GENOTYPIC DIFFERENCES IN LEAF FREE AMINO ACIDS AS OSMOPROTECTANTS AGAINST DROUGHT STRESS IN GRAIN SORGHUM

A.M. Al-Naggar[1], O.O.El-Nagouly[2] and Zeinab S.H.Abo-Zaid[2]

(1) Agronomy Dept., Faculty of Agriculture, Cairo University, Giza
(2) Grain Sorghum Res. Section, Field Crops Research Institute, Agric. Res. Center, Giza

ABSTRACT

Eight grain sorghum lines of variable drought tolerance were crossed in 1999 to make 16 F_1's . Parents and F_1's were field-evaluated for leaf free amino acids content under water stress and non-stress conditions at pre-flowering stage in 2000. The objectives were to study effects of drought stress on the accumulation of free amino acids and to describe differences between drought tolerant and susceptible genotypes in such osmoprotectants following the imposition of water deficit. Mean squares due to genotypes , soil moisture regimes and genotypes X soil moisture regimes were significant for all the studied 18 leaf free amino acids. In general absolute amounts of most free amino acids increased under stress as compared to control . Maximum increase in free amino acids due to water stress occurred in proline content. On average proline showed 6.71 and 3.09 fold increase under stress over well - irrigated parental lines and their F_1 crosses, respectively. Drought tolerant genotypes were significantly higher in total free amino acids (7 and 41 %), proline (183 and 335 %), glycine (35 and 51 %), cysteine (78 and 29 %) , metheonine (14 and 36 %) , aspartic (30 and 23 %)and phenylalanine (6 and 24 %) and lower in glutamic acid (5 and 11 %) , tyrosine (12 and 11 %), tryptophane (81 and 38 %) than susceptible ones under stress at GS2 for parents and hybrids, respectively. Free proline content under stress as compared to control was 19.66 and 21.06 fold in the tolerant line R-90011 and the F_1 A-88005 X R-89016, respectively, while such increases in the susceptible line B-1 and the cross A-1 X RTX-82BDM-499 were only 1.50 and 2.46 fold, respectively. The role of accumulation of more proline and less glutamic acid in tolerant than susceptible genotypes under stress was discussed.

Key words: *Free amino acids, Proline, Glutamic acid, Osmoprotectants, Compatible osmolyte, Water deficit, Drought tolerance, Grain sorghum*

INTRODUCTION

Many compatible solutes are known to accumulate at high levels in plant cells in response to water deficit such as amino acids (e.g., proline), sugar alcohol (e.g., pinitol), other sugars (e.g., fructans) and quaternary ammonium compounds (e.g., glycine betaine) (Delauney and Verma 1993).

It has been suggested that compatible osmolytes do not interfere with normal biochemical reactions and act as osmoprotectants during osmotic stress (Yoshiba *et al* 1997). The most striking change in amino acid composition following the imposition of water deficit was an approximately sixty-fold increase in proline levels (Girousse *et al* 1996). They reported that glutamate levels also increased, although the increase was not as dramatic as that observed for proline. Genes for enzymes involved in the biosynthesis and metabolism of proline have been isolated from various plants and the functions of their gene products have been characterized. Results of investigations indicated that over production of proline results in increased tolerance of transgenic tobacco plants to osmotic stress (Yoshiba *et al* 1997).

In grain sorghum, leaves of water-stressed field-grown plants also accumulated several times greater proline than non-stressed plants (Waldren *et al* 1974). Blum and Ebercon (1976) explored a possible association between free proline amino acid accumulation in water-stressed leaves and the drought resistance of various grain sorghum cultivars. Yavada *et al* (1991) also reported proline accumulation to be associated with drought tolerance in sorghum. On the contrary, Dhopte *et al* (1995) indicated that both tolerant and susceptible grain sorghum genotypes responded similarly with respect to changes in contents of proline, glycine and phenylalanine, under water-stress conditions. However, they found that in a susceptible genotype, the lysine and valine contents were considerably affected, the former being reduced by 16 fold and the latter being increased under stress as compared to irrigation conditions.

The information on leaf free amino acid content of tolerant and susceptible grain sorghum genotypes in response to imposition of water stress are generally limited (Dhopte *et al* 1995).Therefore, the objectives of the present investigation were:(1) to study effects of drought stress on the accumulation of free amino acids, and (2) to describe differences between drought tolerant and susceptible genotypes in such osmoprotectants following the imposition of water deficit.

