

THE ROLE OF SOME SOLUBLE METABOLITES IN OSMOTIC ADJUSTMENT TO REDUCED MATRIC AND OSMOTIC WATER POTENTIAL IN TWO SOYBEAN CULTIVARS

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ABSTRACT:

This work aimed to study the effect of reduced matric- and osmotic water potential on changes of some parameters affecting plant water potential related to metabolism. The experimental plants are two cultivars of soybean (Giza 111 and Giza 21). The study is concerned mainly with internal adjustment in soluble metabolites affecting plant water relation. Such as soluble sugars (SS), hydrolysable carbohydrates (HC) among carbon metabolites and total free amino acids (AA) as nitrogenous components as well as soluble proteins which contribute water conservation in the plant. Statistical treatment of data helped much in clarifying the role of each of the four metabolic components in adjusting to the reduced water potential in the soil (water stress).

INTRODUCTION:

Subjecting plant to stress through effect of plant water deficit on some aspect like changes in morphological or anatomical characteristics of plant is not of main importance (Danin, 1991) but the effect on metabolic process is more essential (Branett and Naylor, 1996 and Roy, 1987). Actually, the effect of water stress should take in consideration the causes of this stress which are mainly the effect of shortage of water available in soil (reduced matric water potential) and increased salinity in the soil (reduced osmotic water potential) (Kramer 1969). However, many plant species adjust to both types of water stress; the mechanisms involved in this respect remain obscure. Osmotic adjustment in response to water stress enables many plants to withstand moderate internal which may affect normal metabolism (Hsiao et al., 1984 and Mccree et al., 1984). Study by Xu, (2002) revealed that a remarkable array of organic compounds and inorganic ions contribute to the solute potentials of the plant cells. Water stress affects the accumulation levels of soluble sugars (Gao, 2006). Both carbon (C) and nitrogen (N) metabolites are involved in osmotic adjustment. Accordingly, specific amino acids may characterized and/or differentiate between different plants subjected to stress (e.g. desert plants) (Farghali et al., 2001). The aim of the work in this study is to identify the response of the two experimental cultivars to decreased matric- and osmotic water potential also to make a comparison between equipotential levels of both especially concerned the metabolic means of adjustment.

MATERIALS AND METHODS:

In this work seeds of two Soybean varieties were tested including c.v. Giza 111 and c.v. Giza 21. These varieties were obtained from Crop Science Department, Faculty of Agriculture, Assiut University.

Adjustments of drought levels (matric water potential, Ψm):

Different levels of soil matric water potential, ψm: 0(control), -0.1,-0.3,-0.5, 0.7, -0.9 and -1.1MPa can be obtained by characteristic curve data of soil moisture desorption curve achieved by the pressure plate technique (Richards 1947).

Adjustments of salinity levels (osmotic water potential, ψ_s) (at a fixed sodium adsorption ratio (SAR=1/20)):

Different osmotic water potential levels were prepared by using solutions of NaCl+CaCl₂ in concentrations that yield different osmotic potentials (Ψ_s) at a definite SAR ratio (1/20): Ψ_s levels were chosen at the same ψ_m levels: 0 (control), -0.1, -0.3, -0.5, -0.7, -0.9 & -1.1 MPa. The concentrations of NaCl and CaCl₂ in solutions prepared are based on calculations explained by El-Sharkawi (1968).

Previously weighed one-liter volume plastic pots lined with double polyethylene bags were used in growing the experimental plants. Each pot was filled with 1000 gm air dry soil (sand/clay 1:2 v/v). Two plants were allowed to grow in each pot as an experimental unit. The soil water content was maintained near field capacity most of the time, during germination and growth, until the treatment was planned to start. This was achieved by a periodic (daily or twice daily, if necessary) adjustment of the weight of the whole System (the pot and its contents) by watering with distilled water using four replicates for each treatment. The plants were kept in the Greenhouse during experimentation to secure mild climatic conditions.

