J. Environ. Sci. & Natural Resources, **6(2): 01-05 , 2013** ISSN 1999-7361

Selection of Drought Tolerant Groundnut Genotypes (*Arachis hypogaea* L.) Based on Total Sugar and Free Amino acid Content

D. E. Jharna[1], B. L. D. Chowdhury[2], M. A. A. M. Rana[3] and S. Sharmin[4]

[1]Dept. of Biochemistry and Food Analysis, PSTU.

[2, 3&4] Dept. of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh

Abstract: For the selection of drought tolerant genotype, a field trial was conducted to monitor the quantitative changes of biochemical parameters such as total sugar and total free amino acid, due to water deficit condition in groundnut **(***Arachis hypogaea* L.) seed. Plants were grown in irrigated and non-irrigated conditions. Mature seeds of forty genotypes and premature seeds of twenty one genotypes were freeze dried, defatted, ground and subjected to analysis. Drought exhibited no definite trend of increase or decrease for total free amino acid and sugar in mature as well as premature seed. Premature seed accumulated higher amount of total free amino acid having mean values 1.01% and 0.88% for control and drought respectively, than did the matured ones (control - 0.41% and drought - 0.43%). In mature seeds, drought augmented total free amino acid in twenty one genotypes and retrenched in the remaining nineteen. The data on immature seed was not much useful for selection purpose. In mature seeds of genotypes BINA-Chinabadam-2, 9267, ICGV-97228, ICGV-96295, and ICGV-95412, drought resulted in maximum elevation of total free amino acid. Accumulation of total sugar was higher in mature than in premature seeds. Drought brought about the increase in total sugar in mature seeds of thirty genotypes and decrease in the remaining ten. On imposing water stress, mature seeds of genotypes ICGV-92120, ICGV-94143, ICGV-96295, ICGV-95399 and ICGV-97228 showed comparatively high increment of total sugar. A particular genotype did not appear drought tolerant when the data on both the mature and premature seed are considered. However, in mature seed of genotype ICGV-97228, water stress induced a desired level of change in accumulation of total free amino acid and total sugar, and hence it may be preliminary considered as drought tolerant.

Key words: Drought, Groundnut, Total free amino acid, Total sugar

Introduction

Groundnut (*Arachis hypogaea* L.) a legume, is commercially grown in many subtropical and tropical regions of the world. In Bangladesh, groundnuts are cultivated mainly in char area and as well as other areas. In many groundnut producing areas in the world, irrigation is unavailable or impractical. Moreover, due to agro-ecological changes, the crop is facing more risk from drought condition ever before. So to ensure its unabated supply, development and cultivation of drought tolerant groundnut varieties could be the only solution. Pre-harvest aflatoxin contamination is a common occurrence in groundnuts that are grown under non-irrigated conditions. When exposed to prolonged drought, tolerant lines generally display lower rates of pre-harvest aflatoxin contamination (Holbrook *et al.*, 1994) indicating that they may possess some degree of resistance to aflatoxin contamination.

Plant tissues show several metabolic alterations in response to water deficit. Metabolic alteration under drought condition, lead to accumulation of low molecular weight solutes such as sugars, free amino acids, betains and polyols. Organic acids namely malate, citrate and lactate are accumulated in plants growing in water deficit condition Low water potential may also cause rapid accumulation and transportation of abscisic acid to different parts of the plant (Skriver and Mundy, 1990). The free amino acids which increase most due to water stress are aspartate, glutamate, proline, alanine and valine. However, accumulation of proline may represent a general response to cold stress (Chu *et al*., 1974), salt (Chu *et al*., 1976) as well as water (Singh *et al*., 1973).

However, the unavailability of reliable tools to screen groundnut genotypes for drought tolerance is the major hurdles in the genetic improvement of groundnut for drought tolerance (Rucker *et al*., 1995). For proper screening, it is worthwhile to monitor the drought induced variation of biomolecules such as total sugar and free amino acid in groundnut. Pace of metabolism is much higher in premature seed than in mature one. Considering this, it is imperative to include mature as well as immature seeds to obtain a better screening. The experiment is therefore undertaken to screen the groundnut genotypes based on the accumulation of total sugar and free amino acid for stress tolerance.

