Effect of Water Stress on Composition of Peanut Leaves

M. Ali-Ahmad and S. M. Basha*[1]

ABSTRACT

Water stress was induced in peanut (*Arachis hypogaea* L. cv. Marc 1) plants by withholding water for 5 to 20 d. Leaves from the water-stressed plants were analyzed to determine the effect of water stress on amino acids, sugars, protein content, and polypeptide composition of peanut plants. The results showed that the total protein content of the leaves significantly increased when peanut plants were subjected to water stress for 5 to 20 d as compared to irrigated controls. Analysis of the leaf protein by SDS polyacrylamide gel electrophoresis showed higher levels of polypeptides in stressed leaves compared to the control leaves. Peanut leaves from water-stressed plants also showed higher amounts of free amino acids and soluble sugars as compared to the irrigated plants. Thus, water stress enhanced accumulation of proteins, free amino acids, and soluble sugars in the peanut plants.

Key Words: Amino acids, protein, polypeptides, seedlings, sugars.

Invasion of peanut by *Aspergillus flavus* Link and *A. parasiticus* Speare causes aflatoxin contamination of peanut (*Arachis hypogaea* L.) seed. Preharvest aflatoxin contamination may not occur when peanuts are supplied with adequate moisture during the growing season. However, late season drought stress predisposes peanut to aflatoxin contamination (Cole *et al.* 1982, 1985; Blankenship *et al.*, 1983, 1984; Hill *et al.* 1983; Wilson and Stansell, 1983). Water stress has been reported to induce significant physiological and biochemical changes in peanut (Basha *et al.*, 1986, 1990, 1995; Cole *et al.*, 1988; Dorner *et al.*,1989; Musingo *et al.*, 1989). Riazi *et al.* (1985) observed that water stress induced an increase in concentration of proline and other solutes in growing regions of young barley leaves. According to Venkateswarlu *et al.* (1989), an accumulation of soluble sugar content in water-stressed peanut nodules may lower the solute potential of nodule cells which could maintain their turgidity in water stress condition. An increase in soluble sugar concentration and amino acids was observed in drought-stressed shoots of alfalfa (*Medicago sativa* L.) relative to its control plants (Schubert *et al.*, 1995).

It has been suggested that free amino acid accumulation in response to water stress indicates a dynamic adjustment in plant nitrogen metabolism (Stewart and Larher, 1980; Hanson and Hitz, 1982). Furthermore,

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increased levels of free amino acids in stressed maize (*Zea maize* L.) contributed to osmotic adjustment, which is an important mechanism of drought response (Hanson and Hitz, 1982). The progressive accumulation of amino acids in drought-stressed plants also has been ascribed to osmoregulation, a signal of senescence and an indicator of drought resistance (Aspinall and Paleg, 1981).

Water stress induced significant accumulations of free proline content in leaves of many plant species (Bandurska, 1991). An increase in proline accumulation and RNAase activity was observed in three barley genotypes under water stress (Gniazdowska-Skoczek and Bandurska, 1994), and the accumulation of free proline under water stress conditions is genetically controlled (Hanson *et al.*, 1979; Steward and Hanson, 1980). Gniazdowska-Skoczek and Bandurska (1994) reported that free proline accumulation in barley leaves under water stress conditions depends upon the leaf water content and genetic predisposition of barley to accumulate this amino acid.

Determining the response of peanut to water stress will be useful in understanding the mechanism involved in drought induced aflatoxin contamination of peanut. Once the components resulting from drought stress are identified, they can be used for screening drought-tolerant and susceptible lines. Developing a reliable procedure for screening drought-tolerant and aflatoxin-resistant lines would significantly enhance the existing breeding programs. In the present study, an attempt was made to determine the effect of water stress on protein and polypeptide composition, free amino acids, and soluble sugar content of peanut seedlings.