MATERIALS AND METHODS

Eight parental lines of grain sorghum (two drought resistant and two susceptible restorer and four susceptible cytoplasmic male sterile,cms lines) were chosen based on previous screening experiments for drought tolerance at preflowering stage (GS2).The four restorer lines (R-89016, R-89022, R-90011 and RTX-82BDM-499) were crossed onto the four cms lines (B-1, B-37, B-102 and B-88005) to make a total of 16 F_1 fertile

hybrids at Agric. Res. Sta. of the Field Crops Research Institute (FCRI), Agricultural Research Center (ARC),Giza in 1999 season.In 2000 season, parents and F_1 s were field-evaluated at the Agric. Res. Sta. of Assiut University, Assiut, under control and water stress conditions at GS2 stage. Water stress was imposed by withholding the second and third irrigation, i.e 30-day stress period from panicle initiation to anthesis (i.e GS2).A split-plot design was used in randomized complete blocks with three replications, with irrigation regimes alloted to the main plots and genotypes to the sub-plots. Except for the irrigation treatments agricultural practices were done as recommended by Grain Sorghum Res. Section, FCRI, ARC. At the end of GS2 stress period samples of the third leaf from the top of the plant were taken from each replicate and were kept in a deep freezer until amino acid determination. Contents (in mg/g dry matter) of the total free amino acids and of each amino acid separately were determined in the leaves at the Central Laboratory of Fac. of Agric., Ain Shams University, according to the derivatization procedure proposed by Landault and Guiochen (1964) using n-butanol and trifluoro-acetic anhydrin . The free amino acids included alanine, valine, threonine, glycine, isoleucine, leucine,serine, proline, cysteine, γ aminobutyric acid, metheonine, aspartic acid,phenylalanine,histidine,glutamic acid,lysine and tryptophane.Data were subjected to a regular analysis of variance of a split plot design according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance (not presented) of leaf free amino acid contents of 24 grain sorghum genotypes (4 females, 4 males and their 16 F_1 crosses) evaluated under two soil moisture regimes (well watered and water deficit) at GS2 stage, revealed significant or highly significant differences among genotypes and among soil moisture regimes for contents of all studied eighteen free amino acids, except among soil moisture regimes for valine. Moreover, mean squares due to genotypes X irrigation regimes interaction were highly significant for all free amino acids, suggesting that content of each free amino acid in leaves of sorghum genotypes varies with water supply.

Effect of water stress on free amino acids

The changes in relative percentages of the individual free amino acid constituents of sorghum genotypes as affected by drought stress are presented in Table (1). It is obvious that over all genotypes, the amino

Table 1. Relative percentages of free amino acids in the third leaf of grain sorghum genotypes evaluated under well watered (control) and soil moisture stress at GS2 (Assiut, 2000).

Amino acid	%		Amino acid	%	
	Control	Stress		Control	stress
Alanine	3.29	2.96	Butyric acid	1.77	1.69
Valine	3.25	2.96	Metheonine	1.85	2.96
Threonine	2.62	1.70	Aspartic	16.72	17.98
Glycine	2.51	2.64	Pheylalanine	7.15	8.27
Isoleucine	1.03	1.36	Histidine	5.15	4.79
Leucine	6.29	5.98	Glutamic	33.66	26.11
Serine	1.22	1.89	Tyrosine	2.08	2.72
Proline	3.58	10.81	Lysine	2.23	2.84
Cysteine	3.52	2.71	Tryptophane	1.99	1.99

acids glutamic, aspartic, phenylalanine and leucine were the most predominant amino acids in control (well irrigation), representing 33.66, 16.72, 7.15 and 6.29 % of the total free amino acids, respectively. Under stress at GS2, the predominant amino acids were glutamic, aspartic, proline, phenylalanine and leucine, representing 26.11, 17.98, 10.81, 8.27 and 5.98 %, respectively. On the other hand, isoleucine, gamma amino butyric, serine and tryptophane at both control and stress conditions, methionine under control and threonine under stress were present in low concentrations, i.e. each of them was represented by less than 2 % of the total free amino acids.

Drought stress imposed on grain sorghum at GS2 stage increased remarkably the relative percentage of the amino acid proline from 3.58 % under control to 10.81 % under stress. The percentage of proline under stress was 3 times greater than the proline percentage under control conditions. Moreover, relative percentages of some individual amino acids increased but with low extent due to stress as compared to the control, e.g. isoleucine, serine, tryptophane, metheonine, phenylalanine, tyrosine and lysine. On the other hand, drought stress resulted in a pronounced decrease in the relative percentage of glutamic acid (from 33.66 % under control to 26.11 % under stress) and to a lesser extent in alanine, valine, threonine and cysteine (Table 1).

