THE INFLUENCE OF INADEQUATE WATER SUPPLY ON METABOLISM IN BIOLOGICAL SYSTEMS

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ABSTRACT

THE INFLUENCE OF INADEQUATE WATER SUPPLY ON METABOLISM IN BIOLOGICAL SYSTEMS WITH EMPHASIS ON PROTEIN SYNTHESIS AND NUCLEIC ACID METABOLISM

Data have been obtained that show the effect of drought on growth itself and how this reduction in growth may be a result of specific changes in total protein production, nucleic acid metabolism and on functional activity of a fraction of nucleic acids. While the drought treatments decreased total protein by only 40 percent, growth was reduced 80 percent. These data suggested that the synthesis of growth-dependent proteins was being hindered. Although total nucleic acid production was not reduced by the lack of water, the function of the nucleic acid fraction responsible for delivering the genetic information to the process of protein synthesis was altered. This fraction of nucleic acid was not, under water-stress conditions, getting attached to ribosomes. This malfunction prevented the synthesis of a proline-rich protein which is probably required for cell wall production during growth. This information provides a specific selection criterion and should aid in the development of plants and perhaps other organisms that can withstand stress from drought.

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PROJECT SUMMARY

The goal of these studies was to determine what basic processes or biochemical reactions are susceptible to stress imposed by reducing the supply of water. This information would be used in the development of drought-resistant strains or management practices to alleviate water stress. The proposed research organism was plants but extrapolation of the findings to other biological materials would be meaningful at this subcellular level. The objectives of the research proposal included an adaptation of the research results to screening procedures. All of these goals and objectives have been obtained.

The initial experiments were designed to survey the effects of various degrees of water stress on certain chemical identities that may reflect the response of metabolic processes. These studies revealed that when stress, as a result of reduced water supply, decreased protein content by 40 percent, growth as measured by dry weight was reduced by 80 percent. The effect of water stress on other constituents, such as total amino acids and nucleotides, was similar to that on dry weight and therefore reflected no specific response to drought. However, total ribonucleic acid (RNA) increased in seedlings subjected to drought conditions.

The RNA that accumulated in water stressed plants was characterized chemically to determine why it was not functional in protein synthesis. Extensive studies showed that the nucleotide composition of this RNA had not been altered by water stress. However, the physical characteristics had been affected. The individual components of RNA were markedly smaller in drought treated than in control plants. The reduced size resulted because of the failure of ribosomal RNA to become attached to messenger RNA. Therefore, information required from the genetic material to direct the synthesis of specific proteins was not being delivered to the ribosomes. The lack of attachment can be used as a selection criterion to screen for plants that are resistant to drought.

The amino acid proline accumulated in plants subjected to water stress treatments. Isotope incorporation studies showed that this accumulation was a result of the

lack of synthesis of a proline-rich protein. Together these results support the conclusion that drought altered the function of messenger RNA in plants and this malfunction prevents the synthesis of a cell-wall-type protein.

Publications that have resulted from this project thus far are:

West, S. H., 1966, How Water Affects Plant Life. Weeds, Trees and Turf Magazine, p. 12-14.

West, S. H., 1966, Sub-cellular Physiology as Affected by Drought. Proc. X International Grassland Congress, Helsinki, Finland, p. 91-94.

Shiralipour, Aziz and S. H. West, 1968, Effects of Water Stress on Amino Acids, Protein and DNA Content of Corn Shoot at Different Ages. Soil and Crop Sci. Soc. of Fla. Proc. Vol. 28, p. 115-122.

Shiralipour, Aziz and S. H. West, 1970, Specific Protein Synthesis Altered by Water Stress. In preparation.

INTRODUCTION

Throughout the worlddrought conditions reduce the growth of plants and often contribute significantly to stand failure. Slight improvements in adaptation to these stress conditions have been made, but progress is slow. The time required for the development of drought resistant plants is prohibitive because of the selection criteria available. Selection is usually based upon survival. Often failure to survive cannot be attributed to one factor of the environment. Furthermore, the environment is variable from year to year and selections that are adapted to an environmental factor that predominated in one season may be lost in another season. A response to the environment at the molecular level is needed if a specific effect is to be resolved.

