SOME EFFECTS OF WATER STRESS ON RIBONUCLEIC ACID AND PROTEIN COMPONENTS OF *TRITICUM AESTIVUM* L.

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in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
May, 1967

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ACKNOWLEDGEMENTS

The author expresses gratitude and appreciation to the members of his graduate committee, Dr. Glenn W. Todd, Dr. Eddie Basler, Dr. John Thomas, Dr. Gene Guinn and Dr. L. Herbert Bruneau, for aid in correcting the manuscript.

Indebtedness is also acknowledged to:

Dr. Walter W. Hansen for his co-operation as Head of the Department of Botany and Plant Pathology in planning and carrying out this research.

Dr. Gene Guinn and Dr. Eddie Basler for valuable suggestions and technical assistance.

Dr. Glenn W. Todd for his greatly appreciated assistance in planning and aiding in obtaining financial assistance and equipment for carrying out the thesis research and for his assistance while on sabatical leave of absence as a Fulbright lecturer.

The National Science Foundation for summer support through the Research Participation Program for College Teachers, that made it possible to carry out a portion of the research.

Mrs. Lillie Stutte, who patiently and energetically assisted in many ways during the course of this work.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table | Page

LIST OF FIGURES

Figure Page

CHAPTER I

GENERAL INTRODUCTION

One of the most important aspects of a plant is the internal water balance or degree of water stress because this controls the physiological and biochemical processes and conditions which determine the quantity and quality of plant growth.

Various methods for determining water stress have been reviewed by Kramer and Brix (1965) and Levitt (1965). They reviewed the measurement of drought resistance and discussed some problems involved in using only one method of determining drought resistance in all types of plants.

Asana (1961) considered drought resistance in crop plants under two separate categories. From review of the results of his tests it appears that none of them proved consistently successful in predicting field performance of different varieties of crop plants. Levitt, Sullivan and Krull (1960) have proposed that adaptation to dry climate is due to (a) ability to stay alive and (b) ability to grow and develop.

Water stress in plants influences such processes as water uptake, root pressure, seed germination, stomatal closure, transpiration, photosynthesis, respiration, enzymatic activity, growth of shoots and roots, shrinkage of tissues, mineral relations, etc.

The purpose of this study was to investigate the relation between the protein and ribonucleic acid components of wheat plants and their

tolerance to water stress in an effort to determine a basis for resistance to water stress which may be used to provide an index for breeding for drought resistance.

This investigation is presented in two parts. Part A consists of the effects of water stress on ribonucleic acid components and Part B consists of the effects of water stress on protein components. A separate literature review will be presented for each part of this study.

PART A

EFFECTS OF WATER STRESS ON RIBONUCLEIC ACID COMPONENTS

CHAPTER II

REVIEW OF LITERATURE

Functions of RNA

Reviews by Hoagland (1960), Berg (1961), Ts'o (1962), and Moldave (1965), have led to a clearly defined hypothesis for the function of RNA in the intermediate reactions of protein synthesis. An outline of this hypothesis is as follows:

Individual amino acids are activated in the presence of ATP, magnesium ions, and specific enzymes (aminoacyl soluble RNA synthesis), to form enzyme-bound aminoacyl adenylates and inorganic pyrophosphate. The carboxyl group of the amino acid is linked to the phosphate group of the enzyme-bound complex and is transferred to amino acid-specific acceptor-sRNA's where it is attached by an ester linkage to the ribose portion of the terminal adenosine residue. The amino acid-binding end of sRNA consists of a cytidylate-cytidylate-adenylate (CCA) sequence. Aminoacyl sRNA's react with template-bound ribosomes (polyribosomes) at specific positions determined by the nucleotide sequence of the template (or messenger) RNA. The aminoacyl sRNA's are specifically aligned in relation to the growing peptide chain, the C-terminal end of which is linked to sRNA. Nucleophilic attack by the α-amino group of the incoming aminoacyl sRNA on the carboxyl carbon atom of peptidyl sRNA results in the formation of a new peptide bond and release of the sRNA that was previously attached to the end of the growing chain. The polypeptide chain is linked through the new amino

acid to its corresponding sRNA. Polyribosomes-bound aminoacyl sRNA and peptidyl sRNA are therefore intermediates in protein synthesis. The incorporation of the aminoacyl moiety of aminoacyl sRNA into the growing ribosome-bound peptide chain requires GTP, glutathione, and at least two enzymes. The messenger and the ribosome then move one coding unit over in relation to each other. A new nucleotide sequence in messenger RNA is thus placed in position to base-pair properly with the next aminoacyl sRNA. As this process is repeated, the polypeptide chain grows from its N-terminal to its C-terminal residue by the sequential addition of aminoacyl sRNA's. The sequence of amino acids is determined by the nucleotide sequence of messenger RNA; the latter is determined by the deoxynucleotide sequence in DNA, which is transcribed in the form of a complementary polyribonucleotide chain, the messenger. Subsequently, the ribosome moves far enough along the messenger molecule to allow a new ribosome to attach. A second identical peptide chain can now be initiated and synthesized in the path of the second ribosome in the same way. Thus, several identical peptide chains can be synthesized simultaneously on a polyribosome complex. Eventually, at the end of the messenger-RNA chain, the ribosome, the completed polypeptide chain, and the terminal, esterified sRNA are released from the polyribosome.

Functions of Ribosomes

In the electron microscope the ribosomes appear as uniform round electron dense particles having an average diameter of 100 to 300 Å. The ribosomes contain 80 to 90 per cent of the total cellular RNA. Some evidence indicates that ribosomes exist in mitochondria,

chloroplasts, and in the nucleus in addition to the cytoplasm. In correlating electron microscopic observations and biochemical studies it has been established that microsomes, which are sedimented by centrifuging the mitochondria-free supernatant fraction tissue homogenate at 100,000 g for an hour or more, are ribosomes still attached to varying amounts of lipoprotein of the endoplasmic reticulum. The ribosomes consist of equal parts of relatively highly polymerized RNA and protein. Both the RNA and protein are composed of subunits of several sizes which are possibly held together by Mg^{++} linkages.

Various types of enzyme activities have been reported associated with the ribosomes. The presence of ribonuclease in ribosomes has been reported by several investigators along with other enzymes such as glucose-6-phosphatase, nucleotide-cytochrome C reductase, peptidase, B-galactosidase and amylase.

Ribosomes are held together in clusters. The electron microscope studies show the individual ribosomes of the clusters of ribosomes or polysomes are fastened together by a nucleic acid strand which is about 10 to 15 Å in diameter. Brief treatment with RNAase releases all the ribosomes as individual particles of sedimentation coefficient 80 S, which is characteristic of single ribosomes.

Bonner (1965) presented evidence to show that ribosomes combine with messenger RNA at chain ends only, while after combination of ribosomes with messenger RNA, he found ribosomes at various positions along the length of the messenger RNA. As synthesis of a peptide chain is finished ribosomes leave the polysome. This indicates that ribosomes read the messenger RNA sequentially and that attachment and release of ribosomes to messenger are associated with initiation and completion of

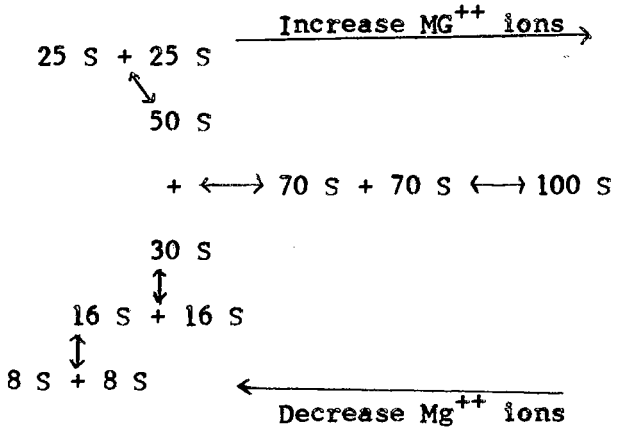
peptide chain growth.

Allen and Schweet (1962) made the observation that ribosomes are very sensitive to RNAase while enzymological release of the labeled protein is not influenced.

The control of protein synthesis by the ribosomes is probably due to inability of the ribosome to proceed to synthesize a new protein molecule unless the previously finished one can be released. Morris and Schweet (1962) reported that both an enzyme and ATP are needed to release labeled protein from reticulocyte ribosomes and that such release is accomplished without apparent degradation of the ribosomes.

Using cultured tobacco cells, Nicolson and Flamm (1965) showed the cells contained two kinds of ribosomes: (1) Those bound to the endoplasmic reticulum requiring deoxycholate for release (bound ribosomes) and (2) those which are readily extracted with Tris buffer (free ribosomes). Both types sediment in the 70 to 80 S range. The bound ribosomes incorporate precursors of both protein and RNA at a significantly more rapid rate <u>in vivo</u> than do the free ribosomes. Deproteinized ribosomal RNA showed that the bound ribosomes are composed of 25, 16 and 8 to 5 S RNA, whereas the free ribosomes contain only 25 S RNA and a heterogeneous fraction sedimenting between 10 and 5 S. The protein/RNA ratio was the same for each type of ribosome.

The effects of Mg^{++} ions on the ribosome complex may be represented by the following: (Davidson, 1965; Spirin, 1964; Nicolson and Flamm, 1965; Ts'o, 1962).



Only the ribosomes bound as polyribosomes are active in protein synthesis in complex with messenger RNA and soluble or transfer RNA. Ribosomes combine with messenger RNA at chain ends only, while after combination of ribosomes with messenger RNA, ribosomes are found at various positions along the length of the messenger molecule. Ribosomes read the messenger RNA sequentially and attachment and release of ribosome to messenger are associated respectively with initiation and completion of peptide chain growth.

Microsomes, which consist of lipoprotein and ribosomes, do not possess the power to reproduce. Bonner (1965) concludes that the RNA of ribosomes is formed by DNA-dependent RNA synthesis on the chromosome. The RNA thus synthesized moves in some way to the nucleolus, where it is complexed with ribosomal protein.

Functions of Soluble RNA

The so-called soluble RNA; (sRNA, adaptor RNA or transfer RNA) accounts for approximately 10% of the total RNA content. Most sources of data give the molecular weight as about 25,000 to 30,000, which corresponds to not more than 80 to 100 nucleotides. In all active molecules of "soluble" RNA, the end nucleotides are arranged in a characteristic sequence, CCA, (Spirin, 1964). Three things distinguish

the sRNA found in cell homogenates fractionated by differential centrifugation: (1) Small size, (2) high content of the 5-ribosyl uracil monophosphate and (3) ability to accept specific amino acids and transfer them under enzymic control to microsomal protein (Smith, 1960). A preparation can be tested for specific biological ability, which is the ability to combine with activated amino acids and transfer these to the ribosomes in the presence of appropriate enzyme systems (Hoagland, 1960).

The soluble fraction of cells contains RNA which is not bound to protein and which precipitates at pH 5. This precipitate includes also the "pH 5 enzymes", among which are the amino acid activating systems (McQuillen, 1962).

Studies have demonstrated that the structural similarities among the sRNA chains go no further than the CCA trinucleotide end group. The secondary structure of sRNA is apparently not involved in the recognition of the activating enzyme since heat treatment sufficient to disorganize the secondary structure does not impair the ability to accept amino acids (Davidson, 1965). There appears to be more than one form of sRNA specific for each amino acid and at least one form of sRNA specific for each amino acid (Von Ehrenstein and Dais, 1963)

The nucleotide sequence adjacent to the CCA end of the RNA chains specific for isoleucine is different from the corresponding sequence in the RNA chains which accept leucine. Indications are also that heterogeneity of nucleotide sequences also occurs among chains specific for a single amino acid. Grunberg-Manago (1963) reports only degradation of a fraction of the chains specific for a particular amino acid by polynucleotide phosphorylase. Mg^{++} seems to have a protective

effect on the secondary structure of sRNA which causes resistance to polynucleotide phosphorylase. From several sources of data, Grunberg-Manago shows the characteristics of sRNA to have a near equivalence in the molar ratio of adenylic to uridylic and guanylic to cytidylic acids.

Transfer of the amino acid is stimulated, as is the incorporation of free amino acids, by the presence of ATP, GTP, and a nondialyzable heat-labile fraction derived from the soluble portion of the tissue extract. By loading the reaction mixture with relatively large quantities of ribosomes and small amounts of amino acyl RNA, almost all of the amino acids can be incorporated into the ribosomes (Berg, 1961).

Functions of Messenger RNA

Messenger RNA (mRNA) is generally found in cells in relatively small amounts not exceeding approximately 5 to 8% of total RNA content. Spirin (1964), reporting works by several investigators, has shown the molecular weight to vary over a wide range from 20,000 to 50,000 to 2×10^6 and even higher. The sedimentation coefficients vary from 4 to 30 Svedberg units with some as high as 45 Svedberg units.

From works done with bacteria it was found that mRNA was a rapidly labeled RNA fraction. It has a base composition reflecting the DNA base composition and possesses long nucleotide sequences complementary to its homologous DNA. It is very heterogeneous with respect to molecular weight which possibly reflects the different sizes of the polypeptide chains to be synthesized. It can be associated under certain conditions with ribosomes, which are sites of protein synthesis.

Turnover rate of mRNA is very high for bacteria, however in higher plants the turnover rate is not as rapid.

The metabolic turnover rate of polyribosomal messenger RNA is more rapid than that of ribosomal RNA. It sediments in gradients between sRNA and ribosomal RNA (Ogata et al., 1963). Its base composition is different from those of the sRNA and ribosomal RNA (Munro and Korner, 1964) and when it is incubated in vitro with appropriate ribosomal systems it stimulates the incorporation of amino acids (Revel and Hiatt, 1964).

Moldave (1965) reviewed studies which suggest that ribosome-bound messenger RNA is resistant to breakdown, and that protection is attributable to the cessation of protein synthesis, thereby preventing the completion of polypeptide chains and release of messenger RNA from the ribosome. The individual ribosomes are fastened together by a nucleic acid strand which is 10 to 15 Å in diameter as determined by the electron microscope. With RNAase treatment the strands were cut, releasing all the ribosomes as individual particles of sedimentation coefficient 80 S, characteristic of single ribosomes of higher organisms.

It is commonly suggested that RNA specifies the sequence of amino acids in the protein formed on a particular ribosome. It has been proposed that mRNA is patterned on the DNA and carries information to a ribosome, enabling it to fabricate a particular protein molecule.

Bonner (1965) presented evidence that a single strand of the double helical DNA of a chromosome is transcribed. The single strand transcription of DNA assures that mRNA will be able to base pair with

molecules of sRNA. It is upon this recognition by base pairing of mRNA by sRNA that the sequencing of amino acids in enzyme molecules is obtained. The liberation of finished protein by the ribosomal system depends on the messenger RNA being intact.

As shown by Hardesty et al. (1963), 80 S ribosomes combine with polysome-labeled mRNA and the attachment requires energy in the form of ATP and it is enzymatic, as determined by a decrease in rate of attachment in the cold. That attachment occurs at the end of the mRNA chain is indicated by the findings of Goodman and Rich (1963) that binding of 80 S ribosomes to polysomes is proportional to the number of mRNA ends rather than to the total length of mRNA. The binding of ribosomes to polysomes is inhibited by the presence of small molecular weight poly A, which presumably acts by being bound to the ribosome, blocking the mRNA attachment site.