It is worthy to note that the relative increase in the percentage of proline and the relative decrease in the percentage of glutamic acid under stress were approximately equal.

Data in Table (2) show that drought stress imposed at pre-flowering stage (GS2) caused a remarkable average increase in the content of total leaf

free amino acid in grain sorghum. Average increase in total free amino acids due to stress at GS2 relative to that of control was 6.0 % for parental lines and 21.0 % for their F_1 crosses.

The general increase in the total free amino acids following drought stress at preflowering stage suggests that the increased supply of low molecular weight compounds, that are effective osmotic solutes could account for the osmotic adjustment in the plant tissues (Jones *et al* 1980). That hybrids showed a higher increase in total amino acid contents under stress than their parents, suggests the superiority of heterozygotes over homozygotes in this regard.

Table 2. Average content (mg/g dry matter) of free amino acids in leaves of grain sorghum genotypes non-stressed and water stressed at GS2, Assiut 2000.

Amino acid	Parents		Deviation (fold)	Hybrids		Deviation (fold)
	Cont	Stress		Cont	Stress	
Alanine	2.03	2.76	1.36	2.49	2.60	1.04
Valine	2.99	2.79	-1.07	2.27	2.43	1.07
Threonine	2.36	1.68	-1.40	1.85	1.35	-1.37
Glycine	2.03	2.84	1.40	1.79	2.00	1.12
Isoleucine	0.88	1.33	1.51	0.73	1.24	1.70
Leucine	4.20	5.93	1.41	4.87	4.65	-1.05
Serine	1.17	1.43	1.22	0.84	1.73	2.06
Proline	1.62	10.87	6.71	2.82	8.72	3.09
Cysteine	2.63	2.93	1.11	2.75	2.02	-1.36
Butyric acid	1.45	1.50	1.04	1.37	1.44	1.05
Metheonine	1.08	2.30	2.13	1.53	2.65	1.73
Aspartic	13.89	16.53	1.19	12.53	14.19	1.13
Phenylalanine	5.94	5.80	-1.02	5.02	7.19	1.43
Histidine	2.99	4.34	1.45	3.94	4.05	1.03
Glutamic	34.42	20.39	-1.69	22.03	23.86	1.08
Tyrosine	1.52	1.97	1.30	1.60	2.61	1.63
Lysine	1.17	2.01	1.72	1.77	2.61	1.48
Tryptophane	1.64	1.72	1.05	1.43	1.35	-1.06
Total	84.01	89.12	1.06	71.60	86.70	1.21

The absolute amounts (mg/g dry matter) of all free amino acids generally increased under stress compared to control, except for valine, threonine, phenylalanine and glutamic acid in parents, and threonine, leucine, cysteine and tryptophane in hybrids, which showed a general trend of decrease under water stress .The reduction in glutamic acid content under stress may be attributed to its conversion into proline as proline (Pro)is synthesized from glutamic acid by two enzymes, P5C synthesase (P5CS) and P5C reductase (P5CR) (Yoshiba *et al* 1997). They suggested that levels

of Pro are regulated at the level of transcriptional regulation of the genes of those two enzymes during dehydration and redehydratrion.

Maximum increase in free amino acids due to water stress occurred in proline content, followed by metheonine and lysine in parents and by serine and metheonine in crosses (Table 2). On average, proline showed 6.71 and 3.09 fold increase under stress at GS2 over control for parental lines and their F_1 crosses, respectively. Among compatible organic solutes, it is probable that proline is the most widely distributed osmolyte (Yoshiba *et al* 1997). Tomato cells cultured under water stress rapidly accumulated about 300 times more proline than non-stressed cells and they adapted to osmotic stress (Handa *et al* 1983, 1986 and Rhodes *et al* 1986). Eubacteria, protozoa, marine invertebrates and many plants including algae (e.g., halophytes, tobacco, spinach, potato, tomato, *Arabidopsis*, alfalfa, field bean, soybean, wheat, barley and rice) can accumulate proline (Delauney and Verma 1993). These observations indicate that many plants have the ability to adapt to water stress and that proline is involved in tolerance to osmotic stress, acting as a compatible osmolyte.

Genotypic differences in free amino acid accumulation

Range of variability in free amino acids (mg/g dry matter) for studied genotypes under control and stress conditions and deviation from control are presented in Table (3). Genotypes varied widely in their response to water deficit for accumulation of different amino acids.

