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Osmotic Adjustment in Cultivars of C3 (Groundnuts, *Arachis hypogaea*) and C4 (Sorghum, *Sorghum bicolor*L.Moench) Species in Response to Water Stress*

Mazahir Sanosi Mohammed[1], Eltayeb Elhag Ali Ahmed[2] and Abdalla El Khider Madani[3]

Department of Biology, Faculty of Education, University of Khartoum, Sudan

Abstract: A field experiment was conducted for two consecutive seasons at the experimental farm , Faculty of Agriculture, University of Khartoum during 2009/2010 and 2010/2011 to study osmotic adjustment in two cultivars of Groundnut (C3 plant) Gibaish and Madani, and two cultivars of sorghum (C4 plant) Tabat and Arfa Gadamak in response to three irrigation intervals: 7 (W1), 14 (W2) and 21 days (W3); W3 is assumed to be stressful. The treatments were arranged in split plots with three replicates. The irrigation intervals were assigned main plots and cultivars to sub-plots. Measurements of osmotic adjustment were computed from measurements of osmotic potentials and relative water contents of leaves of sorghum and groundnuts. Watering treatments had significant effect on osmotic potential (O.P) of cultivars of sorghum and groundnut resulting in significant increases in O.P (becoming more negative) . In sorghum genotypes , results revealed that the mean O.P was -0.3 , -0.3 and -0.7 MPa under W_1, W_2 , and W_3 respectively after 7 weeks of growth . After 15 weeks of

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[1] Former postgraduate student, at present Assistant Professor, Department of Biology, Faculty of Education, University of Khartoum,

[2] Associate Professor of Plant Physiology, Desertification and Desert cultivation Studies Institute, University of Khartoum, Sudan

[3] Associate Professor, Faculty of Education, University of Khartoum, Sudan

growth the mean was -0.3 , -0.3 and -0.5 MPa under W_1, W_2 , and W_3 respectively . In groundnut cultivars the mean O.P was -0.3 , -0.3 and -0.6 MPa under W_1, W_2 , and W_3 respectively after 7 weeks of growth . After 15 weeks of growth the mean was -0.3 , -0.3 and -0.5 Mpa under W_1, W_2 , and W_3 respectively .In this study, the highest (more negative) O.P (-0.8 MPa) was measured after 7 weeks of growth in Tabbat under the 21-day irrigation interval in the first season . Tabat may have, therefore, more capacity for osmotic adjustment .Results of this study have shown that C_4 plants (sorghum may have a higher capacity for osmotic adjustment than C_3plants (groundnut).
Key words: Groundnut, sorghum, water stress, osmotic adjustment

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench), a C4 plant which belongs to the family Poaceae, is the staple food for many people around the world and is grown mainly in low-rainfall arid and semi-arid regions (Mutava, 2009). Groundnut is an important cash crop in many parts of the world. It is an annual legume belonging to the family Papilionaceae. It is an important crop both for oil and food.

Dwindling water resources and increasing competition for water among domestic, industrial and agricultural uses prompts the need for improved agricultural water use efficiency (WUE). This may be achieved through improved irrigation practices, improved rain water utilization and improved crop water use (Ahmed and Ahmed, 2007). There is a large genotypic variability in WUF in both C3 and C4 plants that could be utilized to improve crop WUF. C4 plants, which are adapted to hot, dry environments, have very high WUE (Hopkins and Huner, 2003), high productivity (Vitkauskaite and Venskaityte, 2011) and resistance to water deficit (Osborne and Freckleton, 2009). C4 plants are more efficient water users in comparison to C3 plants (Taiz and Zeiger, 2006) due to their peculiar leaf anatomy (kranz type) and their pathway of photosynthesis.

In these plants, the PCR cycle (Calvin cycle) occurs in the bundle sheath around the vascular bundles and, therefore, they are not required to transport water over long distances in the leaf for photosynthesis

(Hopkins and Huner, 2003). They are, therefore, expected to perform better under stress conditions. On the other hand, C3 plants flourish in cool, wet and cloudy climates where lighting levels may be low, because the metabolic pathway is more energy efficient, and if water is plentiful, the stomata can stay open and let in more carbon dioxide. However, carbon loss through photorespiration is high (Vitkauskait and Venskaityte, 2011).