From the accumulated data, the indications are that the active cellular component in protein synthesis consists of ribosomal clusters held together by mRNA.

Effects of Senescence on RNA Content

Shah and Loomis (1965) showed that RNA concentration was highest in young leaf blades and lowest in the older leaves of sugar beets. The molar proportion of purine components declined with increasing leaf age, while pyrimidine components increased. Young blades had the highest purine/pyrimidine ratio (1.28) while the old leaves were low (0.90).

Oota and Takata (1959) showed a decline in RNA in aging cotyledons

which was matched by a rise in RNA in the growing points of the plants of which they proposed that the transport of RNA out of aging organs was an integral part of the development of senescence.

"The Microsomal fraction", isolated from avocado fruit in which the mitochondria have lost the ability to carry out oxidative phosphorylation *in vivo* and *in vitro*, is still capable of an ATP-dependent incorporation of labeled amino acids into protein. Exactly similar but more detailed results have been obtained with the microsomal fraction from pea cotyledons in normal and induced senescence (Young et al., 1960). One may conclude that the ribonucleoproteins are still capable of functioning *in vivo* but does not because of the lack of ATP. Such a conclusion is consistent with the known stability of isolated ribonucleoprotein particles, their capacity for dissociation into subunits, and their reassociation to form functional particles (Varner, 1961).

Oota (1964) advanced a hypothesis for the fate of ribosomal RNA in senescing cells. He visualizes a stepwise breakdown of ribosomes during cell senescence as follows: Intact ribosomes ⟶ RNA separated from protein moiety -- further degradation of RNA -- free RNA as small size expelled into soluble cytoplasm (termed "transportable RNA"). "Transportable RNA" is speculated to be transported to actively growing portions or RNA-sinks to be utilized there for the reconstruction of ribosomes. Upon ultra-centrifugation, microsomal RNAs from both hypocotyl and cotyledon of beans are resolved into 24, 17 and 5 S components, whereas supernatant RNAs from hypocotyl and cotyledon yield a sharp 4 S peak and an asymmetrically diffused peak at 4 S,

respectively. "Transportable RNA" appears to consist of 4S or smaller particles.

Senescence in plants may be due to some physiological mechanism which creates a distinct catabolic condition. The decline in anabolic activities seems to be a natural consequence of the deterioration of RNA and protein components as senescence advances (Leopold, 1964).

Effects of Water Stress on RNA Content

The role of ribonucleic acids (RNA) in protein synthesis has prompted investigations into the influence of water stress on RNA metabolism (Gates and Bonner, 1959; Kessler, 1961; West, 1962; Todd and Basler, 1965).

Kessler (1961) found in preliminary work that water deficits impair the nucleic acid system which is intimately connected with protein synthesis.

West (1962) found water stress reduced fresh and dry-weight in corn seedlings and that proteins and nucleotides were quantitatively decreased. He suggested that reduced growth in the stressed seedlings may have been a result of a shift in adenosine triphosphate production to guanosine triphosphate and uridine triphosphate. Ribonucleic acid from seedlings grown under water stress contained a significantly higher ratio of guanosine monophosphate and uridine monophosphate to cytidine monophosphate and adenosine monophosphate (West, 1962).

The harmful effect of drought may be on the enzyme activity due to the effect of water stress on the nucleic acid system which is intimately connected with protein synthesis. Kessler (1961) using

incorporation studies with uracil-C-14 on tomato plants, found that the rate of incorporation into RNA was similar in the water-stressed and control plants, indicating that the RNA synthesis system was not hampered. Kessler (1959) also found that water stress reduced RNA content in sunflowers. Gates and Bonner (1959) concluded that the block to net RNA synthesis was caused by more rapid destruction than synthesis of RNA. They showed that the leaves of moisture-stressed tomato plants possess the ability to incorporate ^{32}P-labeled phosphate into RNA even though they do not exhibit any net synthesis of RNA.

Todd and Basler (1965), working with different varieties of wheat exposed to various levels of water stress, found a decrease in nucleic acid content in various subcellular fractions of leaves and crowns. They postulated that death of the plants would occur whenever the nucleic acid content of any given fraction dropped below a certain critical level and suggested that drought injury is primarily due to a destruction of cellular components by hydrolytic enzymes. The most drought tolerant part of cereal plants showed the least change in nucleic acids as well as protein.

Effect of Water Stress on Purine and Pyrimidine Content of RNA

The possibility that adenine is destroyed in drought has been proposed by Kessler (1961), as has been suggested with high temperature (Highkin, 1957). It has been suggested that in heat-resistant varieties the amount of adenine increases with increase in temperature (Galston et al., 1949). Kessler (1961) also proposed the possibility that adenine somehow stabilizes the RNAase-x (ribosome) complex and limits

its destruction.

Kessler (1961) showed that various purine and pyrimidine bases either promote or inhibit the synthesis of either RNA or DNA or both. Such changes in the nucleic acid metabolism should somehow be reflected in the resistance of plants to drought.

A striking difference was found between the nucleotides of water stressed and check corn seedlings. The water stressed seedlings were high in GTP plus UTP and low in ATP, the check seedlings were high in ADP and extremely low in GDP, whereas the relative quantities of ADP and GDP were equal in the stressed seedlings. Water stress alters markedly the structure of corn seedling RNA by increasing the $\frac{G + U}{A + C}$ ratio (West, 1962).

In adenine-treated tomato plants the RNAase activity remained low while the RNA level remained high, close to that of the control plants. Water stressed plants showed increased RNAase activity with lower amounts of RNA (Kessler, 1961). Using olive leaves Kessler and Frank-Tishel (1962) found an increase in the $\frac{G + C}{A + U}$ ratio with water stress, with the most rapid increase from 0 to 20% water loss.

CHAPTER III

METHODS AND MATERIALS

Preparation of Plant Material

The hard red winter wheat (Triticum aestivum L.) varieties Cheyenne (CI 8885) and Ponca (CI 12128) were planted in 6 inch pots with sand, soil and peat moss mixture (2 : 1 : 1). Cheyenne variety is considered to be drought hardy whereas Ponca is considered to be drought susceptible. They were grown in environmental growth chambers and in an Agronomy Department greenhouse at Oklahoma State University. The plants were allowed to grow for three weeks before a water stress gradient was started.

The controlled environment experiments were carried out using four different controlled environment chamber units with temperature settings at 16° days and 10° nights; 21° days and 16° nights; 27° days and 21° nights; and one with 27° days and 21° nights setting until the beginning of water stress, at which time the setting was changed to 41° during the daytime for the duration of the water stress. The light intensity in each of the units was 22,000 lux at plant level. The light and dark periods were of 12 hours each.

The greenhouse experiments were without light intensity and temperature controls. The temperature ranged from 15° during the nights to maximum of 41° during the growth period. A mean of 18° night and 35° day was recorded for the duration of the experiments.

The degree of water stress was determined by the relative turgidity method of Weatherley (1950). This method has been shown to be an excellent indicator of degree of moisture stress in cereal plants (Todd et al., 1962).

All plants were harvested between 10:00 A.M. and 11:00 A.M. in an effort to standardize possible fluctuations in water content and dry matter. Halevy and Monselise (1963) showed a fluctuation in dry weight during a 24 hour period which causes a change in the water percentage.

At harvest the leaf portions were separated from the crowns on all plants used in the tests. The number of plants and the fresh weights were determined on the plant portions at the time of harvest. The dry weights were determined after the plant portions were oven dried.

At harvest the fresh plant materials were treated in boiling 95% ethyl alcohol for one minute, and dried in an oven at 100° for three hours. The material was then ground in a Wiley mill, using a sixty mesh screen, and the meal stored in a deep freeze until used. All of the test were made from this meal.

Extraction of RNA

Several methods for the extraction of RNA from plant material have been investigated. Some different methods have been presented and reviewed by Ogur and Rosen (1950), Smillie and Krotkov (1960), de Deken-Grenson and de Deken (1959), Ingle (1963), Zscheile and Murray (1963) and Guinn (1966).

In this investigation extraction of RNA from plant tissue and estimation of nucleotide content (refer to appendix) was made by modification of a method reported by Zscheile and Murray (1963).

One gram of wheat leaf meal or 0.5 gm crown meal was weighed for extraction of RNA. The RNA was removed quantitatively from the dried material with four extractions of 40 ml each of 0.55 M sodium chloride solution and steaming each extraction in a pressure cooker or an autoclave at 100°. The resultant supernatants on centrifugation were pooled, and equal volumes of 95% ethyl alcohol were added and the mixtures were cooled at about 2° overnight. The resultant suspensions were centrifuged batchwise (1500 X g for ten minutes for each batch), in a 50 ml centrifuge tubes. The supernatant fractions were discarded. The RNA was a white gelatinous precipitate in the bottom of the centrifuge tube.

Hydrolysis of RNA

The RNA was hydrolyzed by adding 1 ml of 0.5 M KOH to the precipitate in the centrifuge tube, and the mixture was incubated 40 hours in a water bath at 30°. The hydrolysate was transferred to a calibrated centrifuge tube after adjusting the pH to about 8.5 with hydrochloric acid and diluted to 10 ml. Centrifugations were at 1500 X g which removed the non-hydrolyzed material. The supernatants (hydrolysates) were used for determining total ribonucleic acid by diluting hydrolysates and determining RNA spectrophotometrically.

Separation of Ribonucleotides

Before nucleotide separation was made on an anion exchange column, a complete ultraviolet spectrum was recorded using a Perkin-Elmer 202 recording spectrophotometer. A spectrum was recorded for each of the

100 ml fractions from the anion exchange column, and the moles of each nucleotide was determined by the formula developed by Zscheile and Murray (1963). The steps in the procedures for anion column preparation and formulas for calculations of molarity of nucleotide content are given in the appendix.

CHAPTER IV

RESULTS

Separation of Nucleotides on Anion Exchange Column

To check the elution pattern of nucleotides from the anion exchange column, 100 ml fractions of elutants were collected in volumes of 5.2 ml. Absorbance was determined on each 5.2 ml volume to determine if the nucleotides were removed from the columns within the 100 ml fractions. It was found that the order of elution was cytidylic acid and 2' and 3' adenylic acid using 0.01 N HCl as the first elutant. These nucleotides are distinguished by the difference in their absorbance at 268.7 mμ and 284 mμ. Cytidylic acid has high absorbance at both wave lengths whereas adenylic acid has a very small amount of abosrbance at 284 mμ and high absorbance at 268.7 mμ (Figure 1). The above findings are in agreement with Cohn (1955) and Zscheile and Murray (1963). All the cytidylic acid was removed from the column in approximately 20 ml of elutant and all the adenylic acid was removed before 100 ml of 0.01 N HCl flowed through the column.

The second fraction using 100 ml of 0.1 N HCl separated uridylic acids and guanylic acids with uridylic acid being eluted first, followed by guanylic acid. Absorbance was determined at 268 mμ and 290 mμ. From the results shown in Figure 1 it may be assumed that the 100 ml fractions are sufficient to remove the nucleotides from the columns into two separate fractions.

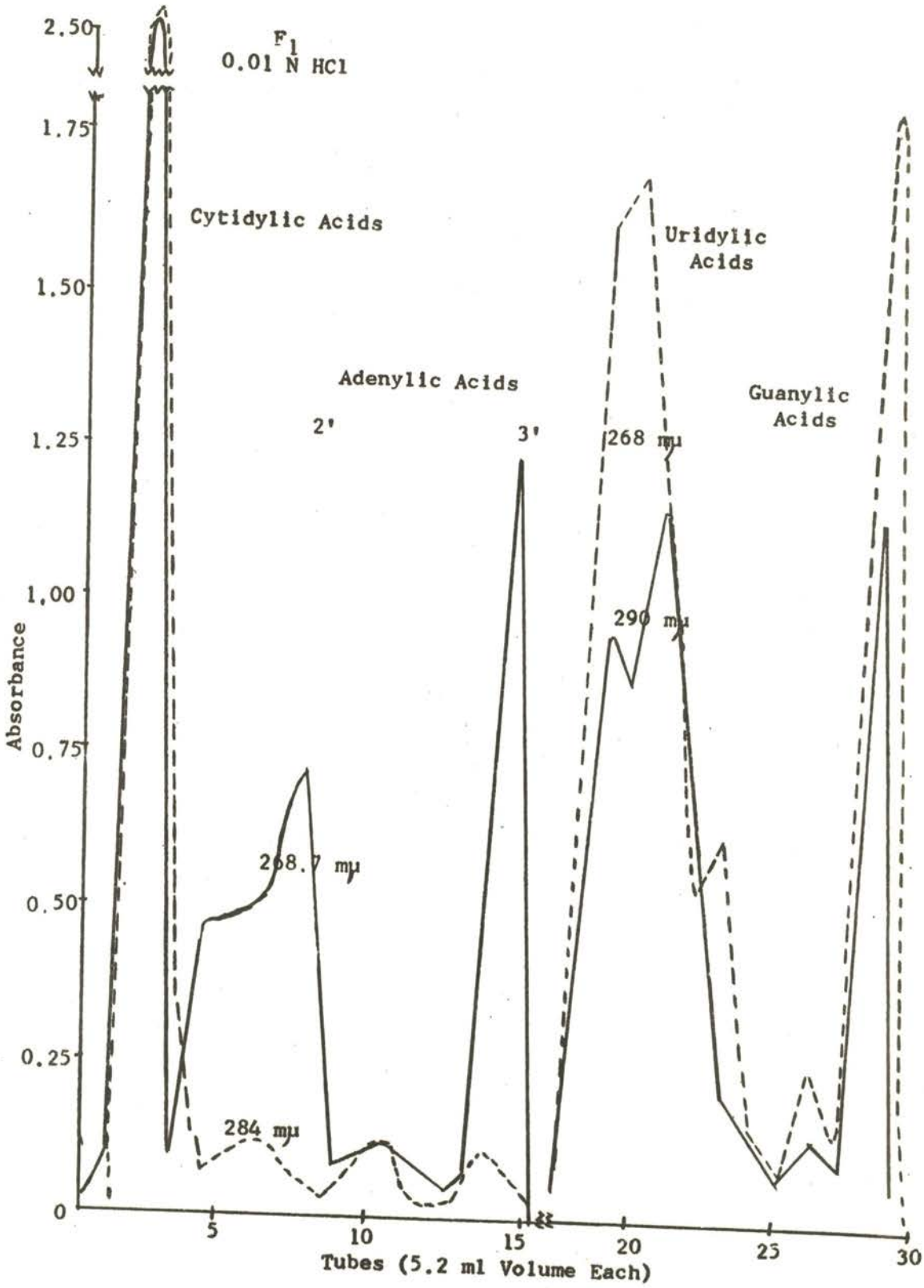


Figure 1. Anion exchange chromatogram elution pattern of RNA alkaline hydrolysate from wheat leaves. Column: Dowex-1-Cl⁻, 400 mesh. 6 cm x 1.1 cm.