Amongst studied amino acids, the widest range was recorded for proline (Table 3). Free proline content in sorghum leaves under water stress at GS2 varied from 2.49 mg/g (in B-1 and B-102) to 20.38 mg/g (in B-37) for parents and from 2.09 mg/g (B-37 X R-89022) to 22.70 mg/g (B-1 X R-89016) for F_1 crosses. Increases of free proline content under stress as compared to control reached 14.17 and 19.66 fold (for the parents R-89016 and R-90011, respectively) and 21.05 fold (for the F_1 cross A-88005 X R-89016). These results are in agreement with results of field performance of parents, which classified the parental lines (R-89016 and R-90011) as resistant to drought based on yield performance under stress, and the hybrid A-88005 X R-89016 is a cross between a resistant and susceptible parent.

Free glutamic acid in grain sorghum leaves recorded highest absolute content under stress in the parents B-102 (26.90 mg/g), R-90011 (26.69 mg/g) and in the crosses A-102 X R-89016 (35.37 mg/g) and A-37 X R-90011 (34.00 mg/g) and the lowest values in the parent B-37 (7.14 mg/g) and the cross A-102 X RTX (12.04 mg/g). The greatest increase in glutamic

Table 3. Range of variation (highest (Hi) and lowest (Lo) values) in leaf free amino acid contents (mg/g dry matter) and in fold deviation from control of grain sorghum genotypes evaluated under control and water stress at Assiut, 2000 season.

Amino acids	Control		Stress		Deviation (Fold)	
	Hi	Lo	Hi	Lo	Hi	Lo
Parental lines						
Alanine	3.57 (1)	1.05 (5)	3.78 (6)	1.86 (9)	2.84 (6)	-1.38 (1)
Valine	7.55 (4)	1.05 (5)	4.30 (10)	1.67 (5)	3.47 (10)	-3.81 (4)
Threonine	7.55 (4)	1.08 (1)	2.92 (3)	0.84 (9)	2.36 (3)	-6.74 (4)
Glycine	3.65 (6)	1.16 (1)	4.21 (2)	1.37 (3)	3.16 (5)	-1.47 (8)
Isoleucine	1.33 (6)	0.49 (2)	1.81 (2)	1.02 (5)	3.69 (2)	-1.29 (8)
Leucine	5.73 (3,6)	1.24 (1)	11.44 (2)	3.09 (1)	2.65 (2)	-1.48 (3)
Serine	2.90 (10)	0.49 (2)	2.15 (4)	1.11 (9)	3.69 (2)	-2.59 (10)
Proline	4.15 (8)	0.53 (9)	20.38 (2)	2.49 (1,3)	19.66 (9)	1.50 (1)
Cysteine	3.82 (2)	1.58 (1)	6.28 (2)	1.37 (3)	2.16 (5)	-1.63 (3)
Butyric acid	2.49 (4)	0.99 (3)	2.04 (5)	0.93 (9)	1.79 (5)	-1.70 (9)
Metheonine	1.82 (2)	0.53 (5)	3.72 (5)	1.37 (1)	7.02)5)	1.39 (9,10)
Aspartic	21.65 (5)	5.06 (4)	22.50 (9)	8.51 (2)	2.36 (4)	-1.82 (2)
Phenylalanine	13.78 (4)	3.87 (5)	10.04 (5)	3.52 (10)	2.59 (5)	-1.31 (3)
Histidine	6.67 (10)	0.79 (9)	8.43 (4)	1.77 (5)	9.43 (9)	-2.78 (5)
Glutamic	45.32 (9)	22.10 (4)	26.90 (3)	7.14 (2)	1.02 (4)	-3.87 (2)
Tyrosine	2.74 (3)	1.05 (5)	3.78 (1)	0.94 (6)	3.05 (1)	-1.35 (4)
Lysine	2.24 (4)	0.97 (5)	3.44 (1)	1.03 (8)	2.31 (1)	-1.30 (4)
Tryptophane	3.15 (3)	0.97 (5)	3.27 (3)	1.02 (5)	1.10 (1)	1.04 (2,3,8,10)
Crosses						
Alanine	5.54 (2x6)	0.86 (4x5)	4.51(2x7)	1.08 (4x8)	5.24 (2x8)	-3.20 (2x7)
Valine	4.09 (3x6, 3x8)	0.86 (4x5)	4.11(2x7)	0.98 (4x5)	4.07 (2x7)	-3.79 (1x6)
Threonine	4.09 (1x6, 3x8)	0.86 (4x8)	2.45 (2x7)	0.86 (3x6)	1.96 (2x7)	-4.35 (1x6)
Glycine	3.49 (2x6)	1.09 (1x5)	3.23 (2x7)	0.87 (4x6)	2.31 (2x7)	-1.47 (1x8)
Isoleucine	1.71 (1x6, 3x8)	0.08 (1x5)	2.06 (1x7)	0.58 (3x8)	11.00 (1x5)	-2.95 (3x8)
Leucine	11.54 (1x7)	1.85 (4x6)	10.38 (1x7)	20.90 (3x6)	4.07 (4x8)	-2.49 (1x8)
Serine	2.57 (3x7)	0.08 (1x5)	3.04 (2x7)	0.73 (4x6)	14.63 (1x5)	-1.10 (3x8)
Proline	8.81(3x5)	0.99 (4x8)	22.70 (1x5)	2.09 (2x6)	21.05 (4x5)	-3.97 (2x6)
Cysteine	4.29 (1x6, 3x8)	0.23 (3x7)	3.13 (1x5)	0.95 (4x6)	7.21 (3x7)	-3.07 (3x6)
Butyric acid	2.38 (3x8)	0.33 (3x6)	2.94 (1x7)	0.43 (3x6)	3.24 (1x7)	-2.47 (3x7)
Metheonine	3.49 (2x6)	0.47 (3x7)	6.37 (1x7)	1.46 (4x6)	6.85 (1x7)	-1.64 (4x8)
Aspartic	18.87 (4x7)	6.93 (3x6)	26.80 (4x5)	7.99 (1x6)	2.12 (3x6)	-1.81 (1x5)
Phenylalanine	8.84 (3x6)	1.72 (4x6)	11.31 (1x8)	4.46 (3x7)	4.20 (4x6)	-1.98 (3x6)
Histidine	6.16 (3x5)	0.68 (1x6)	8.72 (1x7)	0.94 (3x6)	4.20 (1x6)	-3.93 (2x8)
Glutamic	35.80 (4x7)	10.82 (2x6)	34.00 (2x7)	12.04 (3x8)	2.65 (3x5)	-1.85 (4x5)
Tyrosine	5.93 (1x5)	0.82 (4x6)	5.83 (3x6)	0.86 (1x6)	20.4 0(3x7)	-3.37 (1x5)
Lysine	3.72 (2x5)	0.92 (1x6,3x8)	7.15 (3x7)	1.09 (1x8)	5.42 (3x7)	-2.49 (1x8)
Tryptophane	2.34 (3x5)	0.86 (2x5)	2.30 (2x6)	0.78 (3x8)	1.27 (3x8)	-1.26 (4x6)