Materials and Methods

Plant Cultivation and Stress Treatment. Peanut (*A. hypogaea* cv. Marc I) seedlings were grown in vermiculite in a greenhouse at 26-28 C. Seedlings were transferred after 4 wk to a lab bench under 16/8 hr light/dark and 26-28 C with a photon flux density of 125 $\mu M\ m^{-2}\ sec^{-1}$ and subjected to water stress by withholding water for 5 to 20 d. Control plants were irrigated regularly during the 20-d study period. Total protein, free amino acids, soluble sugar content, and polypeptide composition of leaves from stressed and nonstressed seedlings were analyzed from 5-, 10-, 15-, and 20-d stress treatments. All the analysis was carried out in six replications of two independent experiments. Significant differences between stressed and nonstressed treatments from all replications in both experiments were evaluated using the t test (at $P = 0.05$).

Extraction of Total Protein, Free Amino Acids, and Soluble Sugars. Leaves (4 g) were harvested from stressed and control plants and homogenized in 80% ethanol (8 mL) using a Polytron homogenizer and centrifuged for 15 min at 25,000g. The resulting supernatant was analyzed for free amino acids (Yemm and Cocking, 1955) and soluble sugars (Yemm and Willis, 1954), and pellet was used for determining total protein and polypeptide composition. A portion (0.5 g) of the pellet was incubated with 1 M NaOH (5 mL) at 37 C for 60 min and the soluble material was analyzed for total protein (Lowery *et al.*, 1951) using bovine serum albumin as the standard protein.

A second portion (0.5 g) of the pellet was homogenized in 12 mL of imidazol-HCl buffer (170.2 mg imidazol, 1.861 mg EDTA in 50 mL distilled water, pH 6.4) and centrifuged at 20,000g for 25 min. The supernatant was filtered and equal volume of 10% TCA (trichloroacetic acid) was added and refrigerated overnight to precipitate the proteins. Precipitated protein was recovered by centrifugation at 12,000g for 12 min and washed twice with 80% ethanol and once with acetone to remove traces of TCA.

Polyacryamide Gel Electrophoresis. TCA precipitated protein obtained from stressed and nonstressed leaves were boiled in dissociation buffer (100 mL) containing 2% (w/v) SDS, 3% 2-mercaptoethanol, and 1.2% (w/v) Tris. The dissociated protein (100 mg) was subjected to electrophoresis in 12.5% (w/v) acrylamide gel containing 0.1% SDS according to the procedure of Laemmli (1970). The proteins were stained with Coomassie Blue R-250 and destained with 7% acetic acid and 10% ethanol.

Results and Discussion

Total Free Amino Acids. Changes in free amino acid levels from peanut plants subjected to water stress are shown in Fig. 1. Water stress caused a significant ($P < 0.05$) increase in the free amino acid levels of the leaves from peanut plants stressed for 5 d. For example, at 5-d treatment, the control leaves had 0.08% free amino acids while the treatment had 0.12% free amino acids. Likewise, 10-, 15-, and 20-d stress periods also caused a significant ($P < 0.05$) increase in the free amino acid levels of leaves. Comparison of free amino acid levels among the treatments showed that free amino acid buildup reached maximum by 10-d stress period and then remained unchanged at 15- and 20-d stress periods. In the control plants, free amino acid levels increased from 0.08% (5 d) to 0.09% by 20 d while in the water-stressed plants the increases were 0.12 and 0.14%, respectively. These data clearly showed that rapid build up of free amino acids in the leaves is mainly the result of water stress and not due to aging. The observed data are in agreement with the previous studies which showed a marked increase in the amount of free amino acids due to water stress (Stewart and Larher, 1980; Aspinal and

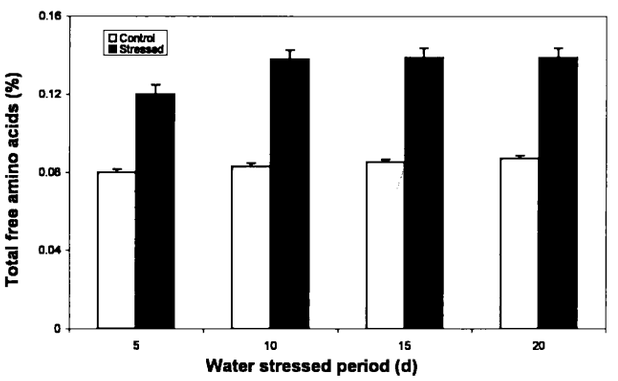

Fig. 1. Effect of water stress on total free amino acids of peanut leaves. Four-week-old peanuts plants were subjected to water stress for 5, 10, 15, and 20 d. Nonstressed plants were watered regularly. Error bars represent standard error of the mean at 5% levels of probability.