Materials and Methods

Thirty seven groundnut genotypes from ICRISAT, Hydrabad, India and 3 local varieties from BARI, Gazipur and BINA, Mymensingh, Bangladesh, were included in the study. All genotypes were of *virginia* type. The plants were grown at the Field Experimental Farm of the Department of Genetics and Plant Breeding (GPB), BAU, Mymensingh. To develop and maintain drought condition, the plants were grown under transparent polythene shed and no irrigation was used. The control plants were grown in an open field and irrigated as and when necessary. Premature seeds having yellow to slightly orange colored seed's coat were collected (both control and drought) and after partial maceration, subjected to freeze drying. Testa was not excluded at premature stage, because of the difficulty involved in its removal from seed. Mature seeds with grey orange to pink colored seed's coat were collected and subjected to freeze-drying immediately. Mature seeds were collected from plants grown under irrigation as well as in water deficient condition. About four grams of freeze dried seed sample was extracted thrice with 10 mL of n-hexane and each time clear supernatant was expelled. Residues were dried at 50^0C temperature, later ground and passed through 0.125 mm strainer. The finally ground samples were kept in refrigerator in air-tight pot.

Extraction of total sugar and total free amino acid

Hundred milligrams of freeze dried defatted groundnut seed sample were taken in a test tube, Five milliliters of 80% ethanol was added and they were mixed well in vortex. Supernatant was collected and the residue was re-extracted with 5 mL 80% ethanol and subsequently filtered. Two extracted volumes were taken together and evaporated in a rotary vacuum evaporator at temperature not exceeding 50^0 C. On completion of evaporation, 1 mL distilled water was added and the contents were sonicated properly. The extract was transferred to an ependrop vial and centrifuged in refrigerated centrifuge (5^0C) at 14500 rpm for 20 minutes. The supernatant was collected carefully without disturbing the sediment. Total free amino acid was determined by following the method of Moor and Stein (1948). Total sugar content of groundnut seed was determined by the anthrone method.

Result and Discussions

Variation on the accumulation of total free amino acid

The level of total free amino acid in mature and premature groundnut seeds grown in irrigated and non-irrigated condition are shown in Table1. The total free amino acid content in mature seeds, produced in irrigated condition, varied from 0.179% to 0.895% (Mean 0.41%), where as those in drought condition, observed variation was from 0.218% to 0.782% (mean 0.43%). Drought caused increment of total free amino acid in twenty one genotypes. However, in remaining nineteen genotypes drought rather induced reduction of free amino acid. Considering the mean value, premature seed accumulated higher amount of total free amino acid. In control it varied from 0.384% to 2.295% (mean-1.01%), whereas in drought condition the variation was from 0.317% to 1.972% (mean 0.88%). As in mature seeds, drought caused elevation of total free amino acid in seven genotypes and reduction in the remaining. In premature seeds, the pace of metabolic rate is much higher than that in mature seed. This might have provided a richer amino acid pool in premature and accordingly the total free amino acid content became much higher than in mature seed. Tayeb and Hassanein (2000) observed that free amino acid contents in the shoots and roots of *Vicia faba* progressively increased as water stress level increased. Madhusudhan *et al.* (2002) observed drought induced increased of free amino acid in the leaves of groundnut. In the present study, groundnut seeds, not leaves, roots and shoots were analyzed and this may explain the deviation of our findings with those of Tayeb and Hassanein (2000) and Madhusudhan *et al.* (2002). It is reported that the effect of drought depends on plant species, tissues, age as well as the nature and duration of stress (Hanson and Hitz, 1982). Under water stress, either protein synthesis diminished or increased breakdown, which leads to elevated level of total free amino acid. But when a plant faces water stress beyond its optimum level, injury to cell and impaired nitrogen metabolism takes place. It is also observed that even with the level of stress, the accumulation of different free amino acids vary significantly. A wide difference in the drought endurance capability of different genotypes might have resulted positive response in some genotypes where as negative, in others. Navar-Izzo *et al.* (1990) also reported that increment of total free amino acid could contribute to tolerance of plant to water stress, through an increase in water potential. On the basis of maximum elevation of total free amino acid due to water stress, the genotypes BINA-Chinabadam-2, 9267, ICGV-96295, ICGV-97228 and ICGV-95412 may be considered as drought tolerant.