1=B-1, 2=B-37, 3=B-102, 4=B88005, 5=R-89016, 6=R-89022, 7=R-90011, 8=RTX-82BDM-499

acid under stress was recorded for the parent B-88005 (1.02 fold) and the cross A-102 X R-89016 (2.65 fold) . On the contrary, the maximum reduction in glutamic acid due to water stress was recorded for the parent B-37 (-3.87 fold) and the cross A-88005 X R-89016 (-1.85 fold). It is worthy to note that the resistant parents (R-89016 and R-90011) showed a remarkable decrease in glutamic acid (of 2.05 and 1.69 fold, respectively) under stress as compared to control. Such decrease in glutamic acid due to water stress in resistant genotypes is expected, probably due to greater utilization of glutamic acid in proline biosynthesis.

Some genotypes showed higher content of more than one amino acid due to water stress at GS2 as compared to control. Maximum number of amino acids (nine) which increased with greatest folds due to water stress, was achieved by the tolerant parental line R-89016 (which was chosen based on its yield performance under stress). This line showed the highest increments in proline, glycine, cysteine, butyric acid, metheonine, phenylalanine, tyrosine, lysine and tryptophane and a decrease in glutamic acid. Some genotypes showed superiority in accumulating only one amino acid due to water stress, e.g. A-1 X R-89016 (serine), A-37 X R-89016 (tryptophane), A-37 X RTX (leucine) and A-102 X RTX (lysine).