Comparison of Wheat RNA With Yeast RNA

After hydrolysis of yeast RNA and separation of the nucleotides with anion exchange column, the absorbance spectrum was determined (Figure 2). The absorbance spectrum for Cheyenne and Ponca wheat leaf RNA hydrolysate was found to be similar to the absorbance spectrum of yeast RNA hydrolysate (Figure 3 and 4). The absorbance spectrum of the hydrolysate would indicate that the extraction and hydrolysis of RNA from plant material gives preparations that were relatively free from large amounts of contaminating materials such as proteins. The absorbance spectra were determined on the hydrolysate and resultant fractions from anion exchange separation for all the plants which were grown in the environmental growth chambers. All test samples were similar to the examples in Figures 3 and 4.

Total RNA Content of Gradient Water Stressed Wheat Plants At Different Growing Temperatures

Todd and Basler (1965) reported a reduction in RNA content as a result of water stress. Similar results were obtained when the RNA content was calculated on a per plant basis, (Figure 5). The reductions of RNA content in wheat plants when subjected to water stress were similar for the plants grown at 27^{o} day - 21^{o} night and 21^{o} day - 16^{o} night. However the plants grown at 16^{o} day - 10^{o} night showed an increase in RNA content per plant after 4 days without additional water followed by a slight drop in temperature after 8 days. At this temperature the increase in RNA content could be the result of less actual water stress on the plants due to less water loss by transpira-

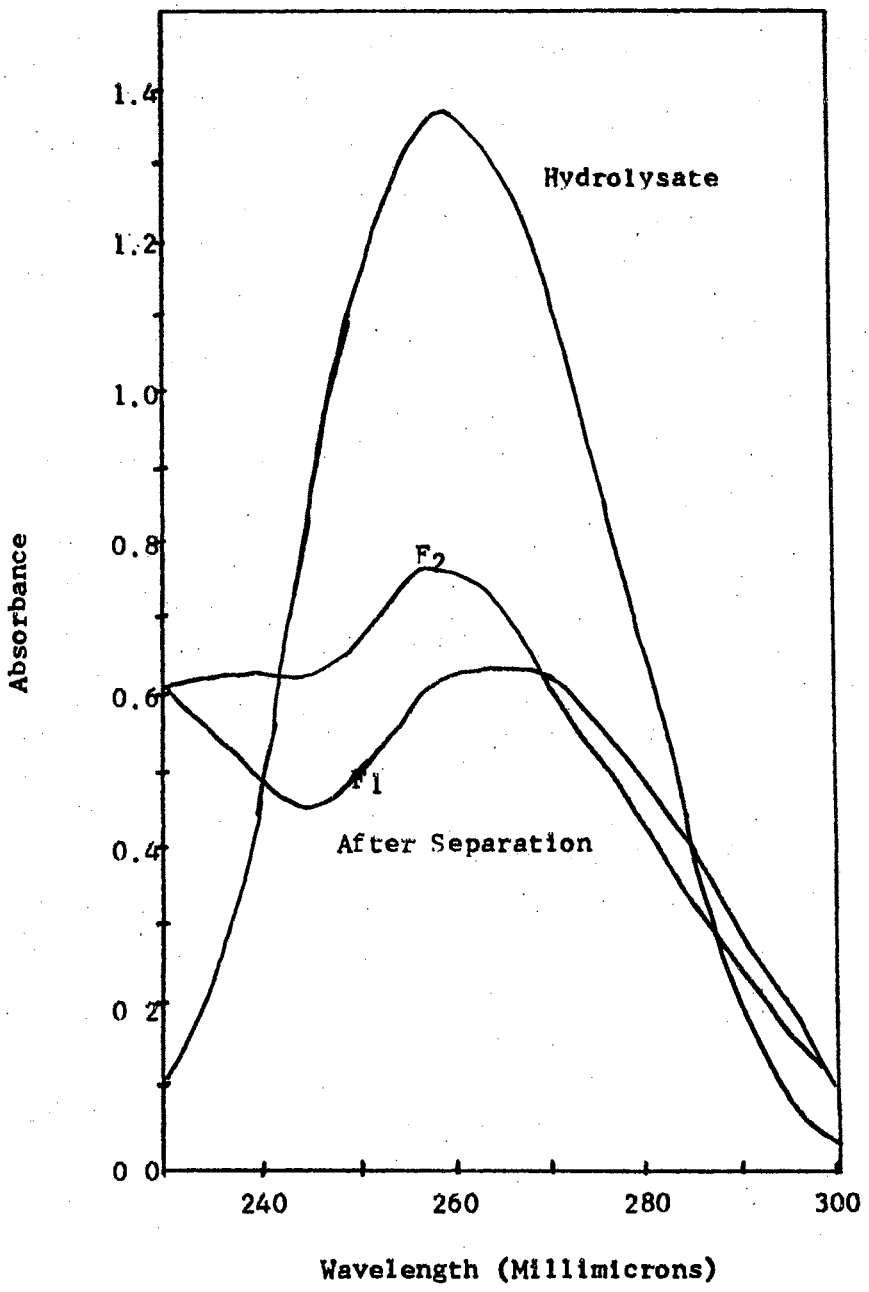


Figure 2. Absorption spectra of 5 mg hydrolyzed yeast RNA and resultant fractions 1 (F_1) and 2 (F_2) after anion exchange separation. (Diluted 100 X)

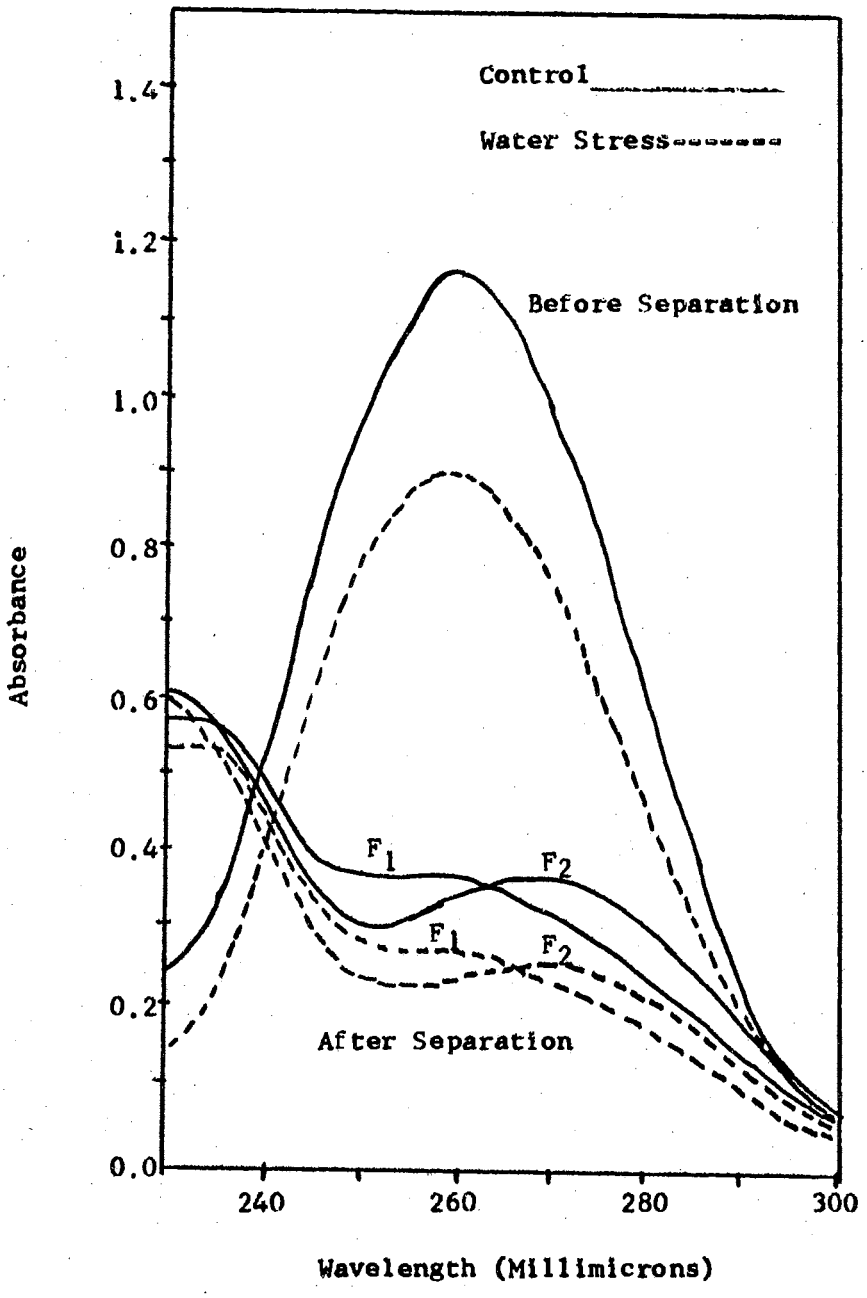


Figure 3. Absorption spectra of total hydrolyzed RNA extracted from 4 weeks old Cheyenne wheat leaves before anion exchange separation and resultant 100 ml fraction 1 (F_1) and 2 (F_2) after separation on anion exchange column. (Diluted 100 X)

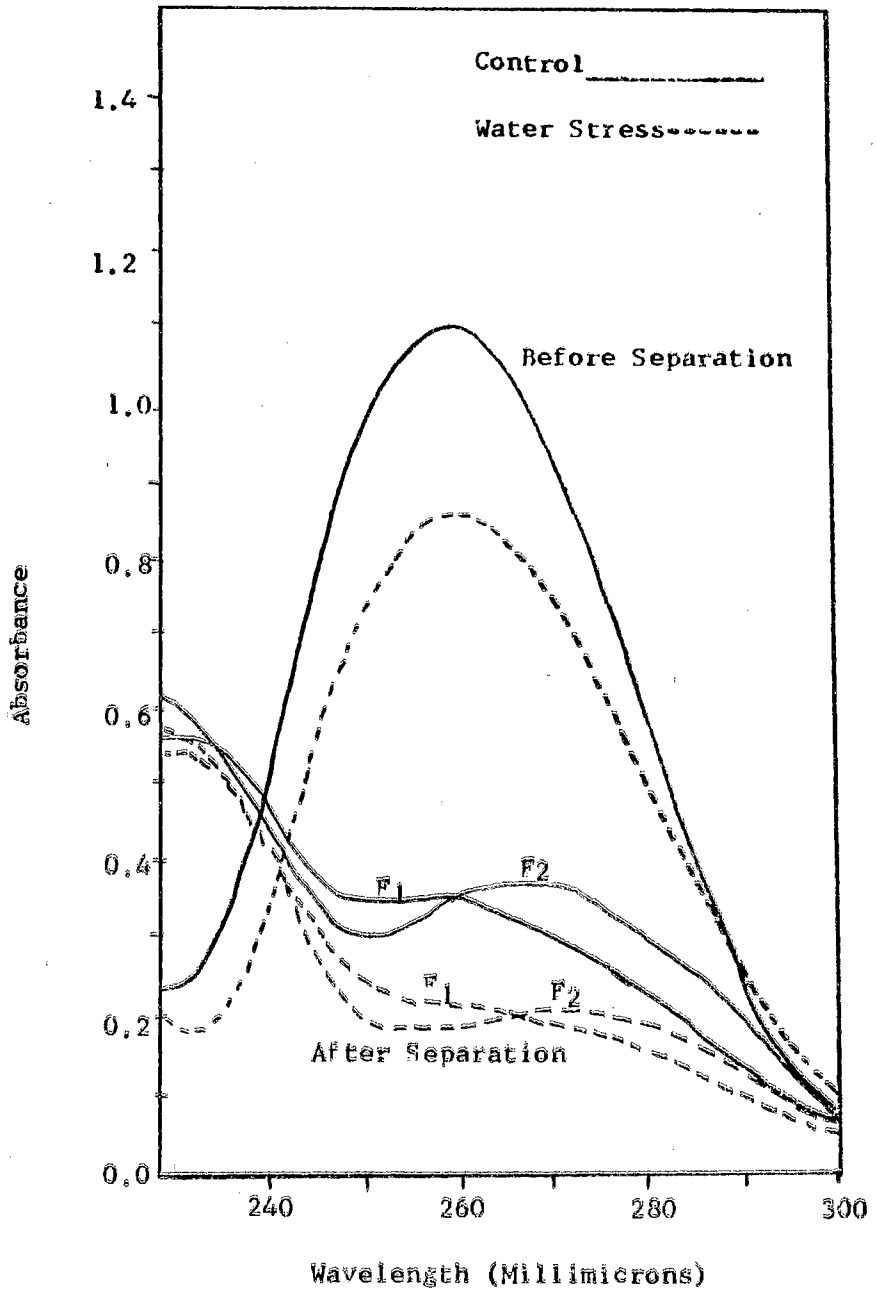


Figure 4. Absorption spectra of total hydrolysed RNA extracted from 4 weeks old Ponca wheat leaves before anion exchange separation and resultant 100 ml fractions 1 (F_1) and 2 (F_2) after separation on anion exchange column. (Diluted 100 X)

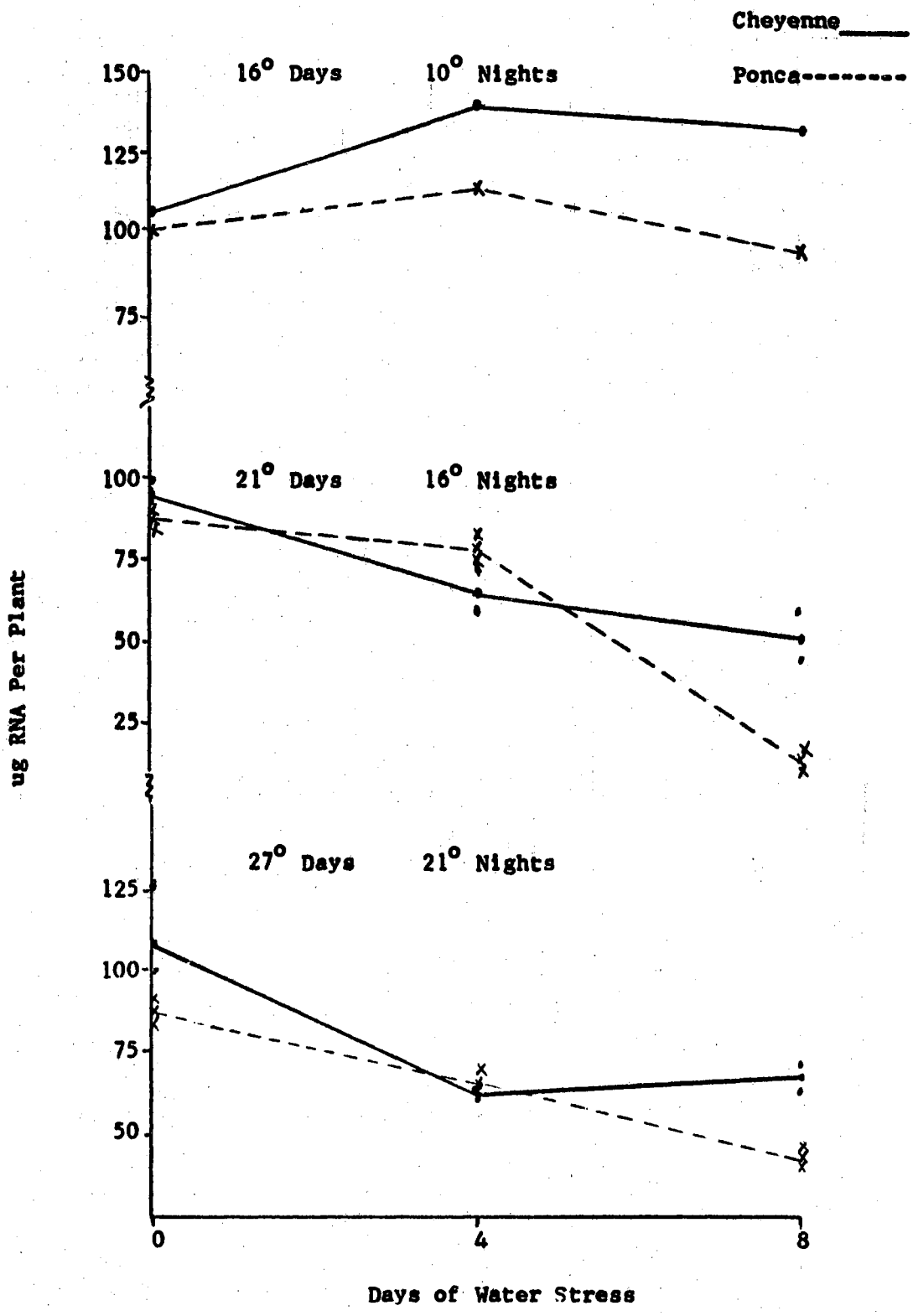


Figure 5. Total RNA per plant from Cheyenne and Ponca wheat leaves grown under different temperature regimes.

tion. In most cases after 8 days water stress the Ponca variety showed a greater decrease in RNA content per plant than did the Cheyenne variety. The water loss appears to decrease the RNA content of Cheyenne more rapidly than Ponca for the first 4 days after which time the RNA content of the Ponca variety is lost more rapidly, while Cheyenne tends to have a decreased rate of RNA loss. This results in a considerably higher RNA content in Cheyenne than Ponca when the plants are subjected to severe water stress.