Preparation of plant extracts for analyses:

The leaves sampled from experimental plants, oven-dried at 80 °c were powered thoroughly (after determination of their oven dry weight). 0.1 gram powder samples taken in a test tube, to which ten ml of distilled water were added and then heated to 90 °c in a water bath (shaking tube every 5 minutes) for one hour and then filtration was done by using filter paper according to El-Sharkawi and Michel (1977). Plant extracts were kept in deep freez. In the extracts several analyses were run including the following:

1)-Determination of water soluble nitrogen metabolite: - Total free amino acids (AA) and soluble proteins (SP) (as nitrogen metabolites) were determined according to procedures described by Dudois et al., (1956) and Lowrey et al, (1951).

2) Determination of carbon metabolites: - Soluble sugars (SS), hydrolysable carbohydrates (HC) were determined according to procedures described by Lee and Takahashi (1966).

3) Determination of acidic fraction: Organic acids (OA) estimated as total acidity of the plants, extract was determined by titration against standard alkaline solution (NaOH 0.001 N) by using phenolphthalein as an indicator Millar (1973).

Evaluation of partial (estimated) osmotic potential (POP) of different constituents of the metabolic fractions:

The estimated osmotic pressure (OP) computation for each ion took place as follows:

Metabolic POP = OP of SS + OP of AA

+ OP of OA

OP (of organic

solutes)

Concentration (g/l) × 22.4

atmospheres. Molecular weight Partial osmotic potential (POP) which includes OP of SS, AA and OA are expressed as % calculated as following: % of soluble sugars OP = OP of SS *100 Data are shown in figures 1(A& B) and POP (atm)

Experimentation was carried out on the vegetative stage only. Statistical analysis of randomized complete block design according to Gomez and Gomez (1984) of experimental data took place. The significant means of any trait studied were compared using LSD at 5% probability level according to Waller and Duncan (1969).

RESULTS:

I) Effect of reduced matric and osmotic water potential stresses on some soluble osmotically active (osmoregulatory) metabolites of plant sap:

The data on analysis of plant extracts for metabolites which may potentially contribute to osmoregulation, namely: soluble sugars and hydrolysable carbohydrates (carbon metabolites), total free amino acids and soluble proteins (soluble nitrogenous compound) are shown in figure 1, 2, 3 and 4.

I) A- Soluble sugars and hydrolysable carbohydrates:

2 (A&B)

In Giza 111, the content of soluble sugars (fig. 2 A, B) had the same trend of response to both water potential stress (osmotic and matric) where the soluble sugars content remains more or less unchanged upto -0.7 & -0.5 MPa under osmotic and matric water stress, respectively. Thereafter, a marked reduction took place. Soluble sugars decrease by about 60 % and 80 % at 1.1MPa of osmotic and matric water stress, respectively. The significant difference of the response among the stress levels appeared early (at -0.1MPa) in the case of matric water stress rather than osmotic.

In Giza 21, there is a remarked progressive increase in soluble sugars with increasing osmotic stress. The magnitude of this effect was higher at higher salt stress. At the level - 1.1 MPa soluble sugars was 2.4 fold than that in the control samples. This clearly refers to an obvious difference between the responses of the two cultivars to osmotic stress. While under matric water stress, the soluble sugars content increased upto -0.5 MPa then there was a marked reduction in sugars content. The differences in the response were significant allover the stress levels of both type of stress.

Statistically, as shown in tables 1 & 2, the soluble sugars play a greater role in adjustment in Giza 111 and this role was significantly pronounced at moderate (-0.7MPa) and high (-0.9&-1.1MPa) levels of osmotic water stress. The same was true in Giza 21 but only at the severest level of stress.

In Giza 111, HC content (fig.1 A, B) remained unchangeable upto -0.3 MPa then a marked and progressive enhancement was noticed at moderate (-0.5& -0.7MPa) and high (-0.9&-1.1) osmotic stress levels. At high water potential levels (-0.1&-0.3MPa) the response of HC was the same in osmotic and matric stress, where the HC content remained more or less unchanged upto – 0.5 MPa after which there was a marginal non significant increase. On the other hand and interestingly, in Giza 21, the HC content dropped earlier at Ψ m = -0.1 MPa and continued to decrease with decreasing the matric water potential. This trend is more or less similar to that under osmotic stress.