To describe the differences between drought tolerant (T) and susceptible (S) genotypes, relative percentages and absolute contents of free amino acids were averaged for two groups of genotypes differing in drought tolerance based on their absolute and relative grain yield/plant under stress (Table 4) in previous experiments. The drought tolerant genotypes at GS2 included the parental lines R-89016 and R-90011 and the hybrids A-1 X R-89016, A-102 X R-90011 and A-88005 X R-89016. The drought susceptible genotypes at GS2 were lines B-102 and B-88005, and the crosses A-37 X RTX, A-102 X R-89022 and A-88005 X RTX.

The advantage of T over S in grain yield under water stress conditions would allow to expect greater total amino acids contents and greater content of each individual amino acid in T than in S parental lines and F_1 crosses.

Consistent with expectation, total free amino acids was 1.07 and 1.41 fold (i.e. 7 and 41 %) greater in T than in S parents and crosses, respectively. Out of 18 amino acids, 7 and 15 amino acids showed greater amounts in T than in S for parents and hybrids, respectively under water stress. Results in Table (4) indicate that superiority of T over S under stress was more pronounced in the hybrids than their parental lines, suggesting a role of hybrid vigor in the change of content of total free amino acids as well as individual amino acids, except glutamic, tyrosine and tryptophane.

Free proline content was appreciably greater in the drought tolerant (T) than in the drought susceptible (S) genotypes by 2.83 fold for parental lines and 4.35 fold for crosses. Glycine, cysteine, methionine, aspartic acid and phenylalanine were significantly higher in T than in S for parental lines by 1.35, 1.78, 1.14, 1.30 and 1.06 fold under stress at GS2. For crosses, alanine, valine, threonine, glycine, isoleucine, leucine, serine, cysteine, butyric acid, methionine, aspartic acid, phenylalanine, histidine and lysine were significantly greater in T than in S by 1.83, 2.41, 1.59, 1.44, 1.89, 1.87, 2.25,

Table 4. Free amino acids (mg/g) averaged over the two most tolerant or most susceptible parental lines and the 3 most tolerant or most susceptible F_1 crosses.

Amino acids	Susceptible			Tolerant			Superiority over susceptible (fold)
	Cont	Stress	Deviation (fold)	Cont	Stress	Deviation (fold)	
Parental lines							
Alanine	2.32	2.57	1.11	1.05	2.14	2.04	-1.20
Valine	4.98	2.45	-2.03	1.27	1.81	1.42	-1.35
Threonine	4.40	2.02	-2.18	1.45	1.49	1.03	-1.35
Glycine	2.28	2.19	-1.04	1.41	2.97	2.11	1.35
Isoleucine	0.87	1.37	1.58	0.94	1.39	1.48	1.01
Leucine	5.35	5.63	1.05	3.03	5.06	1.67	-1.11
Serine	0.83	1.67	2.02	0.68	1.25	1.84	-1.34
Proline	1.25	4.47	3.57	0.79	12.65	16.01	2.83
Cysteine	2.66	1.67	-1.59	2.11	2.97	1.41	1.78
Butyric acid	1.74	1.54	-1.13	1.36	1.48	1.09	-1.04
Metheonine	0.92	2.32	2.52	0.83	2.65	3.19	1.14
Aspartic	10.75	14.53	1.35	19.05	18.92	-1.01	1.30
Phenylalanine	9.88	6.58	-1.50	4.26	6.97	1.64	1.06
Histidine	0.95	8.00	8.42	2.81	4.18	1.49	-1.91
Glutamic	27.65	24.67	-1.12	43.60	23.53	-1.85	-1.05
Tyrosine	2.24	1.98	-1.13	1.10	1.76	1.60	-1.12
Lysine	1.70	2.15	1.26	1.14	1.72	1.51	-1.25
Tryptophane	2.28	2.36	1.04	1.23	1.30	1.05	-1.81
Total	83.02	88.17	1.06	88.11	94.24	1.07	1.07
Crosses							
Alanine	3.89	2.16	-1.80	2.86	3.95	1.38	1.83
Valine	2.28	1.61	-1.42	2.18	3.88	1.78	2.41
Threonine	1.56	1.19	-1.31	1.74	1.89	1.09	1.59
Glycine	2.19	1.70	-1.29	1.51	2.45	1.62	1.44
Isoleucine	0.55	0.81	1.48	0.88	1.53	1.74	1.89
Leucine	4.22	3.79	-1.11	8.83	7.08	-1.25	1.87
Serine	0.39	1.22	3.13	1.32	2.74	2.08	2.25
Proline	3.78	3.94	1.04	3.23	17.15	5.31	4.35
Cysteine	2.97	1.70	-1.75	2.26	2.20	-1.03	1.29
Butyric acid	0.62	1.08	1.74	1.59	1.54	-1.03	1.43
Metheonine	1.99	2.49	1.25	0.97	3.38	3.48	1.36
Aspartic	8.18	13.15	1.61	12.44	16.21	1.30	1.23
Phenylalanine	6.49	6.65	1.02	5.69	8.23	1.45	1.24
Histidine	5.30	2.66	-1.99	4.29	5.07	1.18	1.91
Glutamic	15.5	22.84	1.47	23.51	20.62	-1.14	-1.11
Tyrosine	1.39	2.88	2.07	2.75	2.37	-1.16	-1.22
Lysine	2.26	2.46	1.08	1.69	3.23	1.91	1.31
Tryptophane	1.75	1.92	1.09	1.54	1.39	-1.11	-1.38
Total	65.30	74.25	1.14	79.19	104.91	1.32	1.41