Table 1. Effect of water stress on the total free amino acid content in premature and mature groundnut seed

Sl. No.	Genotypes	Total free amino acid, g. /100g seed (mature)		% Increase/ decrease over control	Total free amino acid, g. /100g seed (premature)		%Increase /decrease over control
		Control	Drought		Control	Drought	
1.	ICGV-92121	0.4333	0.294	- 32.10	1.061	0.662	- 37.65
2.	ICGV-95377	0.485	0.374	- 22.89	1.467	1.016	- 30.74
3.	ICGV-93260	0.476	0.448	- 5.88	1.233	1.106	- 10.30
4.	ICGV-87846	0.417	0.437	+ 4.80	2.294	1.967	- 14.25
5.	3203	0.380	0.323	- 15.00	1.544	0.474	- 69.30
6.	ICGV-97190	0.824	0.782	- 5.10	0.812	0.531	- 34.61
7.	ICGV-88388	0.516	0.435	- 15.70	0.961	0.657	- 31.63
8.	ICGV-93232	0.895	0.452	- 49.47	0.702	0.521	- 25.78
9.	ICGV-94165	0.458	0.322	- 29.69	0.732	0.583	- 20.36
10.	ICGV-192116	0.408	0.302	- 25.98	1.177	1.036	- 11.98
11.	ICGV-97182	0.394	0.548	+ 39.07	0.592	0.961	+ 62.33
12.	ICGV-95399	0.557	0.469	- 15.80	0.709	0.920	+ 29.76
13.	ICGV-96318	0.392	0.317	- 19.13	0.873	1.231	+ 41.00
14.	ICGV-95390	0.493	0.457	- 7.30	1.482	0.668	- 54.92
15.	4595	0.362	0.275	- 23.91	1.258	0.421	- 66.35
16.	9276	0.363	0.684	+ 88.41	1.015	1.113	+ 9.65
17.	ICGV-92118	0.263	0.340	+ 29.28	0.626	0.691	+ 10.38
18.	ICGV-92126	0.260	0.263	+ 1.15	0.809	1.185	+ 37.60
19.	ICGS-1955	0.396	0.472	+ 19.19	0.589	0.528	- 10.18
20.	ICGV-96294	0.392	0.280	- 28.57	0.384	0.317	- 17.44
21.	ICGV-93255	0.320	0.270	- 15.62	1.088	1.972	+ 88.60
22	ICGV-97228	0.332	0.594	+ 78.92			
23	ICGV-94143	0.291	0.410	+ 40.89			
24	ICGV-95412	0.262	0.452	+ 72.51			
25	ICGV-96305	0.370	0.290	- 21.62			
26	ICGV-97229	0.659	0.445	- 32.47			
27	ICGV-93277	0.410	0.291	- 28.95			
28	ICGV-96295	0.366	0.681	+ 86.07			
29	ICGV-95364	0.350	0.413	+ 18.00			
30	6635	0.474	0.615	+ 29.75			
31	ICGV-86707	0.280	0.313	+ 11.79			
32	ICGV-95414	0.421	0.489	+ 16.15			
33	ICGV-94138	0.354	0.569	+ 60.74			
34	DG-2	0.500	0.665	+ 33.00			
35	ICGV-92120	0.374	0.519	+ 38.81			
36	ICGS-76	0.343	0.451	+ 31.49			
37	ICGV-44143	0.418	0.279	- 33.25			
38	ICGV-89259	0.318	0.367	+ 15.41			
39	BINA-Chinabadam-3	0.204	0.218	+ 6.86			
40	BINA-Chinabadam-2	0.179	0.340	+ 89.94			
	Mean	0.41	0.43		1.01	0.88	