Water stress was reported to have a negative effect on most vegetative characters. Water stress is linked to decrease in stem length in plants. Decreased leaf growth, total leaf area and leaf area plasticity were observed under the drought conditions in many plant species, such as peanut (Lisar *et al.*, 2012). Growth rates of C4 plants were found to be less affected than those of C3 plants (Nayyar and Gupta, 2006; Vitkauskait and Venskaityte , 2011). Some plants are able to tolerate drought and salinity stresses by reducing the cellular osmotic potential as a consequence of a net increase in solute accumulation, in a process called osmotic adjustment(Serraj and Sinclair, 2002). Osmotic adjustment refers specifically to a net increase in solute concentration due to metabolic processes triggered by stress.Osmotic adjustment generates more water movement into the leaf and consequently maintains leaf turgor (Blum, 2017). The proposed effect of O.A is to maintain turgor and turgor-dependent physiological process, including maintenance of expansive growth and stomatal conductance. By helping to maintain leaf turgor, O.A enables plants to keep their stomata open and continue taking up CO_2 for photosynthesis under conditions of moderate water stress (Hopkins and Huner,2003). There were significantly greater seed yield in genotypes of sorghum with high osmotic adjustment (HOA) than genotypes with low osmotic adjustment (LOA) as was reported by some workers(Ludlow *et al*. 1990; Morgan, 1995).

A positive relationship between OA and seed yield under drought was reported (Subbarao *et al*. 2000; Blum, 2017).

Groundnuts (peanut) have several mechanisms for adaptation to water and heat stress including stomatal conductance. Parameters like relative water content (RWC), leaf water potential, stomatal resistance, rate of transpiration and leaf temperature influence water relations in peanut during drought (Kambiranda *et al.* 2011). Stressed plants have lower RWC (As low as 30%) than non-stressed plants (85 to 90%) (Babu and Rao, 1983). In peanut, accumulation of soluble compounds in cells increases osmotic potential and reduces water loss from cells. Proline, an amino acid, accumulates whenever there is moisture stress (Kambiranda *et al.* 2011). Existance of OA and genotypic variation in that character in groundnut has been reported (Azevedo Neto *et al*., 2010; Thangella *et al*., 2013).

The present investigation aims at studying the physiological response in term of osmotic adjustment in two C3 (Ground nut) cultivars and two C4 (sorghum) cultivars in response to water stress in order to utilize the large genotypic variability in WUF in both C3 and C4 plants that could be utilized to improve crop WUF.

MATERIALS AND METHODS

A field experiment was conducted during 2009/2010 and 2010/2011 to study the effect of levels of water stress on osmotic adjustment of two cultivars of sorghum(C_4 plant), Tabat and Arfa Gadamak, and two cultivars of groundnut (C_3 plant), Madani and Gibaish. Seeds were obtained from the Department of Botany and Agricultural Biotechnology of the Faculty of Agriculture and the Sudanese Arab Seed Company. The experiments were conducted in the experimental farm at the Faculty of Agriculture, University of Khartoum, Shambat (latitude 15^0 30′ N; longitude 32^o 33′ E).

The soil of the site was heavy clay (48% clay), moderately alkaline (pH 7.8 to 8.5) and of very low permeability (Elamin, 2006). Three irrigation intervals W1, W2 and W3 were adopted. Split-plot design with three replicates was used. Irrigation treatments were assigned to the main plots and cultivars to the sub-plots. Sub-plot size was 12 m^2 (3 m x 4 m) and each sub-plot contained four ridges 70 cm a part. Treatments were assigned to subplots at random. All plots were irrigated weekly for four weeks before imposing the other irrigation intervals.

Five plants were randomly selected from each sub-plot for measuring the means of osmotic adjustment.

Leaf water relations parameters

Quantification of crop water status was made by measuring the following leaf water relation:

Relative water content (RWC) was determined by the method of Barrs and Weatherly (1962) according to the following equation:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where:

FW: Fresh weight of the leaf sample.

Dw: dry weight of the leaf sample.

TW: turgid weight of the leaf sample.

Leaf discs of 0.8cm diameter were punched from 10 leaves, samples collected at random from each plot. The leaf discs were weighed using a sensitive balance to obtain the fresh weight. The turgid weight (TW) was determined after floating the leaf discs on distilled water in Petri dishes for 24 hours at room temperature under dim light. The discs were surface-dried and weighed to obtain the turgid weight. Dry weight (DW) was measured after oven-drying the samples at $80^{o}C$ for 48 hours, and weighing them to obtain the dry weight.