As shown in tables 1 & 2, HC have a highly significant effect on the adjustment under osmotic water stress rather than matric in Giza111. This significance effect appeared at moderate and severe levels of stress. Such highly significant effect was appeared at the level of -0.3 and -0.7 MPa levels of matric and osmotic stress, respectively, in Giza 21.

I) B- Soluble proteins and total free amino acids:

Figure (3-A) indicates that the reduced osmotic water potential induced a marked and progressive increase in soluble proteins (SP) content in Giza 111. The highest content was at reported -0.3 MPa and the lowest was at – 0.1 MPa. Under matric water stress the soluble protein content increased slightly upto – 0.5MPa thereafter a slight reduction was recorded. Generally, there was a tendency to increase in the soluble protein content under both type of water stress and this increase was significant only at the moderate levels.

In Giza 21, (figure 3-B), soluble proteins content decreased gradually and slightly with decreasing the osmotic water potential in the soil, the highest reduction in SP content appeared at the higher doses of the salt. This reduction was significant at the highest levels of stress (-0.9 & -1.1MPa). Applying matric stress exerts a remarked and progressive decreasing trend in protein content the significance of this reduction appeared at moderate and sever stress levels.

Figure (4-A) illustrate that in Giza 111, the total free amino acids (TAA) increased by increasing salt stress. The highest increase in total FAA was found to be at the highest osmotic stress level. While the total free amino acids content increased by the matric stress. This increase was more pronounced at Ψm levels from -0.1 to -0.7 MPa.

In Giza 21, (fig. 4-B) the effect of two type of stress is more or less the same at low and moderate stress levels of both stresses. While at the highest levels of stress (-0.9 and -1.1MPa), the response in the amino acid content differ significantly according to the type of stress (table 6) where a much more significant accumulation of amino acids content under matric stress and the reverse is true in case of osmotic stress at mentioned level.

As shown in tables 1 & 2, adjustment using AA in Soybean Giza 111 was obvious under osmotic stress rather than matric at the levels of -0.9 &-1.1MPa and the vice versa in Giza 21, where a tendency to adjustment by AA appear significantly under matric stress.

II) Organic acids contents:

Organic acids are considered as a transitional and intermediate product of some biological pathways and its importance in plant water relations is due to its contribution to the osmotic activity of the ionic fraction.

In Giza 111, organic acids content (Fig. 5) has the same trend under both osmotic and matric water stress where the organic acids content increased irregularly by osmotic or matric water stress. The highest increase was obtained in plants treated with – 0.3 MPa and – 0.5 MPa in case of osmotic and matric water stress, respectively, and the lowest increase was found to be at the severe potential level (- 1.1 MPa) of both water stress.

In Giza 21, the amount of organic acids dropped quickly and suddenly even at the low level of osmotic potential (-0.1MPa). A slight continued reduction existed upto – 0.3 MPa after which non significant increase was observed at moderate and high levels. Such increase didn't exceed the control value.

In Giza 21, there is a highly significant reduction in the amount of organic acid when the soil matric stress increase. This reduction was pronounced at - 0.9 & - 1.1 MPa.

Tables 1 & 2 show that, under osmotic stress Giza 21 has more capability than under matric stress to use the organic acids as osmotic active components to equilibrate internal water balance at moderate and high stress level. This was pronounced highly significantly at the highest levels of stress. The reverse was true in Giza 111 the significance appeared early at the highest water potential level (low stress levels -0.1 & -0.3MPa) but the negative sign at -0.1MPa level means that the role of organic acids in the adjustment was greater under matric stress than under the osmotic stress.