The purpose of these studies was to determine what basic processes are affected by the stress imposed by reducing the supply of water. This information would be used in the development of drought-resistant strains or management practices to alleviate water stress.

INITIAL RESEARCH PLAN AND RESULTS

The data reported here are from experiments designed to survey the effect of various degrees of water stress on certain chemical identities that may reflect the response of metabolic processes. The quantitative changes of endosperm-scutellum and seedling protein, nucleotides, and RNA during germination and growth under water stress have been measured. The qualitative changes in seedling nucleotides and RNA were determined by ion-exchange chromatography and spectral analyses.

The design of these experiments was such that the effect of a sub-optimal supply of water to plant roots could be observed without the usual complicating factors of loss of turgor and reduced ion absorption. The atmosphere of the growth chamber was maintained near 100 percent relative humidity so that wilting did not occur, and only nutritive ions from the seed were available to all treatments. The quantity of mannitol that moved into the seedlings was not measured. The effect of mannitol on growth is assumed to be only a result of a decreased supply of water.

Reducing available water by increasing the osmotic pressure of the medium surrounding the seeds resulted in a marked reduction in growth rate of the seedlings as shown by the dry- and fresh-weight. The dry-weight increases of the seedlings grown without water stress were similar to those reported elsewhere and after 6 days of incubation were approximately three times as great as the increase of the seedlings subjected to the greatest water deficiency (14.7 atm of osmotic pressure) for the same period. The increase in dry weight of the seedlings occurred at the expense of the endosperm-scutellum dry weight. Treatments that limited water entry and plant growth also reduced the dry-weight loss in the endosperm-scutellum.

Protein accumulated in the seedling during 1 to 6 days of germination and growth at a rate similar to the dry-weight increases. Also like the dry-weight changes, a decrease in endosperm-scutellum protein accompanied the increase in seedling protein. Restricting the water supply slowed protein degradation in the endosperm-scutellum and the formation of protein in the seedling.

Nucleotides increased in seedlings and endosperm-scutella during incubation. The magnitude of increase was greater in the seedlings. The quantity of nucleotides per plant was reduced by water stress, and the level per gram of dry weight was reduced initially. However, the initial

decrease was followed by an accumulation to a level higher than the check treatment. With increasing water stress, more time was required for the accumulation. Throughout the 6 days of growth the nucleotides in the check seedlings remained constant. A disruption in normal nucleotide metabolism as indicated by lower nucleotide content would be expected to adversely affect RNA formation.

The RNA in the seedlings increased during the 6 days of growth and the endosperm-scutellum RNA decreased. The rapid degradation of endosperm-scutellum RNA that occurred during the first 3 days of germination in the check plants was slowed by the water stress. Unlike seedling protein and nucleotides, seedling RNA increased when water was limited.

CHARACTERIZATION OF RNA ACCUMULATED IN DROUGHT

The initial or survey studies showed that growth, amino acids, protein, and nucleotide contents decreased with imposed water-stress treatments, but RNA increased. The following studies were designed to determine what fractions of RNA were accumulating and to characterize that RNA. Protein is an important constituent of the enzymes which catalyze the thousands of chemical reactions required for the growth of a plant. Protein synthesis is dependent upon ribonucleic acid in ribosomes, messenger (mRNA) and transfer RNAs. Ribosomes form the site upon which amino acids are joined. Transfer RNA brings the amino acids to the ribosomes. Finally, mRNA is the vehicle by which the genetic information in the nucleus is conveyed to the ribosomes where this information directs the polymerization of the various proteins.

Zea mays L. seedlings were grown in darkness in a constant temperature of 80°F and 95 percent relative humidity. The test material consisted of one-centimeter sections harvested from the stem immediately above and below the first node of 6-day-old plants. The treatments were: (1) plants grown for 6 days with a continuous supply of water to the roots, (2) similar to treatment (1), except that the roots were supplied with a solution containing sufficient mannitol to provide 9.8 atmospheres of osmotic pressure, (3) plants grown in the mannitol treatment for 4 days and then transferred to the water supply for 2 days.