Measurement of osmotic potential (O.P)

Twenty leaves of sorghum and groundnut were collected before irrigation from each plot at random. Ten g of leaves from each plot were mixed using an electric blender .Ten ml of distilled water were added to each sample and mixed well. The mixture was filtered using filter paper Whattman No.42 in to a conical flask. Fifty μ L were taken from the extract of each sample in Eppendorf tubes and were placed in an osmometer (Model osmomat 030, Gonotec GmbH).Before that the osmometer was calibrated with known concentrations (m mol kg^{-1}) of Na Cl solutions. Osmotic potentials (OP) were calculated according to the following equation (Moinuddin and Khanna-Copra,2004):

$$OP\ (MPa) = -R \times T \times mol\ kg^{-1}$$

Where :

R: the gas constant (0.008314)

T: Temperature measured in the Kelvin scale (298 k in these measurements)

The OP was corrected (OP + 0.1 OP) for the dilution of symplastic sap by apoplastic water, assuming 10% apoplastic water (Kramer, 1983). The osmotic potential at full turgor (Op_{100}) was calculated according to Wilson *et al.*(1979) by the following equation :

$$OP_{100} = \frac{corrected op \times RWC}{100}$$

The experiment above was repeated using leaves of the two cultivars of sorghum and groundnut irrigated every two weeks (W_2), and every three weeks (W_3) to obtain osmotic potentials of stressed plant. The same experiment was carried out in the second season 2010/2011.
Data were statistically analyzed using ANOVA and means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

The capacity for osmotic adjustment (OA) judged by the change in osmotic potentials for the two sorghum cultivars for season one and two is shown in Table (1).Watering treatments had significant effects on osmotic potentials of the two cultivars. The highest (more negative) osmotic potentials were recorded for both cultivars in the two seasons under the longest watering interval of three weeks. In Tabat there were no significant differences when the capacity for OA was measured under the one-or two -week irrigation treatments in both seasons. Also, no significant differences were observed under these two treatments when OA was measured after 7, 11 or 15 weeks of growth. However, when measured under the 21-day irrigation treatment the highest (more negative) osmotic potentials were measured after 7 weeks of growth and their values decreased significantly after 11 and 15 weeks of growth (becoming more positive).

In the second season Table (2) osmotic potentials were observed to be lower under the 7- and 14-day irrigation intervals and higher under the 21-day irrigation interval compared with the first seasons.

In Arfa Gadamak in the first season the highest (more negative) osmotic potentials were recorded under the longest irrigation interval and the lowest (less negative) under the shortest irrigation interval. Osmotic potentials were generally lower in this cultivar compared with Tabat particularly under the 21-day irrigation interval. Again, in this cultivar there were no changes in osmotic potentials with time under both the 7day and 14-day irrigation intervals. However under the 21-day interval, osmotic potentials were highest (more negative) during the first stage of growth (7weeks) and decreased thereafter.

The second season in this cultivar witnessed an increase in the osmotic potentials (becoming more negative) under 7-day irrigation interval and those potentials were not significantly different from those under the 14-day irrigation interval.
The highest (more negative) osmotic potentials were recorded under the 21-day interval.
There was generally no significant increase with time in osmotic potentials under all irrigation treatments in this season

Figure (1) shows the effect of watering treatments on osmotic potential of the four cultivars in the first season (2009/2010). Osmotic potentials were, generally not significantly different for the four cultivars under the 7-day and 14-day irrigation intervals in spite of the lower values of osmotic potentials under the 14-day interval.

Osmotic potentials

Osmotic potentials were significantly higher under the 21-day interval compared to the 7-day and 14-day irrigation intervals for all cultivars.
The highest osmotic potential (more negative) among the four cultivars were recorded for Tabat which was significantly different from the other sorghum cultivar and the two groundnut cultivars which were not significantly different from each other.

Figure (2) shows the effect of watering treatment on osmotic potential of the four cultivars in the second season (2010-2011). Osmotic potentials were almost identical for the four cultivars under the 7-day and 14-day intervals. Again the highest osmotic potentials were recorded under the

21-day irrigation interval. However, there were no significant differences among the four cultivars in osmotic potentials.