Comparison of Nucleotide Composition of Wheat Plants Grown in Control Environment Chambers and Greenhouse

Nucleotide composition of 4-weeks old wheat plants grown at a constant temperature (21^{o}) may be used to indicate what could be expected when the wheat plants are grown at near optimum temperature (Tables I and II). The G + C/A + U ratio is the same for both varieties at about 1.35, which indicate the G + C type RNA of wheat plant tissue. The purine/pyrimidine ratios are about equal to 1. When the plants are subjected to water stress not only does the RNA content decrease but also the type of RNA changes as indicated by an increase in the G + C/ A + U ratios (Table IV). The per cent nucleotide composition of leaves from plants grown in greenhouse are represented in Figure 6 showing an increase in the G + C composition after the 4 days water stress. However after 8 days stress the percentage composition of G + C again resembles that of 0 days water stress. This indicates a more rapid loss of adenylic acid and uridylic acid with a relatively small amount of water stress at about 75% relative turgidity. As the stress increases

TABLE I

NUCLEOTIDE COMPOSITIONS OF 4 WEEKS - OLD WHEAT PLANTS

GROWN IN CONTROLLED ENVIRONMENT AT 21°

Water Stress 0 Days	ug/gm Dry Wt.	*% R.T.	Moles/100 Moles Nucleotide Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Leaves						
Cheyenne	6000	97	19.4	24.6	33.0	23.0
Ponca	8000	91	20.9	24.8	32.6	21.8
Crowns						
Cheyenne	8000	97	18.9	22.2	35.9	23.9
Ponca	14000	91	21.1	22.9	34.1	22.9

*R.T. - Relative Turgidity

TABLE II

NUCLEOTIDE RATIOS OF 4 - WEEKS OLD WHEAT PLANTS

GROWN IN CONTROLLED ENVIRONMENT AT 21°

Water Stress Days	Moles/gm Dry Wt. 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Leaves														
Cheyenne														
0	9.23	1.79	2.27	3.05	2.12	0.84	1.27	1.70	1.07	1.34	1.44	1.36	0.79	1.02
Ponca														
0	13.33	2.78	3.30	4.35	2.90	0.96	1.19	1.56	1.14	1.32	1.50	1.35	0.84	0.99
Crowns														
Cheyenne														
0	14.20	2.55	3.15	5.10	3.40	0.79	1.18	1190	0.93	1.62	1.50	1.39	0.67	1.16
Ponca														
0	23.60	4.75	5.40	8.05	5.40	0.88	1.14	1.69	1.00	1.49	1.49	1.33	0.75	1.18

TABLE III

EFFECTS OF WATER STRESS AND REWATERING ON NUCLEOTIDE COMPOSITIONS OF LEAVES FROM 4 WEEKS - OLD WHEAT PLANTS GROWN IN GREENHOUSE

Water Stress Days	ug/gm Dry Wt.	% R.T.	Moles/100 Moles Nucleotide Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Cheyenne						
0	5200	91	18.6	24.7	35.3	21.4
4	3200	77	16.2	24.5	40.4	18.8
8	2900	52	19.1	26.0	32.6	22.3
*5-2	4800	95	18.7	26.0	33.1	22.2
*7-2	3760	96	18.8	26.0	35.2	20.0
Ponca						
0	5400	94	18.4	25.8	36.4	19.4
4	3995	64	14.7	22.6	42.2	20.5
8	3985	47	18.2	26.5	32.7	22.6
*5-2	5513	96	19.5	25.7	34.0	20.8
*7-2	3600	97	19.1	24.1	35.7	21.1

*5-2; 5 days without water, followed by rewatering and 2 days regrowth.
*7-2; 7 days without water, followed by rewatering and 2 days regrowth.

TABLE IV

EFFECTS OF WATER STRESS AND REWATERING ON NUCLEOTIDE RATIOS OF LEAVES

FROM 4 - WEEKS OLD WHEAT PLANTS GROWN IN GREENHOUSE

Water Stress days	Moles/gm Dry Wt. 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Cheyenne														
0	9.39	1.75	2.32	3.31	2.01	0.87	1.33	1.89	1.15	1.43	1.65	1.30	0.77	1.16
4	5.79	0.94	1.42	2.34	1.09	0.86	1.51	2.49	1.30	1.65	2.15	1.86	0.69	1.30
8	5.12	0.98	1.33	1.67	1.14	0.86	1.36	1.70	1.17	1.26	1.46	1.42	0.82	1.07
*5-2	8.66	1.62	2.25	2.87	1.92	0.84	1.39	1.77	1.17	1.28	1.49	1.16	0.81	1.08
*7-2	5.85	1.10	1.52	2.06	1.17	0.94	1.36	1.87	1.30	1.36	1.76	1.57	0.81	1.17
Ponca														
0	9.93	1.83	2.56	3.61	1.93	0.95	1.40	1.97	1.33	1.41	1.87	1.64	0.79	1.20
4	7.17	1.05	1.62	3.02	1.47	0.71	1.54	2.88	1.10	1.86	2.05	1.84	0.60	1.33
8	7.13	1.30	1.89	2.33	1.61	0.81	1.45	1.79	1.17	1.23	1.45	1.45	0.91	1.04
*5-2	9.93	1.94	2.55	3.38	2.06	0.94	1.31	1.74	1.24	1.33	1.64	1.48	0.97	1.15
*7-2	5.82	1.11	1.40	2.08	1.23	0.90	1.26	1.87	1.14	1.49	1.69	1.49	0.76	1.21

*5-2: 5 days without water, followed by rewatering and 2 days regrowth.
*7-2: 7 days without water, followed by rewatering and 2 days regrowth.

an increase in degradation of guanylic acid occurs. As the total RNA is decreasing (Table III) during gradient water stress the percentage rate of decomposition of the nucleotide must be changing from that of A + U degradation to also G + C degradation as water stress proceeds (Figure 6).

The temperature at which the plants are grown and subjected to a gradient water stress appears to have effect on the nucleotide composition of the RNA. Plants that have been grown at 16^{o} day - 10^{o} night show no apparent change in the RNA composition (Figure 7 A). However, the plants grown at 21^{o} day - 16^{o} night (Figure 7 B) showed a different composition of RNA with water stress. The content of G + C increased as the A + U content decreased. When the plants were grown at 27^{o} day and 21^{o} night the same type of changes occurred (Figure 7 C) as when the plants were grown at 21^{o} day - 16^{o} night. The plants which were subjected to 41^{o} during days showed no significant change in the type of RNA (Figure 7 D), however, the total RNA content showed an increase with water stress (Appendix). RNA increase may be due to the effect of rapid rate of water loss on enzyme activity, such as RNAase, which causes RNA degradation. The 0 days water-stressed plants, when subjected to the higher temperature during the stress period, had greater RNA degradation. Enzyme activity was allowed to occur due to a sufficient water supply.

Cheyenne and Ponca showed the same type of changes in their nucleotide composition, however the changes were greater with ponca than with Cheyenne at the end of 8 days water stress. It appears that Cheyenne tends to react faster to slight water stress, whereas Ponca reacts

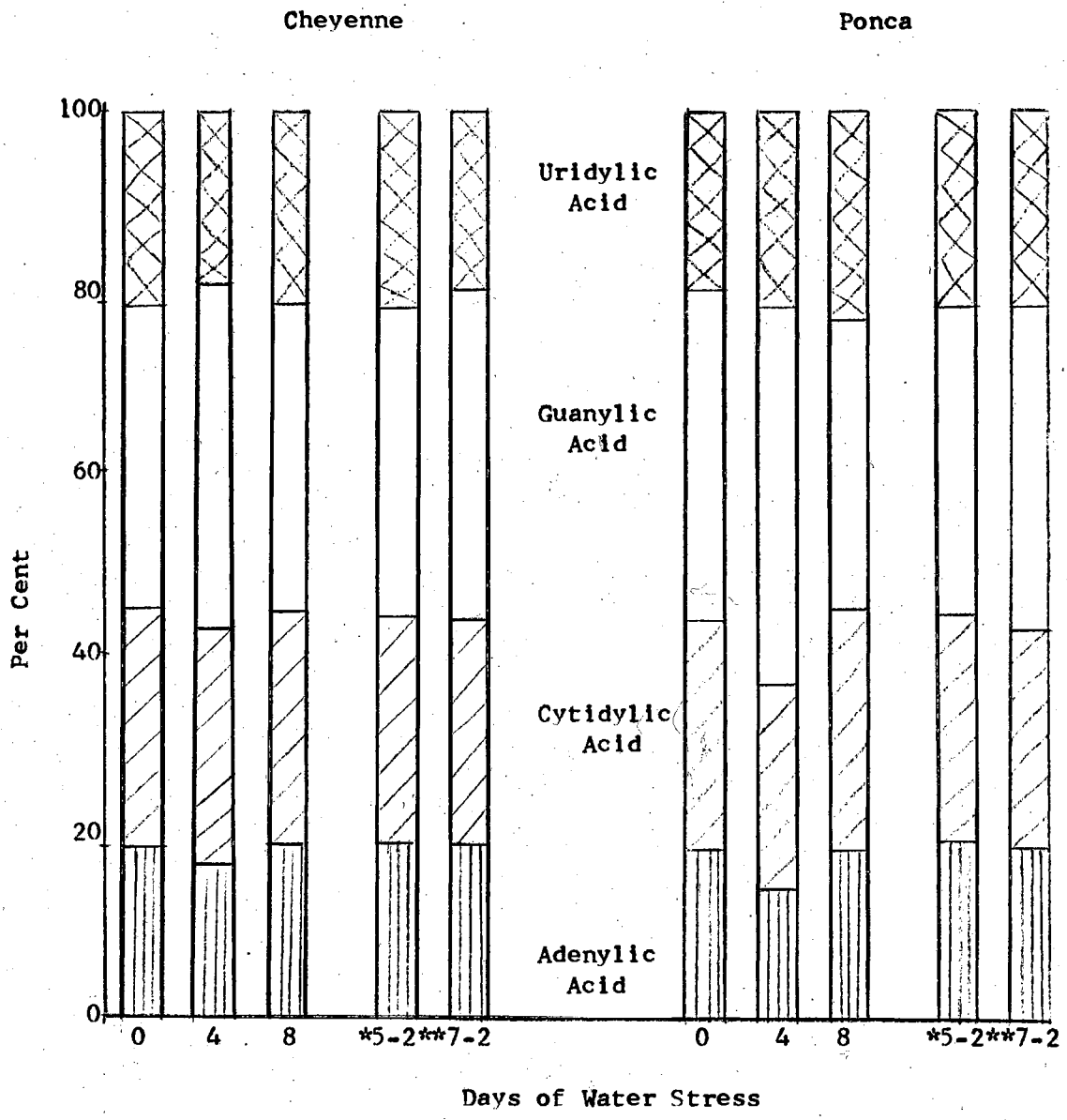


Figure 6. Nucleotide composition of wheat leaves from 4 weeks old Plants grown in greenhouse.

*5 days water stress; rewatered and 2 days regrowth

**7 days water stress; rewatered and 2 days regrowth

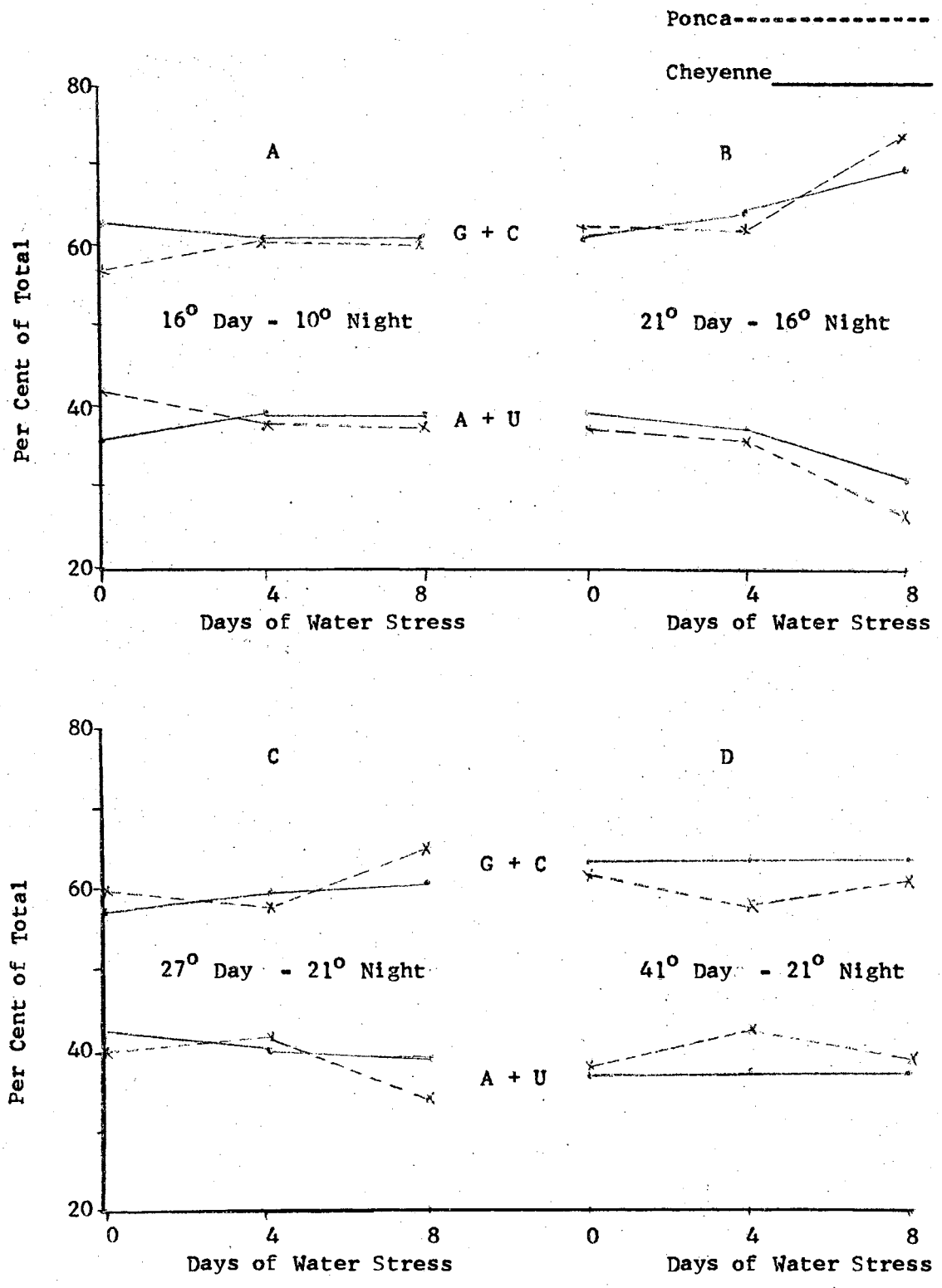


Figure 7. Effect of water stress on G + C and A + U content of total ribonucleic acid when leaf blades from 4 weeks old wheat plants are grown under different temperature regimes.

slower as indicated by changes in nucleotide composition. However, when Ponca reached greater water stress the rate of increase in G + C content of the RNA was accelerated.