III) Effect of matric and osmotic water potential stresses on osmotic pressure of plants leaves:

II) A- Partial (estimated) osmotic pressure (POP) (metabolic OP):

Soluble metabolic fractions (soluble sugars, total free amino acids and total organic acids) play a considerable role at different and variable degrees in contributing to (POP) of the osmotic fractions. The relative role of each, however, apparently differs according to cultivars and the type of water stress (matric or osmotic stress). The partial osmotic potentials (POP) of studied cultivars are shown in figure (7-A, B, C and D) as a circle diagram. The diameter of the circle is proportional to metabolic partial osmotic potential (POP) which includes OP of SS, AA and OA.

Generally, soluble sugars had a prominent role in osmoregulation among estimated metabolites. The contribution reached 99% in case of Giza 21 under osmotic stress. Total free amino acids and total organic acids exchange their degree of contribution where AA had a moderate role in osmoregulation and OA had the minor or negligible role in Giza 111. The reverse was true in Giza 21 (see dark columns in table 3). The contribution of AA in osmoregulation is more pronounced in Giza 111 especially at the lowest osmotic water potential levels and the role of SS much more retreated (table 3). It is worthy to mention that the highest OP of SS and consequently POP was reported at the lowest stress levels of matric and osmotic and the lowest SS osmotic pressure was at the highest stress levels stresses in both cultivars except in Giza 21 subjected to the highest osmotic stress levels, OP of SS was the highest (table 3).

Table (1&2): Comparative effects of reduced Ψ s & Ψ m at equipotential levels on changes in metabolic
components content in Giza 111 and Giza 21, respectively. A negative sign means that the effect of
matric potential (Ψm) is greater than osmotic potential (Ψs), judged by LSD value.

Equipotential	Differences between means in Giza 111									
son	Organic acid	AA	SP	SS	НС					
Ψs _{0.1} - Ψm _{0.1}	-0.26**	1.4	-9	0	-2					
Ψs _{0.3} - Ψm _{0.3}	0.27**	1.5	17	4	-13					
Ψs _{0.5} - Ψm _{0.5}	0.06	1.4	-2	5	68**					
Ψs _{0.7} - Ψm _{0.7}	0.38**	1.0	-4	36**	74**					
Ψs _{0.9} - Ψm _{0.9}	0.05	2.8**	7	20**	98**					
Ψs _{1.1} - Ψm _{1.1}	0.13	2.2**	24**	20**	113**					
L.S.D	0.15	1.9	20	15	30					

Equipotential	Differences between means in Giza 21										
levels compari- son	Organic acid	AA	SP	SS	НС						
Ψs _{0.1} - Ψm _{0.1}	-0.66	-0.1	-8	-16	-26						
Ψs _{0.3} - Ψm _{0.3}	-0.65	-0.3	-6	-10	-62**						
Ψs _{0.5} - Ψm _{0.5}	1.45	0.1	4	17	20						
Ψs _{0.7} - Ψm _{0.7}	1.53	0.1	10**	59	76**						
Ψs _{0.9} - Ψm _{0.9}	2.50**	-1.5**	13**	128**	42						
Ψs _{1.1} - Ψm _{1.1}	1.69**	-2.0**	7	184**	5						
L.S.D	1.6	1	9	100	50						

*Significant at P< 0.05	**Significant at P< 0.01
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Table (3): Changes in the partial osmotic pressure (POP) of organic metabolites according to changes in the osmotic pressure (OP) (atm) of some metabolic fraction (soluble sugars SS, total free amino acids AA and total organic acids OA).

1	Ci 111									C' 11						
	Giza 111							Giza 21								
		1	Ψs		Ψm			Ψs			Ψm					
l urametei Streis level	SS	AA	OA	РОР	SS	AA	OA	РОР	SS	AA	OA	POP	SS	AA	OA	POP
cont	12.4	0.33	0.04	12.81	12.44	0.33	0.04	12.81	11.4	0.05	0.50	12.01	11.4	0.054	0.50	12.0
0.1	11.8	0.87	0.06	12.75	14.44	0.42	0.09	14.95	16.2	0.14	0.35	16.67	14.2	0.090	0.27	14.5
0.3	11.2	0.87	0.12	12.18	14.19	0.42	0.08	14.68	15.9	0.15	0.31	16.39	14.8	0.069	0.21	15.1
0.5	10.7	0.81	0.11	11.62	14.19	0.39	0.10	14.68	13.4	0.09	0.18	13.71	15.4	0.116	0.39	15.9
0.7	9.7	0.78	0.13	10.61	14.93	0.48	0.07	15.48	8.6	0.08	0.10	8.77	15.9	0.116	0.31	16.4
0.9	5.6	0.98	0.07	6.65	16.55	0.33	0.06	16.94	7.3	0.45	0.01	7.80	23.1	0.018	0.36	23.5
1.1	5.1	1.16	0.08	6.35	16.43	0.33	0.06	16.82	6.8	0.60	0.01	7.46	29.7	0.018	0.25	30.0