Drought induced variation of total sugar

Data on the accumulation of total sugar in mature and premature groundnut seed grown under irrigated and non-irrigated conditions are shown in Table 2. The amount of total sugar in mature seeds, produced in irrigated condition, varied from 3.01% to 9.37% with an average value of 5.08%, where as, those in drought condition observed variation was from 2.86% to 11.04% with a mean value of 5.68%. Drought did cause increment (1.88% to 114.10%) of total sugar in thirty genotypes. However, in remaining ten genotypes, drought rather caused reduction of total sugar. Premature seeds accumulated lower amount of total sugar. In control it varied from 2.53% to 5.43% (mean 4.01%) and in drought condition, the variation was from 2.76% to 5.22% (mean-4.23%). As in mature seeds, drought caused elevation (2.40% to 54.44%) of total sugar in fifteen genotypes and reduction in the remaining. In premature seeds, the pace of carbon metabolism rate is lower than that in mature seed. This might have caused decline in total sugar content in premature seed when compared with the mature seed. The concentration of fructan and other soluble carbohydrates decreased in response to drought (Volaire and Gandoin, 1996). Karsten and MacAdam (2001) reported that simple sugar concentration was significantly higher in water stressed grasses in comparison with well watered. Venkateswarlu and Ramesh (1993) observed that total sugar increased in groundnut with water stress increased. The findings of the present study appear to come in line with those of Karsten and MacAdam (2001), and Venkateswarlu and Ramesh (1993), and show opposite trend with Volaire and Gandoin (1996). Sharma *et al.* (1985) observed that PEG induced water stress decreased non-reducing sugar and increased reducing sugar in groundnut seedlings. The decrease of total sugar may be attributed to the fact that sugar might be hydrolyzed towards osmotic adjustment in response to drought, or sugar formation might have impaired due to severe water stress. Degree of water stress, age and varietal difference might have caused discrepancy in sugar accumulation. A wide difference in the drought endurance capability of different genotypes might have resulted positive or negative response in some genotypes. Increment of total sugar often used as a tool for screening purpose. On imposing water stress, mature seeds of genotypes ICGV-92120, ICGV-94143, ICGV-96295, ICGV-95399 and ICGV-97228 achieved highest increment of total sugar (Table 2) and hence considered comparatively drought tolerant.

Table 2. Effect of water stress on the total sugar content in premature and mature defatted groundnut seed

Sl. No.	Genotype	Total sugar, g./100g defatted seed (mature)		%Increase / Decrease over control	Total sugar, g. / 100g defatted seed (premature)		% Increase /Decrease over control
		Control	Drought		Control	Drought	
1.	ICGV-92121	5.64	5.49	- 2.55	5.28	5.00	- 5.41
2.	ICGV-95377	6.20	3.16	- 63.96	4.30	3.83	- 10.82
3.	ICGV-93260	4.84	6.03	+ 24.69	3.10	2.99	- 3.25
4.	ICGV-87846	4.64	5.70	+ 22.63	3.83	4.48	+ 17.2
5.	3203	4.69	2.86	- 38.85	2.53	3.17	+ 25.22
6.	ICGV-97190	4.06	5.33	+ 31.14	3.99	4.38	+ 9.76
7.	ICGV-88388	5.85	5.90	+ 0.82	4.70	4.48	- 4.93
8.	ICGV-93232	8.06	5.00	- 37.99	3.74	4.54	+ 21.44
9.	ICGV-94165	4.25	5.40	+ 27.13	4.20	4.82	+ 14.80
10.	ICGV-192116	4.44	5.36	+ 20.56	4.02	4.11	+ 2.40
11.	ICGV-97182	4.05	5.22	+ 28.89	3.92	4.75	+ 21.19
12.	ICGV-95399	4.17	6.37	+ 52.87	4.11	5.22	+ 26.86
13.	ICGV-96318	4.39	5.06	+ 15.37	4.26	4.44	+ 4.22
14.	ICGV-95390	3.95	5.66	+ 43.20	3.87	4.50	+ 16.19
15.	4595	3.71	5.03	+ 35.60	3.37	4.09	+ 21.24
16.	9276	6.42	8.68	+ 35.17	3.38	3.87	+ 14.47
17.	ICGV-92118	5.60	6.17	+ 10.13	4.42	4.01	- 9.33
18.	ICGV-92126	3.75	4.52	+ 20.52	4.06	4.27	+ 54.44
19.	ICGS-1955	5.73	6.41	+ 11.79	5.43	4.45	+ 17.93
20.	ICGV-96294	5.19	6.05	+ 16.43	3.68	4.54	+ 23.55
21.	ICGV-93255	4.52	5.61	+ 24.02	3.99	2.76	- 30.84
22.	ICGV-97228	4.11	6.03	+ 46.80			
23.	ICGV-94143	3.01	6.08	+102.23			
24.	ICGV-95412	5.27	6.02	+ 14.27			
25.	ICGV-96305	5.78	6.44	+ 11.48			
26.	ICGV-97229	5.53	3.09	- 44.06			
27.	ICGV-93277	4.13	5.37	+ 29.96			
28.	ICGV-96295	5.98	11.04	+ 84.73			
29.	ICGV-95364	5.09	5.19	+ 1.88			
30.	6635	9.37	7.51	- 19.82			
31.	ICGV-86707	5.81	5.96	+ 2.58			
32.	ICGV-95414	4.82	5.82	+ 20.70			
33.	ICGV-94138	3.92	5.22	+ 33.19			
34.	DG-2	6.25	7.17	+ 14.72			
35.	ICGV-92120	4.37	9.36	+114.10			
36.	ICGS-76	4.53	5.22	+ 15.30			
37.	ICGV-44143	5.57	3.64	- 34.61			
38.	ICGV-89259	5.22	5.09	- 2.64			
39.	BINA-Chinabadam-3	3.22	3.22	- 0.19			
40.	BINA-Chinabadam-2	6.83	4.31	- 36.84			
	Mean	5.08	5.68		4.01	4.23	