1.29, 1.43, 1.36, 1.23, 1.24, 1.91 and 1.31 fold under stress at GS2. On the other hand, contents of alanine, valine, threonine, leucine, serine, butyric acid, histidine, glutamic acid, tyrosine, lysine and tryptophane in parental lines were lower in T than in S by 20, 35, 35, 11, 34, 4, 91, 5, 12, 25 and 81 % under stress at GS2. Moreover, for crosses glutamic acid, tyrosine and tryptophane were lower in T than in S by 11, 22 and 38 % under stress.

The grain yield advantage of T over S in parental lines under stress may be a result of their higher ability to accumulate greater amounts of free glycine (35 %), proline (183 %), cysteine (78 %), methionine (14 %), aspartic acid (30 %) and phenylalanine (6 %) in their leaves. Similarly, the higher grain yield from T than from S crosses under stress conditions may result from the capacity of T hybrids to accumulate higher leaf concentration of free proline (335 %), alanine (83 %), valine (141 %), threonine (59 %), glycine (44 %), isoleucine (89 %), leucine (87 %), serine (125 %), cysteine (29 %), butyric acid (43 %), methionine (36 %), aspartic acid (23 %), phenylalanine (24 %), histidine (91 %) and lysine (31 %), compared to S hybrids.

On average, genotypes classified as the most drought tolerant in terms of absolute and relative grain yield had a higher total amino acids, higher contents of proline, glycine, cysteine, methionine, aspartic acid, phenylalanine and lower glutamic acid, tyrosine and tryptophane as compared with those classified as the most susceptible genotypes, when grown under drought stress conditions at GS2 stage (Table 4).

The observed elevation in the proline content in drought tolerant grain sorghum plants with the imposition of drought stress coincides with reports on different plant species. Proline apparently has a specific protective role in the adaptation of plant cells to water deprivation (Handa *et al* 1986). Possible role of proline may be (a) to neutralize toxic free amonia produced in water-stressed leaves (Frota 1972), (b) to serve as a substrate for respiration and an energy source for the recovering plant (Blum and Ebercon 1976) and (c) to reduce stress-induced cellular acidification, i.e. to mediate osmotic adjustment, stabilize subcellular structures and scavenge free radicals (Hare and Cress 1997). The accumulation of proline appears to be an excellent means of storing energy since the oxidation of one molecule of proline can yield 30 ATP equivalents (Atkinson 1977).

Proline synthesis has also been implicated as a mechanism of alleviating cytosolic acidosis, a condition often associated with stress (Kurkdjian and Guern 1989). A decrease in intracellular pH has been implicated as a factor capable of eliciting proline accumulation in plants (Chou *et al* 1991) and removal of excess H^+ due to proline synthesis may

prevent a depression in respiration in salt-or water-stressed soybean seedlings (Krackhardt and Guerrier 1995).

Three genes for enzymes involved in the biosynthesis and metabolism of proline have been cloned to date and their expression has been analyzed (Yoshiba *et al* 1997). In the future, application of genetic engineering to the breeding of environmental stress-tolerant transgenic plants should lead to improvements in crop production in unfavorable environments, such as those with insufficient water.

Glutamic acid was expected to decline sharply, especially in drought tolerant genotypes with the impostion of drought stress, since it acts as a precursor for proline and other amino acids. Previous studies indicated that most of the proline accumulated in plants, in response to stress is the result of exchange synthesis from glutamate (Delauney and Verma 1993 and Verbruggen *et al* 1993). But the variable changes noticed in the relative percentages of glutamic acid under stress may be attributed to the active transformation to and from carbohydrates via α-ketoglutaric acid (Ashour 1991). The interconversion of the amino acids and α keto acids by either oxidative deamination or transamination, e.g. from alanine and aspartic, could also have affected the relative percentage of glutamic acid (Cantarow and Schepartz 1967 and Rhodes *et al* 1986).