Figure 8 shows the same type of response of water stress on the nucleotide composition of the crowns as was shown with the leaves, however the response appears to be delayed. This indicates the greater resistance of the crowns to water stress. The Cheyenne crowns appear to be more resistant to water stress than Ponca crowns. In most cases it would appear that there may be translocation of RNA from the leaves to the area of the crowns (Refer to Appendix)

The nucleotide composition of RNA during water stress may be evaluated if one examines the results from 4 weeks-old wheat plants grown in growth chambers and 6 weeks-old wheat plants grown in greenhouse (Figures 9 and 10 respectively). The C/A and G/U ratios show increase with an increase in water stress. These changes suggest a more rapid degradation of adenylic acid and uridylic acid than guanylic acid and cytidylic acid as the total RNA content of the plants are decreasing.

In general the same type responses were found with the plants which were grown in the greenhouse. However, after 8 days of water stress, the difference in their ratios were not as great as the plants grown under controlled environment.

The resistance of the plants to changes in their nucleotide composition when grown in the greenhouse under conditions of water stress may reflect the effects of varying temperatures (Figure 10).

The RNA structure may become more stable in those cases where water stress is also combined with extreme variations of temperature

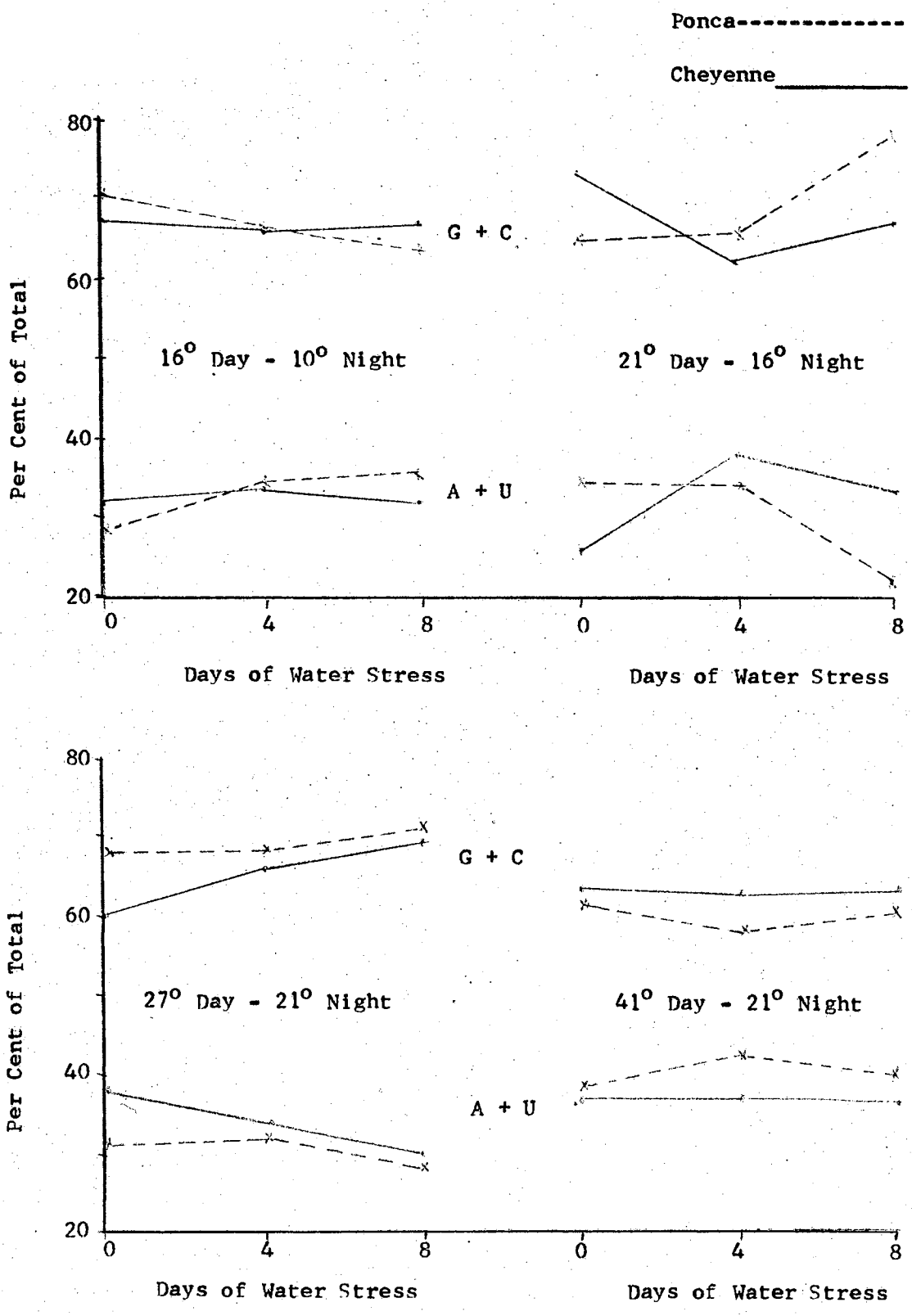


Figure 8. Effect of water stress on G + C and A + U content of total ribonucleic acid when 4 weeks old wheat crowns are grown under different temperature regimes.

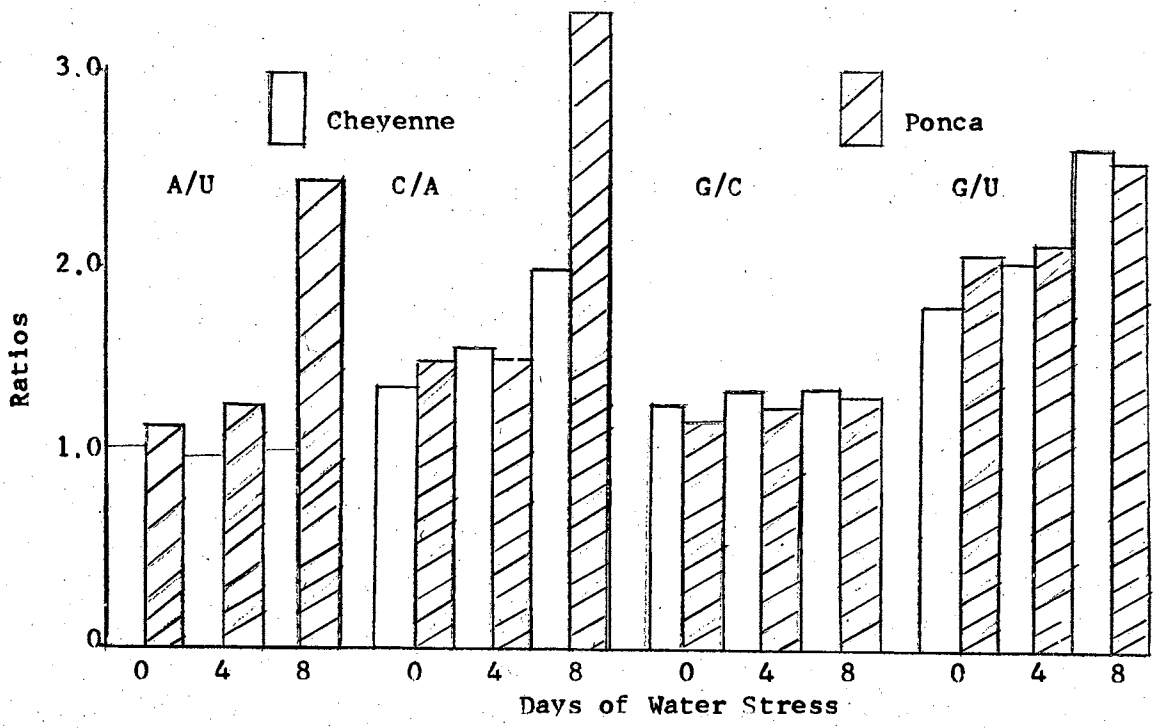


Figure 9. Nucleotide ratios of 4-weeks old wheat leaves from plants grown at 21° during days and 16° during nights. (Average of 2 determinations)

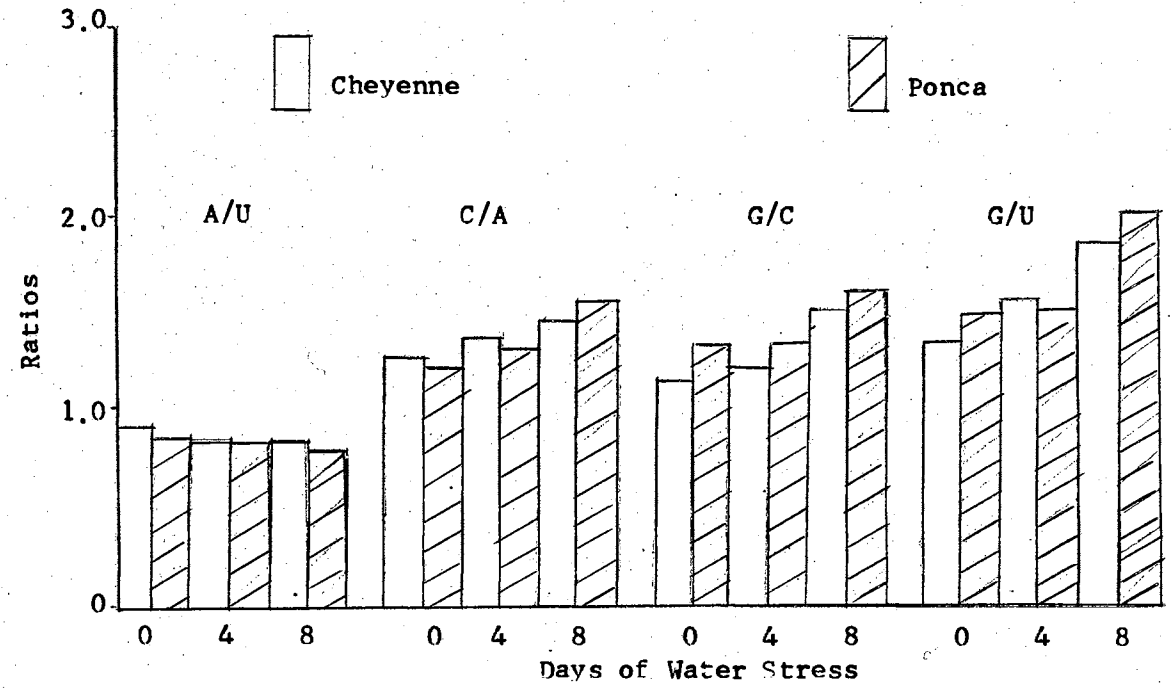


Figure 10. Nucleotide ratios of 6-weeks old wheat leaves from plants grown in greenhouse. (Average of 3 determinations)

(Kessler and Frank-Tishel, 1962).

CHAPTER V

DISCUSSION

The method of Zscheile and Murray (1963) for determining nucleotide composition of RNA in plants proved to be a rapid method for determining the nucleotide composition of wheat RNA. The method makes possible the determination of the nucleotide composition of several samples at the same time by using several columns. The separation can be accomplished in about 8 hours. The reproducibility is good when using the same source of RNA and new anion resin for each determination.

At near optimum growth temperatures the RNA content of wheat plants decreases under the effects of water stress. This is in agreement with Gates and Bonner (1956), Kessler (1961) and Todd and Basler (1965). If this loss is due to an enhanced RNA hydrolysis by water stress, as suggested by Kessler, then the nucleotide composition of the RNA from water stressed plants would be indicative of the more resistant RNA components within the plant cells. The hydrolysis rate of ribosomal RNA is usually considered to be lower than that of free RNA. The RNA in the integrated 80 S form is apparently less exposed than RNA in subunits which in turn is less exposed than free RNA (Ts'o, 1962). It would appear therefore that the soluble RNA and messenger RNA would be the types of RNA which would be most affected by increased hydrolysis, leaving as a result of high water stress, mostly polyribosome type RNA. Such polyribosomes would have bound messenger RNA which would be protected to some extent from hydrolysis

(Moldave, 1965).

The nucleotide composition of total wheat leaf RNA was found to be a "G C" type. In this respect the wheat leaf RNA was similar to barley (Zscheile and Murray, 1963), tobacco (Reddi, 1957), corn (West, 1962), olives (Kessler and Frank-Tishel, 1962) and cotton (Katterman and Ergle, 1966).

There appears to be an interaction of temperature with the degree of water stress at the temperature which is normally considered to be desirable for growing wheat plants. There is a more drastic change in the RNA nucleotide composition toward an increased G + C type RNA than when the plants are grown at a higher or lower temperature. Wheat plants which have lost approximately 50% of their water content still have the ability to recover rapidly as shown in Table III, page 31. This indicates that the synthetic system for new RNA synthesis is not destroyed at this level of water stress.

The nucleotide composition of non-stressed wheat plants (Table II) is in agreement with Mihaelovic', Grujic' and Hadzijer (1964), who attempted to show a difference in the total nucleotide composition of RNA between low-yielding wheat varieties and high-yielding wheat varieties. They showed the purine/pyrimidine (A + G/C + U) ratio to be 1.07 and the 6-amino/6-oxo bases (A + C/G + U) to be 0,94 using young wheat shoots 10 to 12 days old. A significant differenc was not found in the nucleotide composition of drouth hardy and drouth susceptible varieties of wheat except under extreme water stress conditions.

According to West (1962) water stress causes an increase in the G + U/A + C ratio of corn seedling RNA. The same type of trend may

also be observed with wheat RNA. However, the use of such a ratio to distinguish between drought hardy and drought susceptible wheat varieties appear to be invalid. Kessler and Frank-Tishel (1962) noted that with different types of higher plants the G + C/A + U ratio increases with water stress. They suggested that the varieties in which the RNA composition retained a higher G + C/A + U ratio would be more drought resistant than the varieties that had lower G + C/A + U ratio. From the results presented in this paper no such correlation could be found that would be useable as an index in determining drought resistance.