Conclusion

Genotype ICGV-97228 showed an optimum level of drought induced increment of total sugar as well as totals free amino acid in mature seed and provisionally accepted as drought tolerant groundnut genotype. However, the results on immature seeds did not create any positive impact on selection.

References

Chu, T. M.; Aspinall, D. and Palleg, L. G. 1974. Stress metabolism. VI- Temperature stress and the accumulation of proline in barley and radish. *Australian Journal of Plant Physiology,* 1:87-97.

Chu, T. M.; Aspinall, D. and Palleg, L. G. 1976. Stress metabolism. VII- Salinity and proline accumulation in barley. *Australian Journal of Plant Physiology,* 3:219-228.

Hanson, A.D. and Hitz, W.D. 1982. Metabolic responses to water stress. *Annual Review of Plant Physiology,* 33:163-303.

Holbrook, C. C.; Wilson, D. M.; Rucker, K. S.; Kvien, C. K. and Hook, J. E. 1994. Possible role drought tolerance in reducing aflatoxin contamination of groundnut. Agronomy Abstract, pp. 223.

Karsten, H. D. and MacAdam, J. W. 2001. Effect of drought on growth carbohydrates and soil water used by Perennial Rye grass, Tall Fescue, and white clover, *Crop Sci.* 41:156-166.

Madhusudhan, K. V.; Giridarakumar, S.; Ranganayakulu, G. S.; Reddy, P. C. and Sudhakar, C. 2002. Effect of water stress on some physiological responses in two groundnut (*Arachis hypogaea* L.) Cultivars with contrasting drought. *J. Plant Biol.,* 29 (**2**): 199-202.

Moore, S. and Stein, W. H. 1948. Methods in Enzymolozy. Academic Press, New York. 3. 468p.

Navari-Izzo, F.; Quartacci, M. F. and Izzo, R. 1990. Water-stress induced changes in protein and free amino acids in field-grown maize and sunflower. *Plant Physiology and Biochemistry*, 28: 531-537.

Rucker, K. S.; Kvien, C. K.; Holbrook, C. C. and Wood, J. E. 1995. Identification of groundnut genotypes with improved drought avoidance traits. *Groundnut Science,* 22:14-18.

Sharma, K. and Singh, G. 1985. Seedling growth and Sugar metabolism of groundnut under polyethylene glycol induced water stress. *Indian J. Ecol.*, 12 (**2**): 252-256.

Singh, T. N.; Palleg, L. G. and Aspinall, D. 1973. Stress metabolism. I-Nitrogen metabolism and grown in the barley plant during water stress. *Australian Journal of Biological Science,* 26:45-56.

Skriver, K. and Mundy, J. 1990. Gene expression in response to abscisic to abscisic acid and osmotic stress. *Plant Cell,* 2:503-512.

Tayeb, M. A. and Hassanein, A. M. 2000. Germination, Seedling growth, some organic solutes and peroxidase expression of different *Vicia faba* lines as influenced by water stress. *Acta-Agronnomic-Hungarica,* 48 (**1**): 11-20.

Venkateswarlu, B. and Ramesh, K 1993. Cell membrane stability and biochemical response of cultured cells of groundnut under polyethylene glycol induced water stress. *Plant-science Limerick,* 90 (**2**): 179-185.

Volarire, F. and J. M. Gandoin. 1996. The effect of age of the sward on the relationship between water-soluble carbohydrate accumulation and drought survival in two contrasted populations of cocksfoot (*Dactylis glomerata* L.). *Grass Forage Sci.,* 51:190-198.