Phenylalanine was observed to increase more in T than in S genotypes under drought stress, while tyrosine was decreased. Similar results were noted by Thompson *et al* (1996) in turnip leaves, Singh *et al* (1973) in barley leaves and Ashour (1991) in soybean leaves. The pathway of phenylalanine and tyrosine biosynthesis were reported by several investigators (Cantarow and Schepartz 1967 and Miflin and Lea 1977).

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الإختلافات الوراثية في الأحماض الأمينية الحرة بالورقة كحاميات اسموزية ضد الإجهاد المائي في الذرة الرفيعة للحبوب

أحمد مدحت النجار (١) ـ عثمان عثمان النجولي (٢) ـ زينب سيد حسن (٢)

(١) قسم المحاصيل-كلية الزراعة-جامعة القاهرة-الجيزة

(٢) قسم بحوث الذرة الرفيعة للحبوب ، معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية – الجيزة

تم في موسم ١٩٩٩ تركيب ١٦ هجين F_1 بين ٤ سلالات عقيمة الذكر و ٤ سلالات معيدة للخصوبة من الذرة الرفيعة للحبوب مختلفة في تحملها للجفاف وفي موسم ٢٠٠٠ تم تقييم الـ ٨ آبـاء و الـ ١٦ هجين في تجربة حقلية بمزرعة كلية الزراعة جامعة أسيوط تحت الري الكامل أو الحرمان من الري في مرحلة ما قبل التزهير. كانت الأهداف هي دراسة تأثير تقسية الجفاف علي محتوي الأحماض الأمينية الحرة و تقديـر الإختلافات بين التراكيب الوراثية المتحملة و الحساسة للجفاف من الذرة الرفيعة من حيث محتواها من هـذه الأحماض عند تعرضها لتقسية الجفاف في مرحلة ما قبل التزهير . أظهرت النتائج أن متوسـطات المربعـات الراجعة للتراكيب الوراثية و لأنظمة الري و للتفاعل بين التراكيب الوراثية و أنظمة الري كانت معنوية لكـل الأحماض الأمينية الحرة الثمانية عشر المدروسة . لقد زادت بصفة عامة الكميات المطلقة لمعظم الأحمـاض الأمينية الحرة تحت ظروف الجفاف مقارنة بمعاملة الكنترول .و قد ظهرت أقصى زيادة في الأحماض الأمينيـة الحرة في محتوي حمض البرولين .فقد أظهر البرولين في المتوسط زيادات قدرها ٦,٧١ ، ٣,٠٩ ضعفا تحت ظروف الجفاف عنها تحت ظروف الري الكامل بالنسبة للآباء و لهجن الجيل الأول ، علي التوالـي. كـانت التراكيب الوراثية المتحملة للجفاف أعلي معنويا في محتوي الأحماض الأمينية الحرة الكلية (٧ ، ٤١ %) و

البرولين (١٨٣ ، ٣٣٥ %) و الجليسين (٣٥ ، ٤٤ %) و السستيئين (٧٨ ، ٢٩ %) و المثيونين (١٤ ، ٣٦ %) و الإسبارتيك (٣٠ ، ٢٣ %) و الفينايل ألانين (٦ ، ٢٤ %) و أقل معنويا في الجلوتاميك (٥ ، ١١ %) و التيروسين (١٢ ، ١١ %) و التربتوفان (٨١ ، ٣٨ %) عن التراكيب الحساسة تحت ظروف الجفاف في مرحلة ما قبل التزهير (بالنسبة للآباء و الهجن علي التوالي). و قد وصلت الزيادات في محتوي البرولين الحر تحت ظروف الجفاف مقارنة بالكنترول في السلالة المتحملة R-90011 و الهجين المتحمل -A 88005xR-89016 إلي ١٩,٦٦ ، ٢١,٠٦ ضعفا علي التوالي بينما الزيادات في السلالة الحساسة B-1 و الهجين الحساس A-1 X RTX كانت ١,٥، ٢,٤٦ ضعفا فقط ، علي التوالي. و قد نوقش دور تراكم برولين أكثر و جلوتاميك أقل في التراكيب الوراثية المتحملة عن الحساسة تحت ظروف الجفاف.