Leslie (1961) found that ribosomes from human liver cells contain histones. When separated from the RNA, these histones showed RNAase activity. RNAase activity of ribosomes was also reported by Setterfield et al. (1960), Kessler and Engelberg (1962) and others using plant material. If the RNAase is bound to the RNA of the ribosomes one could postulate that water stress would cause an uncoupling of the RNAase (histones or basic protein) from the RNA which would result therefore, in a more rapid hydrolysis due to the free RNAase. Such a finding would be consistent with the increased RNAase activity found by Kessler (1961) in water stressed plants.

The nucleotide composition of pea seedling ribosomes contain about 53 per cent G + C (Wallace and Ts'o, 1961). From a hypochromic effect it was suggested that base pairing similar to that in DNA may also occur in ribosomal RNA. Wheat leaf RNA appeared to have near symmetry (G to C and A to U) in base ratios to about 50% water loss. With greater water loss the ratios become more asymmetrical (Figures 9 and

10).

It has been shown that uracil induces the synthesis of protein when the ratio of U/G is increased in inactivated ribonucleic acid (Kessler, 1956). In Figure 10 it was shown that wheat plants grown in a greenhouse and under controlled environment had a decrease in uracil content and an increase in guanine content. This could cause the loss of protein synthesis when the plants are subjected to severe water stress.

PART B

EFFECTS OF WATER STRESS ON PROTEIN COMPONENTS

CHAPTER II

REVIEW OF LITERATURE

Functions and Distribution of Protein In Plant Cells

The properties of proteins and protein complexes as they exist in the cells and tissues of plants are primary determiners of the morphological and physiological characteristics of the plants.

Most of the protein of the leaf is enzymes (Pirie, 1959). It is now widely accepted that enzymes are proteins (Davies et al., 1964). An examination of the ratios in which different enzymes occur in leaves grown under different physiological conditions could give information on the nature of leaf protein and whether "storage Protein" occurs in the leaf or whether all the protein is made up of enzymes. The problem of investigating leaf protein, however, is that leaves that have been taken from the plant and exposed to adverse conditions for only a few hours may show a distribution of proteins significantly different from that in the leaves when they were harvested.

In young leaves growing under good conditions as much as 40 per cent of the dry matter may be protein. As the leaf matures the protein content decreases. This may be caused by a change in the ratio of fibrous tissue to tissue rich in plastids (Pirie, 1955). In tobacco leaves the phosphatase, invertase, and peroxidase content does not diminish proportionately to the decrease in total protein (Axelrod and

Jagendorf, 1951). According to Pirie, (1955) approximately 30 to 50 per cent of the protein in a recently matured leaf is believed present in the chloroplasts, 4 per cent in the mitochondria, approximately 10 per cent in the micorosmal fraction and the remainder in the cytoplasm except approximately 5 per cent which is attached to the cell wall and nuclear material.

From the investigation of the subcellular fractions of wheat leaves Todd and Basler (1965) reported a distribution of protein as follows: 15 per cent in debris and nuclei; 20 per cent in chloroplasts; 2 to 4 per cent in mitochondria and 60 per cent in supernatant fraction which would include ribosomes and cytoplasm.

Fraction I Protein

In Leaves

Wildman and Bonner (1947) first described the presence of a single electrophoretically homogeneous protein which constituted about 75 per cent of the total proteins of spinach-leaf cytoplasm. They termed this protein "Fraction I", which was believed to occur only in dicotyledonous plants. This protein was characterized as having a sedimentation constant of about 18 Svedberg units.

It has been shown that fraction I protein was associated mostly with the chloroplasts in spinach leaves and that it showed ribulose 1,5-diphosphate (RuDP) carboxylase activity (Lyttleton and Ts'o, 1958). That about 90 per cent of the total RuDP carboxylase activity in chloroplasts was associated with a preparation which was largely fraction I protein was observed by Park and Pon (1961).

Mendiola and Akazawa (1963) found fraction I type protein in rice

leaves using gel filtration (Sephadex) and starch-gel electrophoresis to characterize the protein. The major component of the soluble protein was able to catalyze the fixation of $^{14}CO_2$ using ribose-5-phosphate as substrate in the system. The main product of $^{14}CO_2$ fixation was 3-phosphoglyceric acid. The enzymes reported associated with the fraction 1 protein are phosphoriboisomerase, phosphoribulokinase and 1,5-diphosphate carboxylase (Wildman, 1961; Haselkorn et al., 1965; Mendiola and Akazawa, 1964; Boardman, 1962; and others). Boardman (1962) isolated from etiolated leaves a purified protein-protochlorophyll complex which had similar physical properties to those of fraction 1 protein. Trown (1965), using Sephadex G-200 for separation and purification of proteins, concluded that fraction I protein is crude RuDP carboxylase which may also make up the protein moiety of protochlorophyll holochrome. He was also able to separate RuDP carboxylase from phosphoriboisomerase and phosphoribulokinase. RuDP carboxylase was found to be an 18 S protein.

From electron microscope studies Haselkorn et al. (1965) concluded that the fraction 1 protein consists of a cube about 120 Å along each edge, containing 24 subunits. The protein contained RuDP carboxylase activity. Kreutz (1965) suggested that enzymes are attached to the subunits.

Basic Proteins And Their Functions

The basic protein (histone) has long been recognized as the companion-protein of DNA in animal and plant cells. The basic proteins of cell nuclei have recently been reviewed by Murray (1965). Bonner (1965)

has reported extensively on the function of histones in relation to chromatin as a gene control mechanism. Schwimmer and Bonner (1965) used basic protein (nucleohistone) as template for the replication of DNA.

The presence in ribosomes of basic proteins (histones) which are latent ribonucleases have been reported by Leslie (1961). He proposed the hypothesis that each RNA template has a specific stabilizing histone which acts as depolymerase on other unprotected RNA's.

Watson (1963) found no ribonuclease activity prior to ribosome breakdown and suggested the basic proteins have primarily a structural role functioning to hold the ribosomal RNA in the correct position for peptide bond formation. It was found by Setterfield et al. (1960) that the basic protein fraction from ribosomes of buds of pea seedlings represented about 29 per cent of the protein of the ribosomes.

From the results of experiments with corn scutellum Hansen and Swanson (1962) suggested that the biochemical basis for respiratory senescence in plant cells may be in those events leading to large increases in ribonuclease and/or other basic proteins.

Bound Water In Proteins

A single complex protein molecule can bind to its polar groups some 20,000 water molecules, particularly to hydroxyl, carboxyl, amino and amide groups (Stocker, 1960). The intra- and inter-molecular bridges and the hydration shell of the molecules have been recognized as dependent on the chemical structure of the protein. A relationship between hydration and the state of bonding exists in that the hydration

shell covers and protects the points of bonding and the bridges. It also hinders the formation of new bridges and renders difficult the destruction of existing bridges. The existing protein structures are therefore stabilized by hydration. There is no sharp distinction between water which is bound by hydration and free water. The charge and dipole forces rapidly decrease with distance and so a very strongly bound inner layer is surrounded by successively less strongly bound layers.

Todd and Levitt (1951) used the term bound water for the water held at room temperature in an evacuated desiccator containing a disiccant but driven off in an oven at 80°. Bound water was considered by Levitt to be that portion of water held by dry matter in equilibrium with a definite low vapor pressure. The greater part of the bound water is due to solutes and colloids in the cell wall (Levitt, 1965).

A theory has been proposed which predicts the amount of hydration possessed by any given protein based solely on the amino acid composition of that protein (Fisher, 1965). The predicted values agree closely with those experimentally determined in such cases where direct measurements are available. Using the amino acid composition of individual proteins, Fisher (1965) calculated the first monomolecular layer of water which is strongly bound to protein. From the calculated values of 34 individual proteins the average value was 0.28 grams water vapor per 1 gm protein which corresponds to only about 20 per cent of the final amount of water absorbed by a protein.

From studies on ribonucleoprotein particles from mammalian ribosomes, Petermann and Hamilton (1961) found that ribosomes carry several

times their own weight of water and pointed out that they must be spongelike in form. At pH of about 8 the excess protein is dissociated from the ribosomal RNA. Walter (1966) concluded that the hydration of proteins is largely dependent upon pH values or the influence of electrolytes.

Effects of Water Stress on Proteins

The resistance to water stress in plants is primarily a matter of the colloidal-chemical properties of the protoplasm and of metabolism. In the more resistant mesophytes the chief characteristic is the presence of very intensive metabolic activity and of an ability to maintain synthetic reactions at a high level during wilting. Of great importance among colloidal-chemical properties are a high degree of hydration of the colloids, high viscosity and elasticity of the protoplasm and the quantity of bound water (Henkel, 1961).

Petrie (1943) quoted Oparin as stating that a decrease in water content causes release of adsorbed enzymes into the continuous phase where they effect hydrolysis. Petrie and Wood (1938) found that when the water content and nitrogen supply of the leaves of gramineous plants were varied, the values of the protein content could be almost exactly predicted in terms of the contents of amino acids and water. They pointed out that while water is a factor in the relation between proteins and amino acids on a dry-weight basis, they did not know whether this applied also on a cencentration basis. Aspinall et al. (1964) found that in barley, the organ which is growing most rapidly at the time of water stress is the one most affected.

Upon examining the ability of different leaves to recover from stress Vickery (1956) concluded that recovery was correlated with the protein content of the leaf and that in low protein leaves the enzymes necessary for recovery had been depleted. Todd and Yoo (1964) found that protein content of detached wheat leaves decreased with desiccation and that different enzymes are lost at different rates. In the soluble fraction, the enzymes saccharase and phosphatase were lost at a faster rate than peroxidase and peptidase in relation to total protein. During heat and water stress young corn plants showed a 3 fold drop in nitrate reductase activity on fresh weight basis (Mattas and Pauli, (1965). The nitrate content increased with increased water stress. Shah and Loomis (1965) found that water stress decreased the protein content in the soluble and insoluble fractions of sugar beet leaves at different stages of leaf maturity.

Free amino acids in barley plants under conditions of deficient water have an increase in proline, valine, and arginine with a reduction of alanine, glutamic acid and gamma-aminobutyric acid (Savitskaya, 1965). Younis et al. (1965) found different relative concentrations of free amino acids in corn seedlings after they were subjected to different temperature regimes with water stress which indicated changes in protein composition.

CHAPTER III

METHODS AND MATERIALS

Preparation of Plant Material

Winter wheat varieties, Red Chief, Cheyenne and Ponca, were grown in controlled environment chambers. The temperature was maintained at 20° to 22° constant temperature. The light intensity at pot level was 11,000 lux using a grow lux light source.

The plants were grown in 6 inch clay pots using vermiculite as soil medium. The plants were watered regularly with Hoagland's complete nutrient solution until the water stress period was started. The water stress was maintained on the experimental plants to provide plants with a relative water content of about 60 to 70% (slightly wilted and 20 to 30% (wilted).

A control was harvested at the same time as the experimentals. The last mature leaves were selected for protein analysis from plants with the youngest leaf 2 to 4 cm long. The crown portion used in the analysis consisted of about 3 cm portion of the plant above the roots.

After harvest of the plant portions the tissue was homogenized in cold tris-glycine buffer (0.1 M, pH 8.3) using a glass hand homogenizer maintained at 2° in a cold room. Ten leaves or 10 crowns were pooled for each test. The homogenates were centrifuged for 20 minutes at 27,000 X gravity. The final supernatant fraction consisted of 5 ml each. One to 2 ml of this supernatant fraction was used for separation

of protein fractions on Sephadex and 0.1 to 0.2 ml was used for polyacrylamide gel electrophoresis separation.

Separation of Soluble Proteins Using Dextran Gel (Sephadex)

The dextran gel Sephadex G-100 was used and columns of 1.1 cm X 80 cm were prepared by a procedure similar to that described by Flodin (1962) and Mendiola and Akazawa (1964). The elution pattern from the gel columns was continuously minitored by a Turner Model 111 Fluorometer which was connected to a recorder. The eluate from the column was then collected in 5 ml fractions. This provided continuous written record so that on completion of an automatic run a desirable fraction could be readily located.

Proteins were determined by ultra-violet absorbance (Warburg and Christian, 1942), fluorometric measurements and Folin-phenol protein tests (Lowry et al., 1951) on each 5 ml fraction. Fluorescence analysis appears to be more sensitive and specific than U.V. absorption. Fluorescence is considered quite selective for the proteins since the highly U.V. absorbing nucleic acid derivatives are only slightly fluorescent. A 254 mμ primary interference filter (activating filter) and a secondary (emission) narrow pass filter which peaks at 360 mμ were used in the Fluorometer for increasing the specificity for proteins. Ultra-violet absorbance was determined using a Perkin-Elmer Model 202 recording spectrophotometer. A spectrum from 190 mμ to 350 mμ was recorded for each fraction that contained protein.

Separation of Soluble Proteins Using Polyacrylamide Gel Electrophoresis

Properties of Polyacrylamide: Polyacrylamides are synthetic polymers formed from low molecular weight chemicals obtainable in high purity. The pore size of polyacrylamide gels can be varied through a wide range by adjustment of the monomer concentration, and preparation of the gels is a simple and rapid procedure. These gels are transparent to visible radiation through a wide range of monomer concentrations. Polyacrylamide gel lattices are carbon-carbon polymers with pendant amide groups, are relatively inert chemically and have few or no ionic side groups (Davis, 1964; Williams and Reisfeld, 1964; Steward and Barber (1964).

Preparations of Polyacrylamide Columns: The method used in preparing the gel columns was similar to the method described by Ornstein (1964) and Davis (1964). The chemicals used and their formulations are given in the appendix (Basic Proteins, page 92; Acidic Proteins, page 93).

The procedures used in preparation of the columns were those suggested by Buchler Instruments, Inc. who manufactured the instrument used in carrying out the polyacrylamide gel electrophoresis experiments.

Staining of Separated Proteins: A solution of 1% amidoschwarz (amido black) in 7% acetic acid was used to stain the protein bands. The excess background stain was removed electrophoretically by placing the gels into glass tubes (1.0 cm x 26 cm) with one end constricted to retain the gels in the tubes. The gels were removed from the separation tubes and destaining tubes with the aid of a small pump which forced water behind the gel, causing the gel to be pushed out of the tubes.

Following the destaining process the gels were stored in glass tubes containing 1% Formalin solution.

CHAPTER IV

RESULTS

Dextran Gel Separation of Proteins

Using the florescent properties of proteins, a record of the units of florescence was automatically recorded as the proteins were eluted from the Sephadex column (Figure 11). A relative protein elution profile was determined, using known proteins, which was used for the approximate estimation of the size of unknown proteins (Whitaker, 1963). Utilization of this method of separation of proteins shows that the wheat leaf soluble protein can be separated into two groups which shall be referred to as fraction A and fraction B (Figure 11b)[1]. Fraction A was eluted with the void volume of the column equal to γ globulin and therefore is presumed to have a molecular weight greater than 100,000, which is the exclusion limit of separation of the proteins on Sephadex G-100. Fraction B protein may be considered to have a maximum molecular weight of approximately 20,000 compared to trypsin (Figure 11) and probably also contains small peptides and amino acids which would not be separated from the 5,000 to 20,000 molecular weight proteins using Sephadex G-100.