These experiments showed that drought conditions reduced fresh weight to approximately one half that of the control plants, but when the stress was released, growth

was resumed at a rapid rate. Total cytoplasmic RNA increased per section and per cell. A large part of the increase was attributed to an increase in ribosomes. The level of RNA was not significantly decreased in the stress-release plants. These data should be compared to those that have shown a positive correlation between RNA content and the capacity to make protein. Furthermore, the level or content of ribosomes have been shown in other studies to regulate the rate of protein synthesis. Our data show that drought-stressed plants synthesized more ribosomes upon which protein could be made, yet growth was reduced.

The author surveyed some of the characteristics of the ribosomes that accumulated in plants subjected to drought conditions to determine why they were not functional in the period of slow growth. Conceivably the composition of nucleotide bases in the RNA may reflect changes sufficient to render the ribosomal units non-functional in protein synthesis. To check this feature, ribosomes were isolated from the control and water-stress plants, the RNA was hydrolyzed, and chromatographed on a Dowex-1 column. These experiments showed that the quantities of each of the 4 nucleotides were similar in plants from each treatment. In other tests, P^{32} was added to the incubation solution and the amounts of the isotope occurring in each nucleotide showed also that the base composition was not altered by the water-stress treatment. These data show: (1) that the ribosomes which accumulated in the water-stress condition were not abnormal in base composition, (2) that there was no preferential synthesis of nucleotides, and (3) that there was no preferential incorporation of nucleotides into RNA.

The above studies indicated that the single ribosome unit was not altered by the drought condition and therefore, would not account for the effect on growth. In previous studies the number of single 70s ribosomes that clustered together into aggregates appear to be related to the rate of protein formation. The ratios of the amounts of RNA occurring in fractions containing particle sizes 70s, 70s x 2, 70s x 3, and those greater than 70s x 3 show a positive correlation between ribosome cluster size and growth rate. Slow growth of plants under drought stress was associated with a high level of single 70s particles. The plants in the control treatments contained a higher level of clusters larger than single 70s particles than that of the stress plants. The rapid rate of growth in the drought-released plants resulted in levels of clusters markedly higher than that of the control plants. Electron-microscope studies using permanganate fixing and platinum shadowing revealed more large clusters in the grid fields in fast growing plants than in those growing under stress.

Clusters of ribosomes may be formed by the attachment of messenger RNA (mRNA) to individual ribosomes as visualized in various reports. If this is a necessary step in the process of protein synthesis, measurements of clusters are some measure of amounts and participation of the mRNA in growth. In addition, mRNA has been described as a rapidly P^{32} labelled fraction of RNA, in contrast to ribosomes. Therefore, providing test plants with the isotope for a brief period and then isolating the RNA fractions with their associated counts is still another way of correlating mRNA with growth and with polysome formation. Representative data from these experiments are shown in Table 5. These data show that the RNA fractions that contain more than one ribosome contain high levels of P^{32} and suggest that growth is indeed associated with polysome formation.

These data further indicate that stress from drought adversely affects mRNA. This information provides a specific selection criterion and should aid in the development of strains of herbage plants that can withstand stress from drought. Plant materials can now be screened and selections made on the basis of susceptibility of mRNA to water-stress. In addition, methods of inducing resistance to drought can now be accurately followed.

PROTEIN SYNTHESIS ALTERED BY DROUGHT

The regulation of growth in biological systems is considered an expression of highly organized and ordered protein synthesis. Drought, in most plants, invariably reduces growth. This reduction in growth appears to be partially associated with an alteration of protein metabolism (Barnett and Naylor 1966, Ben-Zione et al 1967, Henckel 1965, Shah and Loomis 1965, and West 1962). The development of drought resistance in plants might be facilitated if the mechanism of growth regulation were by a specific protein and if this mechanism were elucidated. The previous work on this project showed that the apparatus for polymerizing proteins was altered by water stress. Since the altered portion was that involved with the direction of various proteins, our research was aimed at determining if water-stress affected specific proteins.

Research reported earlier by our laboratory and by others has shown that drought stressed plants have a high level of the amino acid proline. The research reported here was designed to determine if the accumulation of the amino acid proline during drought is a result of protein degradation or of the reduced synthesis of a specific proline-rich protein.