Paleg, 1981; Hanson and Hitz, 1982; Schubert *et al.*, 1995). Accumulation of free amino acids in the stressed leaves observed in the present study may be attributed to the lowering of osmotic potential which may play a significant role in osmoregulation.

Soluble Sugars. Figure 2 shows the effect of water stress on soluble sugar content of peanut leaves. After 5 d of water stress, soluble sugar content increased significantly ($P < 0.05$) in the leaves of water-stressed plants (8%) compared to the irrigated control (5%). Likewise, the soluble sugar content of leaves from 10-, 15-, and 20-d water-stressed plants was significantly ($P < 0.05$) higher compared to the irrigated controls. However, the differences between the control and stressed plants appeared to be more pronounced in 10-d stressed plants as compared to the other periods. To assess the effect of aging on soluble sugar levels, comparisons were made of soluble sugar levels at different periods (5 to 20 d). The data showed that in the irrigated control the soluble sugar levels increased from 4 to 5% between 5- to 20-d treatments while in the stressed plants the sugar levels increased from 6 to 9%. This would again indicate that water stress significantly enhanced sugar accumulation compared to aging. A previous study reported accumulation of glucose and sucrose in shoots of alfalfa plants when subjected to drought stress (Schubert *et al.*, 1995). Similarly, Irigoyen *et al.* (1992) reported high accumulation of soluble sugars in drought-stressed nodulated alfalfa plants that may provide nodules an osmotic mechanism to prevent excessive water loss. The increased soluble sugar content of peanut leaves associated with water stress may be part of an adaptive process contributing to osmotic adjustment.

Total Protein. Like free amino acids and soluble sugars, the protein content of the leaves from water-stressed plants was significantly ($P < 0.05$) higher than the irrigated control (Fig. 3). Comparison within each stress period showed that the protein levels were significantly ($P < 0.05$) higher at all stress periods than the controls. For example, protein contents of control plants at 5- and 20-d treatments were 0.4 and 0.45%, respectively, while in the stressed plants it was 0.6 and 0.8%, respectively. In addition, protein increases were higher between control and treatments at 10-, 15-, and 20-d plants as compared to the 5-d treatment. This would indicate that the protein buildup occurred up to 10 d of water stress and then remained unchanged. Increases in both the free amino acids (Fig. 1) and protein (Fig. 3) suggest that under water stress conditions synthetic activity is enhanced to accommodate increased metabolic activity for maintaining the osmotic balance.

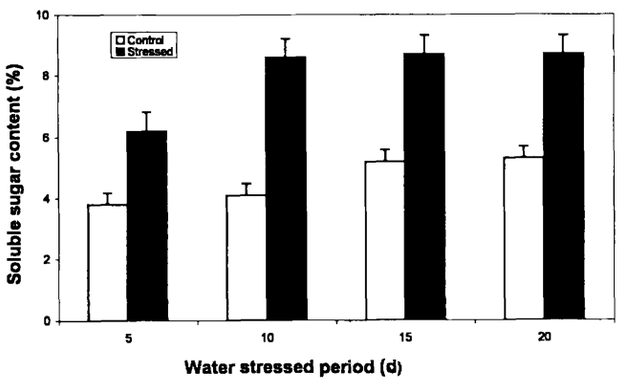

Fig. 2. Effect of water stress on soluble sugar content of peanut leaves. Four-week-old peanuts plants were subjected to water stress for 5, 10, 15, and 20 d. Nonstressed plants were watered regularly. Error bars represent standard error of the mean at P = 0.05.