Sephadex G-100 columns were utilized in determining if the effects of water stress on the wheat plants caused any observable changes in the amount of protein which was found in fraction A and fraction B.

[1]Figure 11b is representative of several replications.

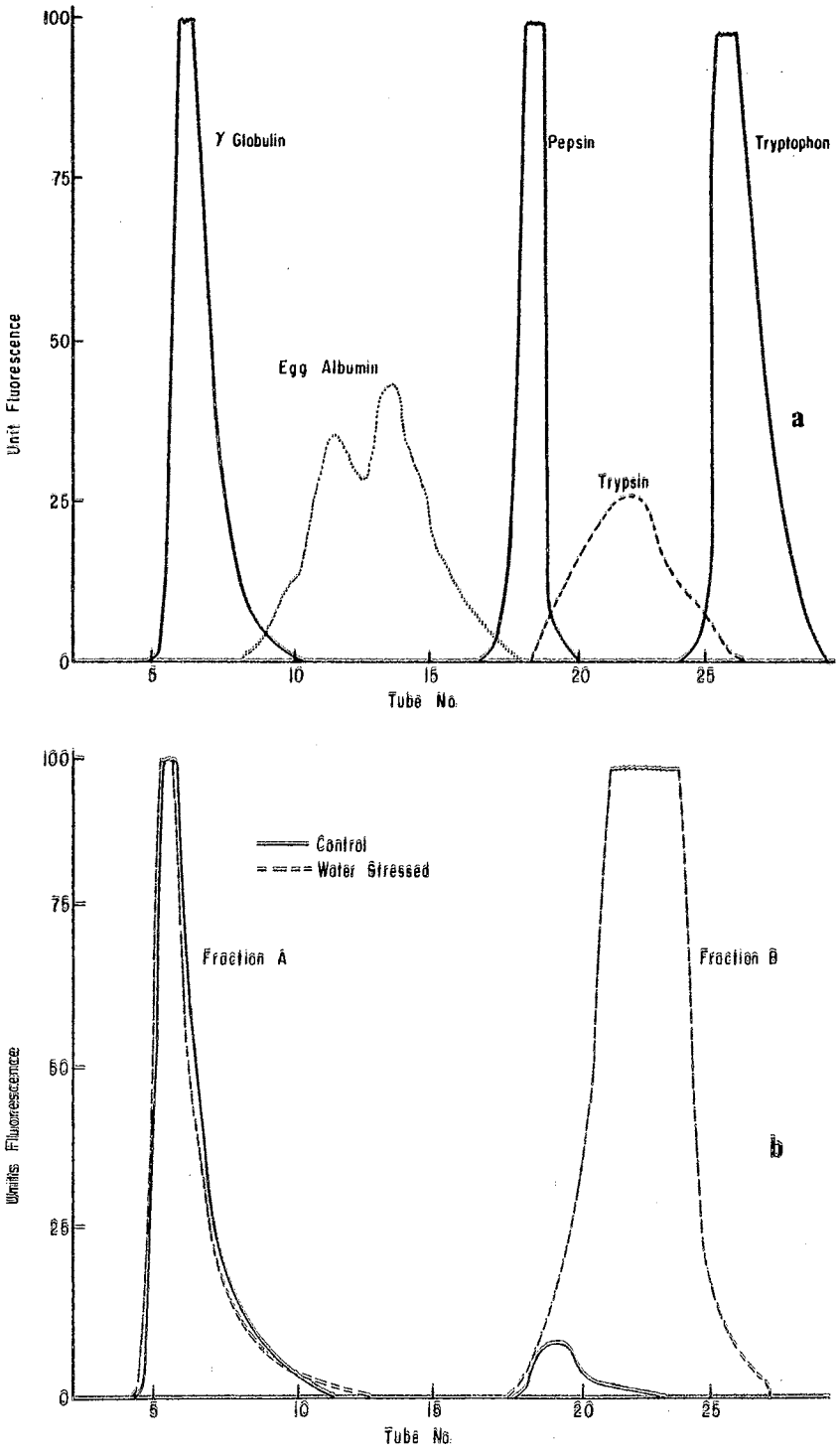


Figure 11. Protein elution patterns from Sephadex G-100 1.1 cm x 80 cm column. (a = Patterns of known proteins; b = Pattern of Fraction A and B proteins).

Using units of fluorescence as a test method, (Figure 11b) it was shown that fraction A protein was decreased and fraction B protein was increased (Figures 12 and 13) by water stress.

Each 5 ml fraction from the Sephadex column was tested for protein content and related to a standard protein source. The change in the amount of wheat leaf proteins in fraction A and B was related to the total protein content before separation (Figure 12). The results showed a decrease in fraction A proteins with an increase in the fraction B proteins. The total amount of protein showed relatively little change as a result of water stress when utilizing this method of investigation.

Similar results were also found in the crowns of the three varieties of wheat used except that the rate of change or magnitude was as great (Figure 13). In Red Chief and Cheyenne varieties there was an increase in total protein in the wilted plants after an initial drop in total protein with the slightly wilted plants.

In figure 14 the ratios of fraction A and B proteins determined spectrophotometrically shows the same response for wheat leaves and crowns. These similarities reflect a decrease in fraction A and an increase in fraction B with increased water stress. The response may be further illustrated by utilizing the ratios of fluorescence as an indication of the response to water stress. Figure 15 shows a decrease in ratios with increased water stress. A relationship to the drought hardy varieties may be indicated by the initial ratios of protein and the rate at which fraction A protein decreases with water stress. The rank from most drought resistant to least drought resistant is Red

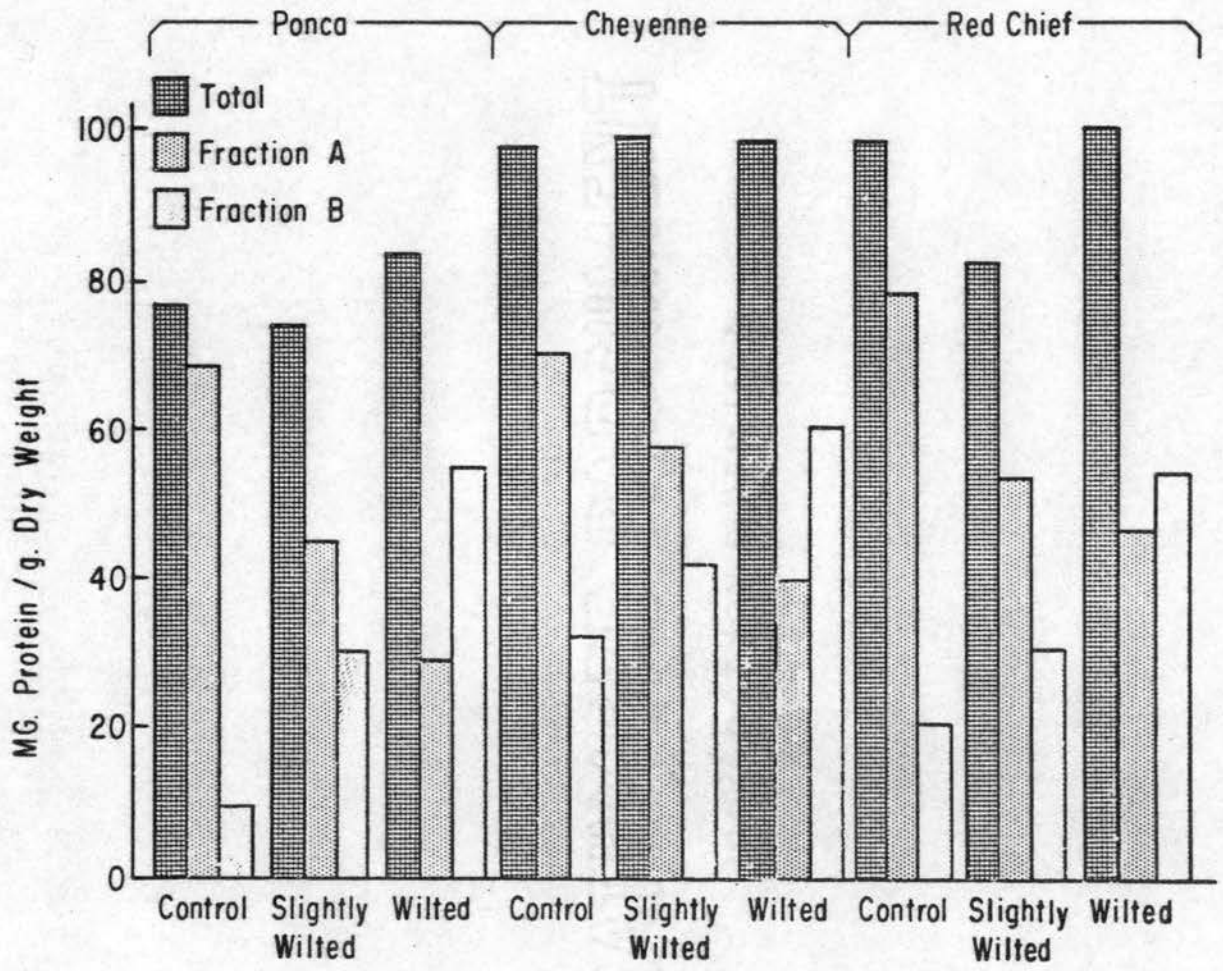


Figure 12. Comparison of the total soluble wheat leaf proteins with Fraction A and Fraction B proteins using Sephadex G-100 gel for separation. (Protein was estimated by Lowery Folin-phenol method).

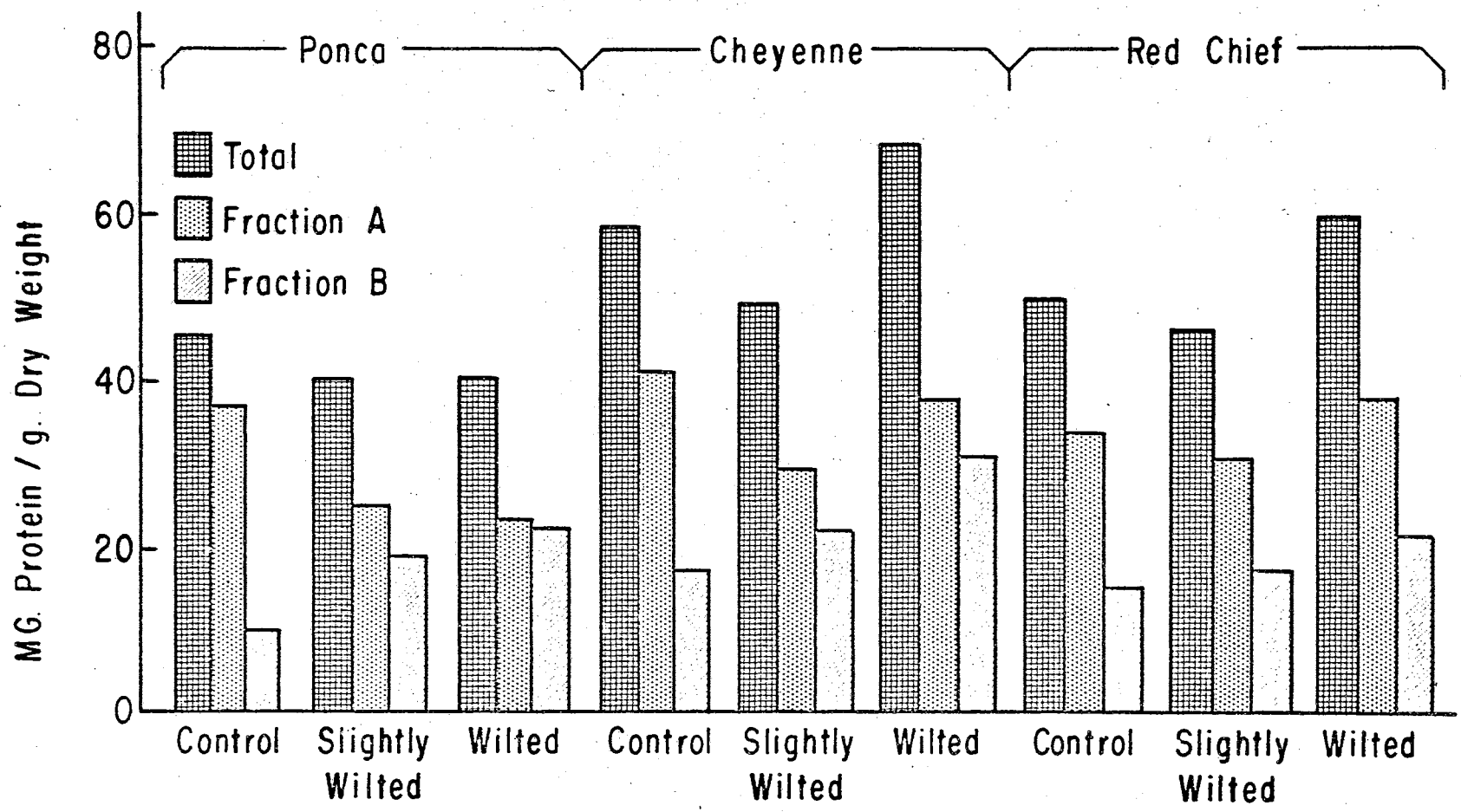


Figure 13. Comparison of the total soluble crown proteins with Fraction A and Fraction B proteins using Sephadex G-100 gel for separation (Protein was estimated by Lowery Folin-phenol method).