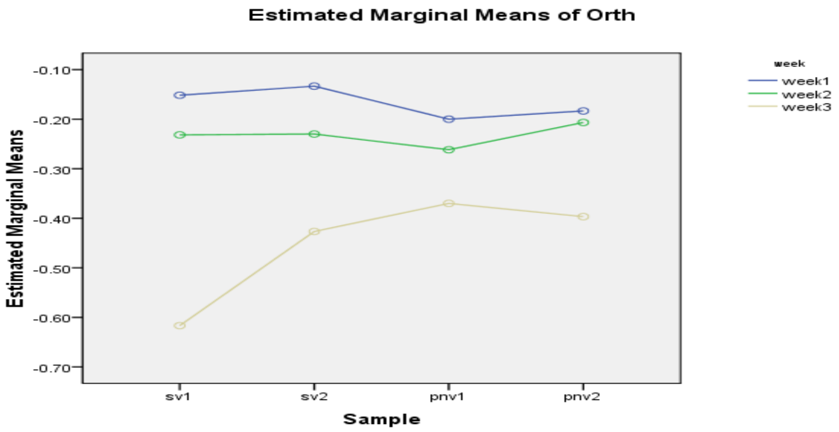

Fig (1): Effect of watering treatment on osmotic potential of the four cultivars in the first season (2009/2010).

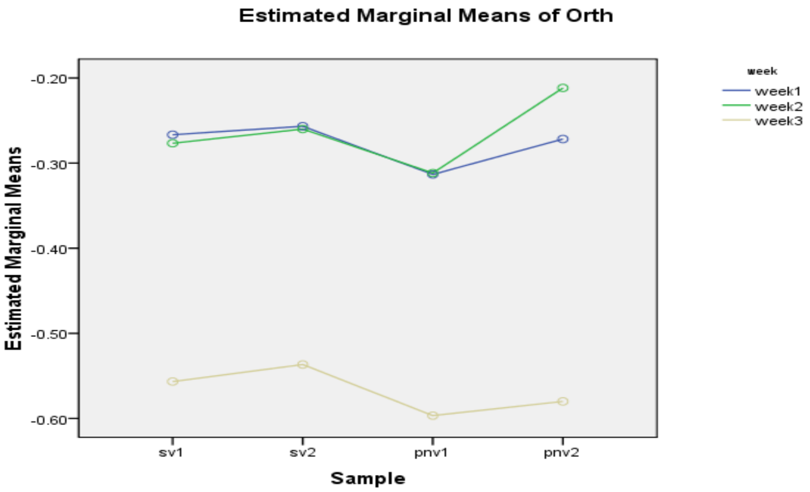

Fig (2):Effect of watering treatment on osmotic potential of the four cultivars in the second season (2010/2011).

Table 1**:** Osmotic adjustment in the leaves of two sorghum cultivars in response to three watering intervals in seasons one (2009/2010) and two (2010/1011).

Variety	Season one									Season two								
	W_1			W_2			W_3			W_1			W_2			W_3		
	Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tabbat	-0.2b	-0.2b	-0.1b	-0.2b	-0.2b	-0.2b	-0.8a	-0.4a	-0.5a	-0.3b	-0.2b	-0.3b	-0.3b	-0.2b	-0.3b	-0.6a	-0.5a	-0.5a
Arfa Gadamak	-0.1b	-0.1b	-0.2b	-0.3b	-0.3b	-0.2b	-0.5a	-0.3b	-0.4a	-0.2b	-0.2b	-0.3b	-0.3b	-0.2b	-0.2b	-0.6a	-0.6a	-0.5a
Mean	-0.2	-0.2	-0.2	-0.3	-0.3	-0.2	-0.7	-0.4	-0.5	-0.3	-0.2	-0.3	-0.3	-0.2	-0.3	-0.6	-0.6	-0.5

-Means within columns and rows with the same letter are not significantly different.
W_1: watering interval of 7 days
- W_2: watering interval 14-days
W_3: watering interval 21-days .
1: 7weeks of growth.
2: 11 weeks of growth.
3: 15 weeks of grow

Table 2: Osmotic adjustment in the leaves of two groundnut cultivars in response to three watering intervals in seasons one (2009/2010) and two (2010/2011).

