relationships between the amino acids and proline contents and the time of stress were linear and exhibited highly significant correlation coefficients. The regression equations of these relationships indicated daily accumulation of amino acids by 8.5 and 9.4 mg/g D wt. in Composite 5 and 12.5 and 10.7 mg/g D wt. in Giza 2 at –2.02 and –4.04 MPa stress treatments respectively. Also, proline accumulated by 8.9 and 9.7 mg/g D wt. in Composite 5 and by 9.2 and 11.5 mg/g D wt. in Giza 2 under the two stress treatments respectively. It is also notable that Composite 5 attained greater amounts of both amino acids and proline than Giza 2 cultivar, but the former cultivar showed lower rate of increase in both compounds per day under each –2.02 and –4.04 MPa stress treatments. This may indicate greater sensitivity in the Giza 2 to the imposed stress in comparison with Composite 5 cultivar. The relationships between the contents of amino acids and proline in the two cultivars under the imposed stress treatments and along the time of experiment were linear. The regression equations of these relations are :

Amino acids = –15.6 + 0.91 proline, r = 0.974** for Composite 5 (** = $P < 0.01$)

Amino acids = – 13.9 + 0.94 proline, r = 0.976** for Giza 2 (** = $P < 0.01$)

These equations indicate an increase by 1.1 mg/g D. wt. in proline per unit increase in the other amino acids in the two cultivars, which demonstrates the great importance of proline accumulation in response to the osmotic stress. Similar important role of proline in osmotic stress adaptation was recorded by many authors *e.g.*[21, 24].

In conclusion, under non-stress conditions Composite 5 cultivar exhibited greater contents of proline, amino acids and proteins in addition to the greater WSD in comparison with Giza 2. But the latter cultivar showed accumulation rates in proline and amino acids in response to osmotic potential and to the subsequent internal disturbances in water content (expressed as WSD). Aloni and Rosenshtein[25] concluded that proline accumulation at the time of dehydration signals drought stress in tomato plants. Amino acids accumulation under lower osmotic stress was not a result of inhibition of protein accumulation in the two cultivars. The high rate of accumulation of most nitrogenous compounds was under a range of osmotic stress from 0 to -3 MPa corresponding to a range of WSD from 0 to 27 and 19% in Composite 5 and Giza 2 respectively. At this range of osmotic stress Composite 5 showed greater contents of proteins, amino acids and proline, which may relate the adaptive response directly to the difference in WSD between the two cultivars. Working with this, determination of the percentages difference in WSD and content of proline between the two cultivars indicated about 30 and 38% higher WSD and proline content respectively in Composite 5 than in Giza 2. This would indicate higher accumulation of proline by about 8% in Composite 5 than in Giza 2. The previous conclusion in addition to the high content of amino acids, proline and protein confirm the higher tolerance to osmotic stress by Composite 5 than by Giza 2. The time of stress brings greater WSD in Composite 5 than in Giza 2 cultivar in addition to the greater rate of accumulation of proline and amino acids. This indicates that Giza 2 cultivar is more suitable for cultivation in dry areas for its high tolerance in one hand and for metabolizing large amounts of proteins under high state of plant WSD on the other.

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علاقـة الجهد الأسموزي الخارجي بعجز الامتـلاء وبعض مركبات النيتروجـين في صنفـين من الـذرة

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المستخلص . درست نسبة عجز الامتلاء ومحتوى صنفين من أصناف الذرة المصري (كمبوسيت٥ وجيزة٢) من المركبات النيتروجينية (الأحماض الأمينية الحرة والبرولين والبروتينات الذائبة) تحت ظروف اختلاف جهود أسموزية ، تتراوح ما بين الصفر و -١٠ ميجابسكال بالإضافة إلى تعريضها لأزمنة مختلفة (من يوم إلى سبعة أيام) للجهود الأسموزية صفر ، -٢٠٢ ، ٢ ، -٤٠٤ , ٤ ميجابسكال . وقد أشارت النتائج إلى أن هناك علاقات خطية معنوية ما بين عجز امتلاء صنفي الذرة والجهد الأسموزي الخارجي وكذا زمن الإجهاد . وكان هناك عجز امتلاء أكبر في صنف كمبوسيت ٥ منه في صنف جيزة٢ بالإضافة إلى معدل يومي غير متشابه لزيادة عجز الامتلاء تحت ظروف الإجهاد في كلا الصنفين .

تراكمت الأحماض الأمينية والبرولين والبروتينات الذائبة بمعدل أكبر تحت ظروف الإجهاد من الصفر إلى -٢٠٢ , ٢ ميجابسكال عن ظروف الإجهاد من -٢٠٢ , ٢ إلى -٤٠٤ , ٤ ميجابسكال في كلا الصنفين . هذا وقد استمر تراكم البرولين والأحماض الأمينية مع زيادة الإجهاد ولكن تثبط تراكم البروتينات الذائبة بعد اليوم الثالث للإجهاد .

كان تراكم البرولين والأحماض الأمينية بمعدل أكبر مع الزيادة في عجز الامتلاء في صنف جيزة ٢ . وأدى النقص في الجهد الأسموزي بمقدار الوحدة إلى زيادة في البرولين بحوالي ٨,١٥ مليجرام لكل جرام مادة جافة في صنفي كمبوسيت٥ وجيزة٢ على الترتيب .