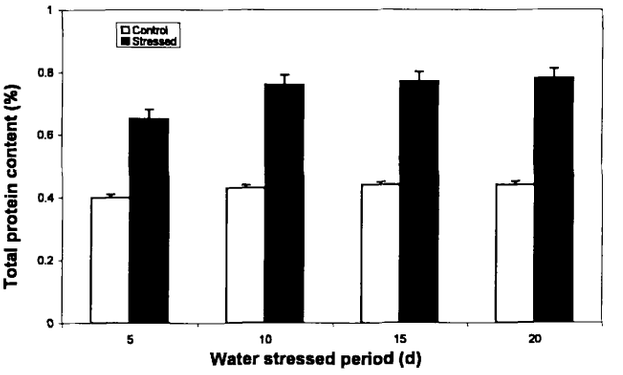

Fig. 3. Effect of water stress on total protein and content of peanut leaves. Four-week-old peanuts plants were subjected to water stress for 5, 10, 15, and 20. Nonstressed plants were watered regularly. Error bars represent standard error of the mean at P = 0.05.

Polypeptide Composition. To determine the differences in the protein composition between water-stressed and nonstressed plants, leaf samples were subjected to polyacrylamide gel electrophoresis under denaturing conditions. The SDS-PAGE resolved peanut leaf proteins into several polypeptides with molecular weights between 14,000 and 70,000 Da (Fig. 4). As seen in the Fig. 4, the protein bands (molecular weight around 70 kDa) in the 5-d water-stressed plant samples (S) were darker than the ones from the control (C), indicating stressed leaves contained more protein than the control leaves. A similar trend was observed in plants stressed for 10, 15, and 20 d, indicating that the water-stressed samples generally contained more protein than the controls. This is consistent with the total protein data (Fig. 3) which showed increases in leaf protein content due to water stress. Comparison of polypeptide profiles among the treatments showed that, except for quantitative differences, no major variations were observed in the polypeptide composition between the water-stressed and control plants.

Conclusions

The observed quantitative increases in protein, free amino acids, and soluble sugars of peanut leaves due to water stress suggest that, like other plants the peanut plant also adapts to water stress by altering synthesis of proteins, amino acids, and sugars to maintain its osmotic balance. The rate and levels of accumulation of these metabolites may determine the ability of a genotype to withstand the level of water stress. Studies are in progress

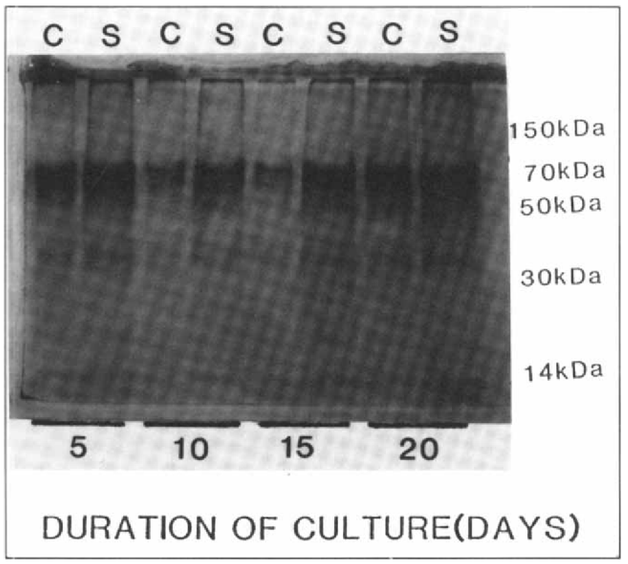

Fig. 4. SDS Polyacrylamide gel electrophoretic pattern of peanut leaves from water-stressed (5, 10, 15, and 20 d) and irrigated control plants. Leaf protein (100 mg) was extracted, dissociated into polypeptides, resolved on 12.5% SDS slab gel, and visualized by staining with Coomassie Blue R-250. C = control, S = water stressed.

to measure differences in the response of drought-tolerant and susceptible genotypes when exposed to various stress levels for identifying the plant components enhancing the drought tolerant characteristics of a genotype. In addition, studies are being conducted also to establish an *in vitro* cell culture system for studying the response of peanut cells to water stress, and to identify the stress metabolites involved in drought-tolerance and aflatoxin-resistance characteristics.

Acknowledgments

This research was supported by the USDA/CSREES, project nos. FLAX 9302654, 9602817 and 97004.

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Accepted 29 April 1998