Carbon metabolites



Figure (1): The average concentration (mg/ml sap) of soluble sugars (SS) in investigated cultivars under equipotential levels of two different types of water stress (matric and osmotic water potential stresses).



Figure (2): The average concentration (mg/ml sap) of hydrolysable carbohydrates(HC) in investigated cultivars under equipotential levels of two different types of water stress (matric and osmotic water potential stresses).



Soluble nitrogenous compounds

Figure (3): The average concentration (mg/ml sap) of soluble proteins (SP) in investigated cultivars under equipotential levels of two different types of water stress (matric and osmotic water potential stresses).



Figure (4): The average concentration (mg/ml sap) of total free amino acids (TAA) in investigated cultivars under equipotential levels of two different types of water stress (matric and osmotic water potential stresses).



Figure (5): The average concentration (mg/ml sap) of organic acids in investigated cultivars under equipotential levels of two different types of water stress (matric and osmotic water potential stresses).



Figure (6): The percentage of osmotic pressure (OP) of some metabolic fractions in Giza 111



Figure (7): The percentage of osmotic pressure (OP) of some metabolic fractions in Giza 21

DISCUSSION CONCLUSIONS:

The plant can adjust osmotically to water stress through accumulation of some organic solutes as well as ionic ones (Chaves et al., 2009). The differences in the magnitude of osmoregulation among the experimental plants may be in response to either low water potential or metabolic disturbance in internal water status of plants. Changes in partial osmotic potential in the experimental plants largely depend on changes in metabolic faction (soluble sugars, total free amino acids and total organic acids). Generally, the role of amino acids and organic acids is very little when compared with that of soluble sugars, thus the latter play the predominant role in osmoregulation especially at the lowest osmotic water potential levels in case of Giza 21 and minor role at high matric water stress in Giza111. The highest POP due to amino acids was recorded in Giza 111 at the highest osmotic stress level.

Accelerated HC conversion to sugars, accelerated respiration rate accompanied by a drop in starch level,

and stimulation in soluble carbohydrates may be observed in Giza 21. A conversion catabolic in nature, at the lowest water potential levels of the osmotic stress this may be a trial to the stabilize protoplasmic colloids by increasing soluble sugars accumulation and thus preventing their denaturation by replacing water molecules from hydration shells (Street and Helgi, 1977), despite the catabolic nature. While in Giza 111 a decrease in soluble sugars is accompanied by an increase in hydrolysable carbohydrates (through enzymatic conversion) (Shinozakiand Yamaguchi-Shinozaki, 2007) which indicates an anabolic source of adjustment. This reflects the tendency of Giza 111 to increase their osmotic potential which serves to reduce its tranpirational water loss as well as increasing the potential gradient between roots and root medium (El-Sharkawi et al., 1988), such adaptive mechanism reflects the superiority of Giza 111 as salt tolerance.

In Giza21, under matric water potential the content of hydrolysable carbohydrates is more or less unchanged upto -0.3MPa accompanied by a significant increase in soluble sugars content at the corresponded levels that explains the active biosynthesis when plant is not suffering from any stress (normal condition) but beyond this level plant start to suffer from high rate of catabolism as both of SS and HC decreased thus the level of -0.3MPa is considered as the maximum level of adjustment (critical level in limit of adaptation).