Variety	Season one									Season two								
	W_1			W_2			W_3			W_1			W_2			W_3		
	Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)			Time of sampling(weeks)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Gibaish	-0.2b	-0.2b	-0.2b	-0.2b	-0.3b	-0.3b	-0.4a	-0.4a	-0.4a	-0.3b	-0.3b	-0.3b	-0.3b	-0.2b	-0.3b	-0.6a	-0.6a	-0.6a
Medani	-0.2b	-0.2b	-0.2b	-0.2b	-0.2b	-0.2b	-0.5a	-0.5a	-0.3b	-0.3b	-0.2b	-0.3b	-0.2b	-0.2b	-0.2b	-0.7a	-0.5a	-0.6a
Mean	-0.2	-0.2	-0.2	-0.2	-0.3	-0.3	-0.5	-0.5	-0.4	-0.3	-0.3	-0.3	-0.3	-0.2	-0.3	-0.7	-0.6	-0.6

-Means within columns and rows with the same letter are not significantly different.
W_1: watering interval of 7 days
- W_2: watering interval 14-days
W_3: watering interval 21-days .
1: 7weeks of growth.
2: 11 weeks of growth.
3: 15 weeks of grow

DISCUSSION

The osmotic potential (OP) in the leaves of the two sorghum cultivars was significantly increased with increasing water stress level in both seasons. In the first season, in both Tabat and Arfa Gadamak, the highest (more negative) osmotic potentials were recorded under the longest watering interval (21-days). In the present study, Tabat exhibited a higher capacity for osmotic adjustment(OA) compared with Arfa Gadamak in the first season only with no significant differences in the second season. This result is in agreement with that of Girma and Krieg (1992) who reported similar values of OP in sorghum. Osmotic adjustment in sorghum was reported to be due to net solute accumulation including carbohydrates and proline (Bazi *et a*l., 2009; Zhou *et al*., 2013).

Osmotic potentials in the leaves of the two groundnut cultivars were significantly increased with increasing water stress levels in both seasons. The volume of OA measured in the shortest irrigation interval in both cultivars was the lowest and similar in both seasons and was highest (more negative) in the longest irrigation interval than the other treatments (W_1,W_2). In both seasons, in Gibaish, the volume of OA did not change between the growth stages (7-11-15 weeks) under the 7-day and 21-day intervals of irrigation. Medani recorded the highest (more negative) value for OA (-0.7MPa) in the second season. These results agree with those reported by Collinson *et al.*(1997) for OA in leaves of peanut in well-watered and under stress condition. Also, Stirling *et al.*(1989) reported that substantial OA between -0.8 -1.6Mpa occurred in expanding leaves of peanut which allowed expanding leaves to maintain higher turgor levels during periods of stress.

Osmotic adjustment results primarily from an active accumulation of inorganic solutes and organic solutes. It is an avoidance strategy with regard to water stress allowing plants to keep stomata open despite of the more negative leaf water potentials. It is also beneficial for plants under stress as they become able to extract more water from the soil and thereby have the capacity to maintain turgor during drought. A positive turgor is required for cell expansion and growth under water-stress condition and is crucial for the continuation of many biochemical and physiological processes of plants.(Cosgrove,1981; Jones *et al.*1981; (Peacock *et al.*1988; Zlatev and Lidon, 2012; Blum, 2017).

In spite of reports in the literature of the existence of genotypic variation for OA in sorghum (Assefa *et al.*, 2010; Zlatev and Lidon, 2012) and Groundnut (Azevedo Neto *et al.*, 2010; Thangella *et al.*, 2013), results of this study were not inconclusive. No variation was detected in the two groundnut cultivars in the two seasons while in sorghum Tabat exihibited higher capacity for OA only under the severe water stress treatment (21 days) in the second season. This may have been due to the limited number of cultivars (Only two for sorghum and two for groundnut) investigated in this study.

In this study the two C_4 cultivars (Tabat and Arfa Gadamak),exhibited a higher capacity for OA compared with the two C_3 cultivars (Gibaish and Medani) particularly under severe water stress. It has been reported that sorghum is more drought tolerant than many other crops due to many of its characteristics including OA (Assefa *et al.*, 2010). The capacity for OA depends on the species involved and the nature of the stress imposed, since mild or rapidly imposed stress induces little or no active adjustment, while progressive severe stress may stimulate substantial solute accumulation (Ong *et al.*1985). On the other hand, drought impact on growth of both C_3 and C_4 plants is an object of many researches (Liu and Stutzel,2004;Nayyar and Gupta 2006; Sliogeryte *et al.*2009). Growth rates of C_4 plants were found to be less affected than those of C_3 plants (Nayyar and Gupta 2006; Vitkauskait and Venskaityte ,2011). C_4 plants are known to be more efficient water users compared with C_3 plants (Taiz and Zeiger.,2006) this is due to, in part, to their peculiar leaf anatomy (kranz type) and the operation of the C_4 pathway of photosynthesis.