Drought treatments resulted in a 58 percent reduction in fresh weight per shoot and 40 percent in dry weight as compared with the control treatment. The reduction for fresh and dry weight of the root and shoot was 58 percent and 30 percent respectively. The plants grown under control conditions for 1 day after 4 days of drought made a marked recovery in both fresh and dry weights. All compounds were compared on a per cell (mg DNA) (Ingle et al 1964) basis. Drought affected weights and protein content of roots and shoots similarly. Protein content per cell was not changed by the treatments; however, drought reduced the total protein per seedling.

Total free amino acids in the shoots and roots of the seedlings grown under drought conditions were not significantly increased as compared with controls. In the 24 hours after release from drought treatment, the change in total amino acids was negligible. While proline, phenylalanine, glutamine and asparagine increased in the shoots of the drought treated seedlings, the increase in proline was greater than that for any other amino acid. Many workers (Barnett and Naylor 1966, Chen et al 1964, Kemble and Macpherson 1954, Mathes 1956, Saunier 1968, Steward et al 1966) have reported increases in free amino acids under drought conditions. In one case free proline increased 10 to 100 fold (Barnett and Naylor 1966). The increase in amino acids has been attributed to a degradation of protein (Kemble and Macpherson 1954, Mathes 1956). However, our results which show no significant decrease in protein, or increase in total amino acids tend to refute this suggestion. The lack of decrease in protein in drought treated plants does not preclude the possibility that some protein was being degraded while others were being formed.

Instead of degradation of protein we propose that the accumulation of certain amino acids, especially proline, in the drought treated seedlings was partially, if not completely caused by their continued synthesis or transfer from seed to shoot and root with a reduction in the rate of incorporation into protein. This is supported by our observation that ^{14}C labeled proline, an amino acid which consistently accumulated in drought conditions was incorporated into cytoplasmic protein of shoot at only about one-third the rate in drought treated plants as in the controls. In a similar comparison of treatments in roots, the proline-^{14}C incorporated into the cytoplasmic proteins of drought treated plants was about one-half that in control plants. Furthermore, plants that had been in drought conditions for 4 days incorporated more proline-^{14}C when released from drought for 1 day than did the plants in continuous control treatments. These data further demonstrate that some protein synthesis was inhibited during

drought conditions and was rapidly reinitiated when drought stress was released, and that these proteins are especially rich in proline. The reduction in the rate of incorporation of ^{14}C-proline in stressed seedlings can not be attributed to isotopic dilution, since maximum specific activities of proline pools in control and stressed shoots and roots are very similar.

In contrast to proline, serine did not accumulate under drought conditions and its incorporation into protein was neither reduced by drought nor appreciably increased after the plants were released from drought.

From the data it is possible to propose that plant cells under drought stress actively accumulate proline not only because of lack of incorporation into protein, but also for other, as yet undetermined, requirements of the stressed plants. The total uptake of proline-^{14}C by the cells of shoots from plants under drought conditions was about 3 times greater than in the control treated plants and the uptake in the drought treated roots was about 2 times that in the control roots. The level of proline in the shoots was reduced when water was supplied to the drought treated plants. Again, in contrast to proline, the total uptake of serine-^{14}C by the roots and shoots of drought stressed plants was less than by control plants (comparison to the control treatment). Plants under drought conditions may preferentially synthesize proline and this may also contribute to the accumulation of that amino acid.

Results from studies on the effect of drought on nucleic acid metabolism are consistent with findings concerning accumulation of certain amino acids and lack of incorporation into protein discussed above. Marcus and Feeley (1965) showed a low level of amino acid incorporation into protein in the cotyledon of the unimbibed peanut seed. After water was added, imbibed seeds incorporated the amino acids at a much higher rate. Furthermore, they showed that the unimbibed seed possessed the entire apparatus for protein synthesis except messenger RNA was not adequate or was in an inactive form. Polysomes were formed and protein synthesis was resumed upon addition of water in the cotyledons and embry (Marcus and Feeley 1965). West (1966) attributed a reduced polysome formation under water stress conditions to a possible messenger RNA malfunction. In our experiments the addition of water caused rapid incorporation of proline-^{14}C into protein, possibly due to the formation of activation of specific messenger RNA and eventually polysome formation.

The partial inhibition of proline incorporation into protein by water stress appears to be completely reversible through the addition of water and the treatment does not affect the genetic apparatus since the plant recovers. The amounts of proline-^{14}C and serline-^{14}C incorporated into protein are inverse to their quantities as free amino acids.

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