Although the distinctive role of soluble proteins as cell matric water binders and thus prevents protein denaturation, no significant increase in their content in all samples except in Giza 111 under osmotic stress (table 1) where the increase in TAA leads to SP synthesis (Farghali, 1998). An obvious different response was exhibited in Giza 21 under matric stress. The AA content increased highly significantly and progressively at the expense of the soluble protein content this confirmed the catabolic status of this cultivar under drought condition so such great accumulation of AA may be due to intensive break down of storage complex

(insoluble proteins) into amino acids (SPAA) having osmotic activity. Such inverse proportionality is there for considered as an indicative of an adjustive mechanism to drought stress through decreasing soluble proteins content to increasing free amino acids as osmotica being beneficial in maintaining the viscous properties of cytoplasm (Werner and Finkelstein, 1995), despite its break down nature. Under osmotic stress the decrease in amino acids accumulation leads to restrict soluble proteins synthesis which may be not effective in adjustment under such condition or aspect of stress, thus using proteins as salt tolerance depend on the nature of plants or cultivars (Ashraf and Harris, 2004).

Accordingly, the data presented in this study leads to the following conclusions:

There is a great variation in the correlation between carbohydrates and protein content among the two soybean genotypes exposed to the various levels of osmotic potential:

1) - In cv 111, salt stress resulted in a great reduction in carbohydrates corresponded to a great accumulation in organic acid amount and consequently protein. 2) – The reverse held true in salt sensitive genotype where salt stress induce a great accumulation in carbohydrates (which approached 2.5 fold) and dramatic reduction in organic acid, amino acids and consequently proteins. This led us to conclude the following:

1) - Differences in the accumulation of carbohydrates are a valid trait to discriminate the genotypic variation among species and genotypes. Consequently, the two soybean genotypes were genetically varied.

2) – The salt tolerant genotype adjusted a great equilibration in the metabolism of carbohydrates, amino acids and consequently nitrogenous compounds. The genotype directed most of synthesized carbohydrates into the manufacturing nitrogenous components for growth and osmoregulation.

3) – The salt sensitive genotype accumulate aconsiderable amount of carbohydrate at the expense of nitrogenous compound which indicate a great troubling in the metabolism of this genotype.

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دور بعض المواد الايضيه الذائبة في الانضباط الاسموزي لسلالتين من نبات فول الصويا تحت النقص في الجهد المائي للتربة

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استهدف موضوع البحث دراسة دور المواد الايضيه الغير متأينه في الانضباط الاسموزي تحت تأثير نقص الجهد المائي (سواء بواسطة نقص الماء أو زيادة الملوحة) لسلالتين من نبات فول الصويا، والفرق بين تأثير كلاً من الجفاف والملوحة على تراكم وتحول هذه المواد ودورها في التوازن المائي الداخلي ومدى تأقلم هذه السلالات للظروف الصحراوية. وقد اشتملت هذه الدراسة على ما يلي:

تحليل وتتبع محتوي الأوراق من المواد الايضيه التي تساهم في الانضباط الاسموزي للخلايا (السكريات الذائبة والقابلة للتحلل، البروتينات الذائبة والأحماض الامينيه الكلية الحرة) بهدف دراسة مصادر الانضباط في اسموزية النبات.

تقييم إحصائي لمعنوية تأثير ودور العوامل المنفردة وكذلك مقارنه بين مستويات الإجهاد المتساوية. و*قد أظهرت الدراسة ما يلي:*

تستطيع النباتات محل الدراسة أن تراكم بعض المواد الايضيه بدرجات متفاوتة لما يفرض عليها من نقص الجهد المائي كمحاوله للحفاظ علي الضغط الاسموزي المتزن. يستطيع جيزا ٢١ أن يراكم كلا من الحماض الامينيه تحت زيادة الملوحة و السكريات الذائبة تحت نقص الماء علي حساب كلا من البروتينات الذائبة و المواد الكربو هيدراتيه علي الترتيب مما يشير إلي وجود تأثير هدمي وليس بناء داخل الخلية وعليه يمكن اعتبار جيزا ٢١ .