CONCLUSIONS AND RECOMMENDATIONS

The capacity for OA was detected in both C4 (Sorghum) cultivars and C3 (Groundnut) cultivars. Perhaps due to the limited number of cultivars investigated, no genotypic variation in the capacity for OA was detected. It is, therefore, recommended that future investigations should include a larger number of cultivars.

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الاتزان الأزموزى فى بعض أصناف الفول السوداني (*Arachis hypogaea*) والذرة الرفيعة (*Sorghum bicolor* L. Moench) استجابة للإجهاد المائي *

مزاهر سنوسى محمد[1] والطيب الحاج على أحمد[2] و عبداللة الخضر مدنى[3]

قسم الاحياء، كلية التربية، جامعة الخرطوم

مستخلص البحث: أجريت هذه التجربة في المزرعة التجريبية بكلية الزراعة، جامعة الخرطوم خلال موسمي 2009 - 2010 و 2010 – 2011 لدراسة استجابة الاتزان الازموزى لصنفين من الذرة الرفيعة (نباتات رباعية الكربون): طابت وأرفع قدمك، و صنفين من الفول السوداني (نباتات ثلاثية الكربون): غبيش ومدني لثلاث فترات ري: 7 أيام (ر1) و14 يوم (ر2) و 21 يوم (ر3)، حيث أعتبرت الفترة الأخيرة مسببة للإجهاد المائي. نفذت التجربة بتصميم القطع المنشطرة بثلاثة مكررات حيث وضعت معاملات الرى فى الاحواض الرئيسة والاصناف فى الاحواض الثانوية. حسبت قم الاتزان الازموزى من قياسات الجهد الأزموزى ومحتوى الماء النسبى لأوراق الذرة الرفيعة والفول السودان .كان للمعاملات المائية أثر معنوي على الجهد الأزموزي (ج.أ) لاصناف الذرة الرفيعة والفول السوداني مما أدى الى إرتفاعات معنوية فى الجهد الأزموزي (أصبح أكثر سالبية). في الطرز لاصناف الذرة الرفيعة أظهرت النتائج أن متوسط الجهد الأزموزي كان 0.3- و 0.3 و 0.7 – ميجاباسكال تحت المعاملات ر1 و ر2 و ر3 على التوالي. وفى اصناف الفول السوداني كان متوسط الجهد الإزموزي 0.3 – و 0.3- و 0.6- ميجاباسكال تحت ر1 و ر2 و ر3 بعد 7 أسابيع من النمو. وبعد 15 أسبوعا من النمو كان المتوسط 0.3 – و 0.3- و 0.6- ميجاباسكال تحت ر1 و ر2 و ر3 على التوالي. وجد فى هذه الدراسة أن أعلى (الأكثر سالبية) جهد أزموزي (0.8 – ميجاباسكال) تم قياسه بعد 7 أسابيع من النمو فى الصنف طابت تحت المعاملة المائية الري كل 3 أسابيع وعلى ذلك فإن طابت قد تكون لها مقدرة أكبر على الإتزان الازموزي. أوضحت الدراسة أن النباتات رباعية الكربون (الذرة الرفيعة) قد تكون لها مقدرة أكبر على الإتزان الأزموزي مقارنة بالنباتات ثلاثية الكربون (الفول السوداني).

كلمات مفتاحية: الفول السوداني، الذرة الرفيعة، الإجهاد المائي، الإتزان، الأزموزي .

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* جزء من أطروحة تقدم بها المؤلف الأول لجامعة الخرطوم لإستيفاء مطلوبات درجة الدكتوراه.

[1] استاذ مساعد، كلية التربية، جامعة الخرطوم

[2] أستاذ مشارك ، معهد دراسات التصحر واستزراع الصحراء - جامعة الخرطوم.

[3] استاذ مشارك، كلية التربية، جامعة الخرطوم