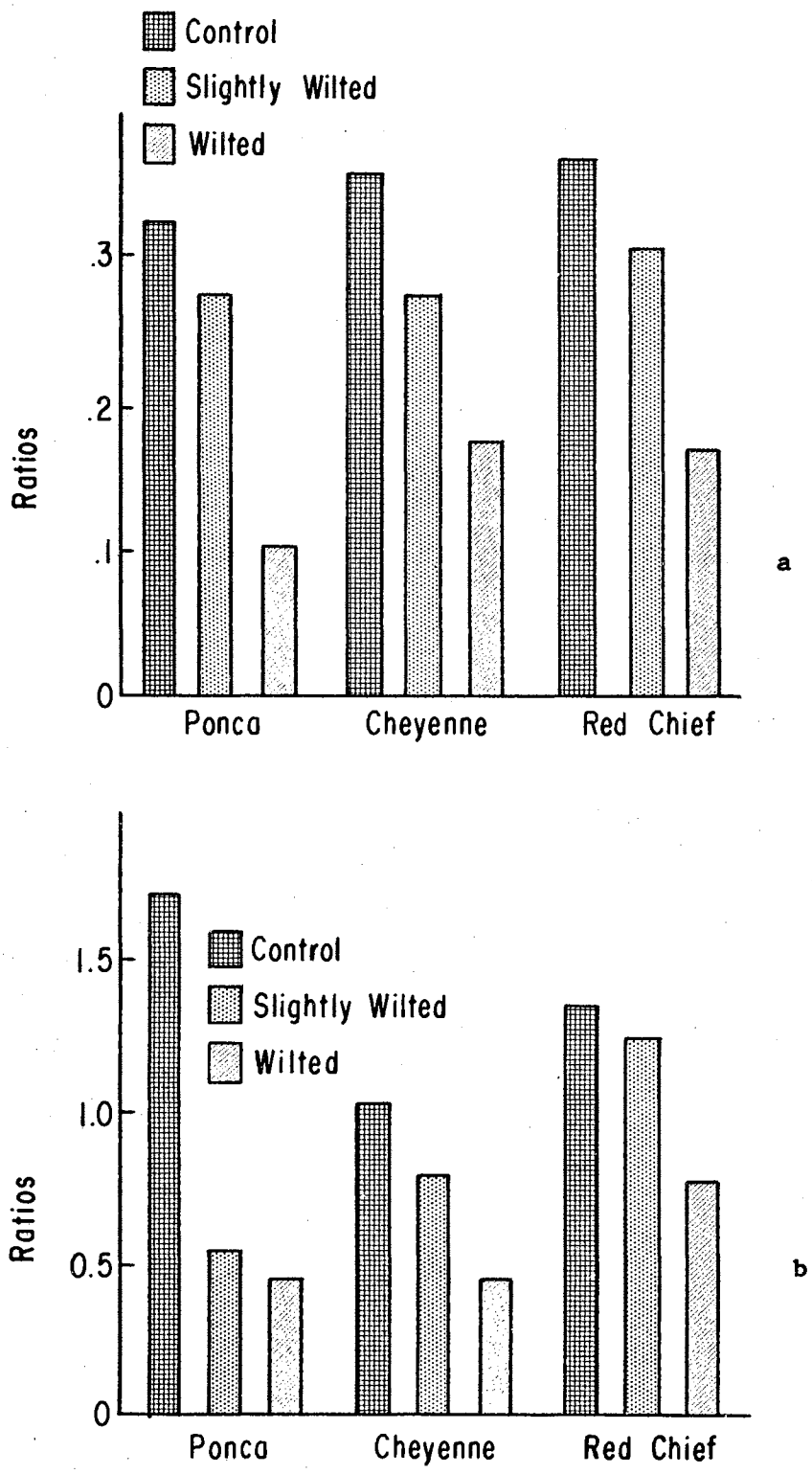


Figure 14. Ratios of Fractions A/B proteins determined spectrophotometrically from water stressed wheat plants. (a - Leaves; b - Crowns)

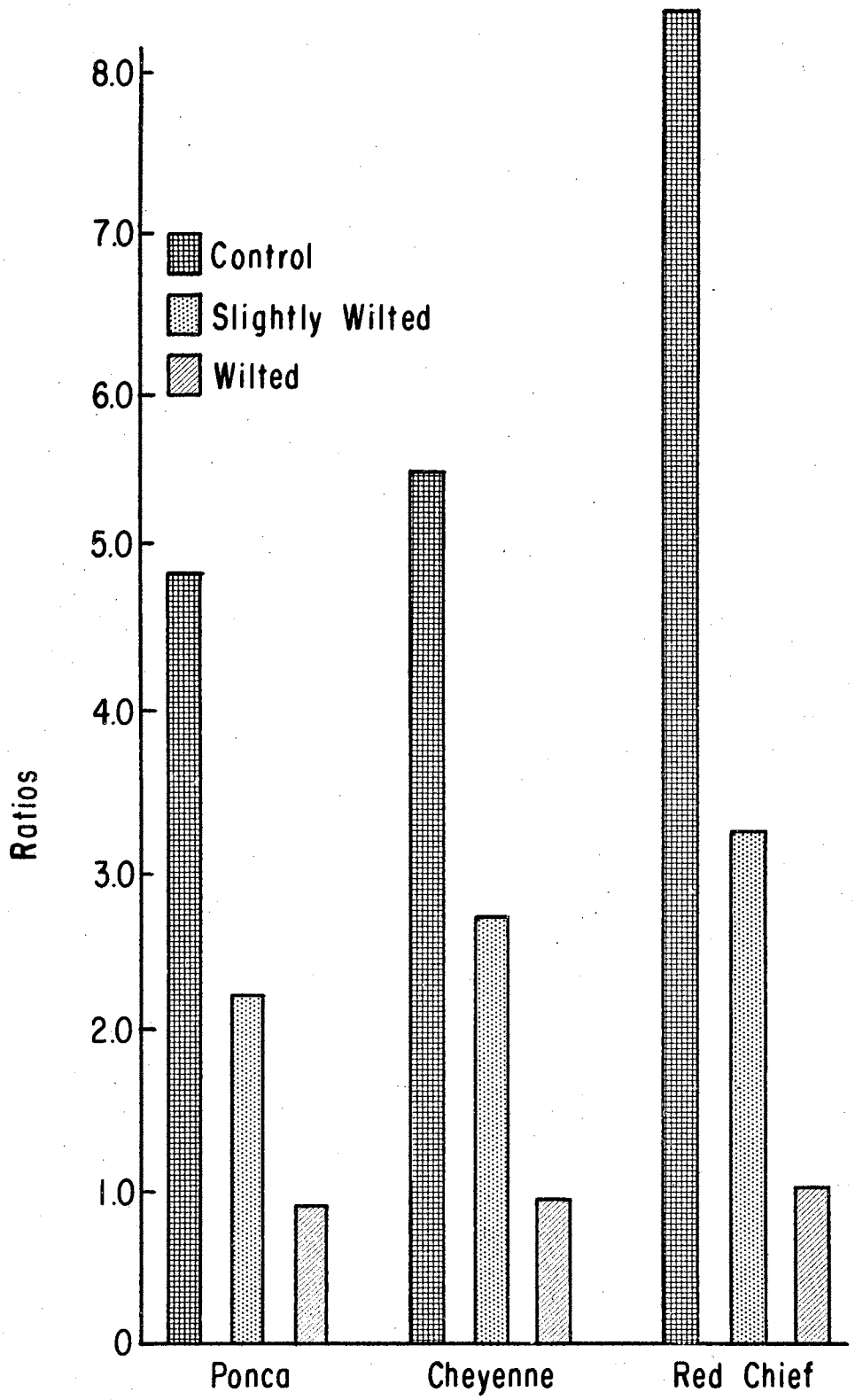


Figure 15. Ratios of fluorescence of Fraction A/B proteins from water stressed wheat leaves.

Chief, Cheyenne and Ponca. The higher ratios of Fraction A/B for each level of water stress would be in the same order.

Acrylamide Gel Electrophoresis Separation of Proteins

Electrophoretic separation on acrylamide gel is readily accomplished with a very high level of reproducibility. When using the same protein source and the same conditions of separation, it is possible to get visually identical results.

Figure 16 shows a representative separation profile of (a) egg albumin, (b) wheat germ, (c) and wheat leaf proteins. The photographs of the stained gel tubes show the relative amounts of protein by the intensity of the bands, the darker bands represent greater amounts of proteins and the lighter bands a lesser amount of protein. Bands which were visible in the stained gels but not visible in the photograph are illustrated by the very light diagram bands. The diagrammatic interpretation beneath the photographs are used in all subsequent illustrations of the protein separation profiles.

Wheat Plants Grown Under Controlled Environment

When plants were grown under constant light and temperature conditions, a decrease in the large molecular weight protein near the origin and an increase in the smaller molecular weight materials near the front was observed when the plants were water stressed (Figure 17). The number of protein bands increased with increasing water stress as well as the intensity of some of the bands. Red Chief, with increased water stress, showed primarily an increase in intensity of the bands in

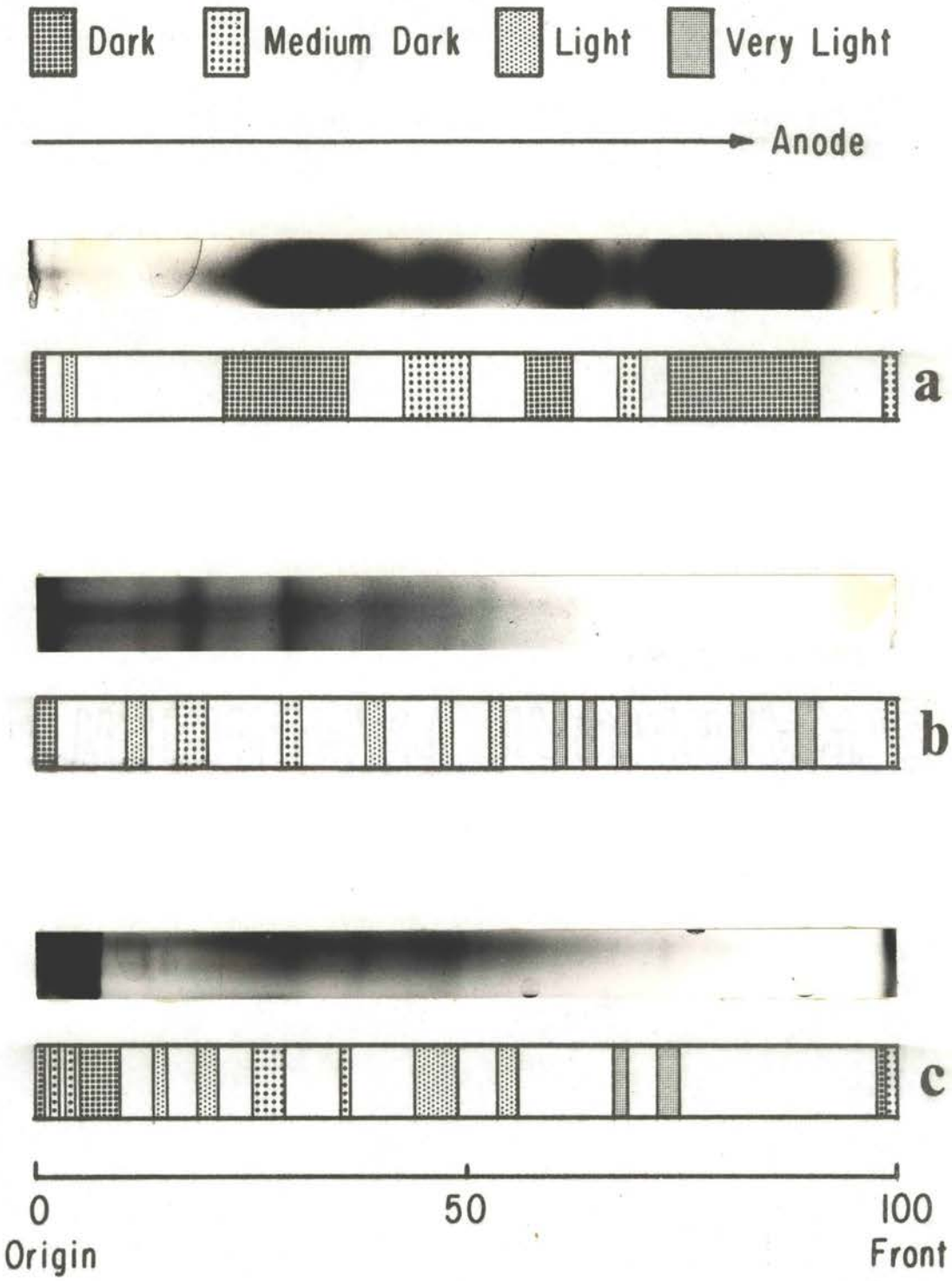


Figure 16. The electrophoretic separations of soluble proteins in pH 8.3 Tris-glycine buffer on 7.5% Acrylamide gel. (a - egg albumin; b - wheat germ; c - wheat leaf)

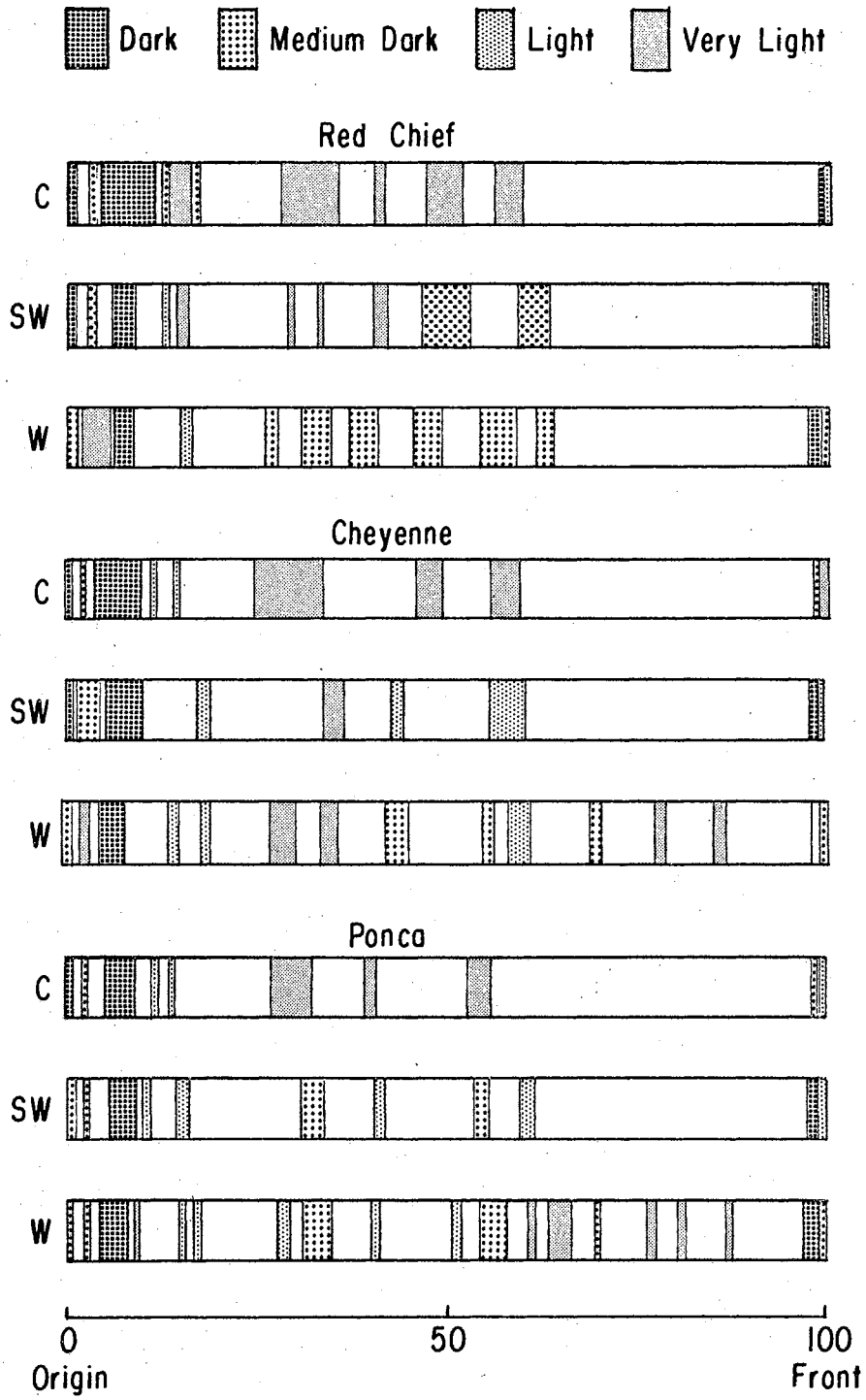


Figure 17. Diagrammatic interpretations of the electrophoretic separations on 7.5% Acrylamide gel (pH 8.3) of the soluble proteins from water stressed wheat leaves grown under controlled environment. (C=control; SW=slightly wilted; W=wilted)

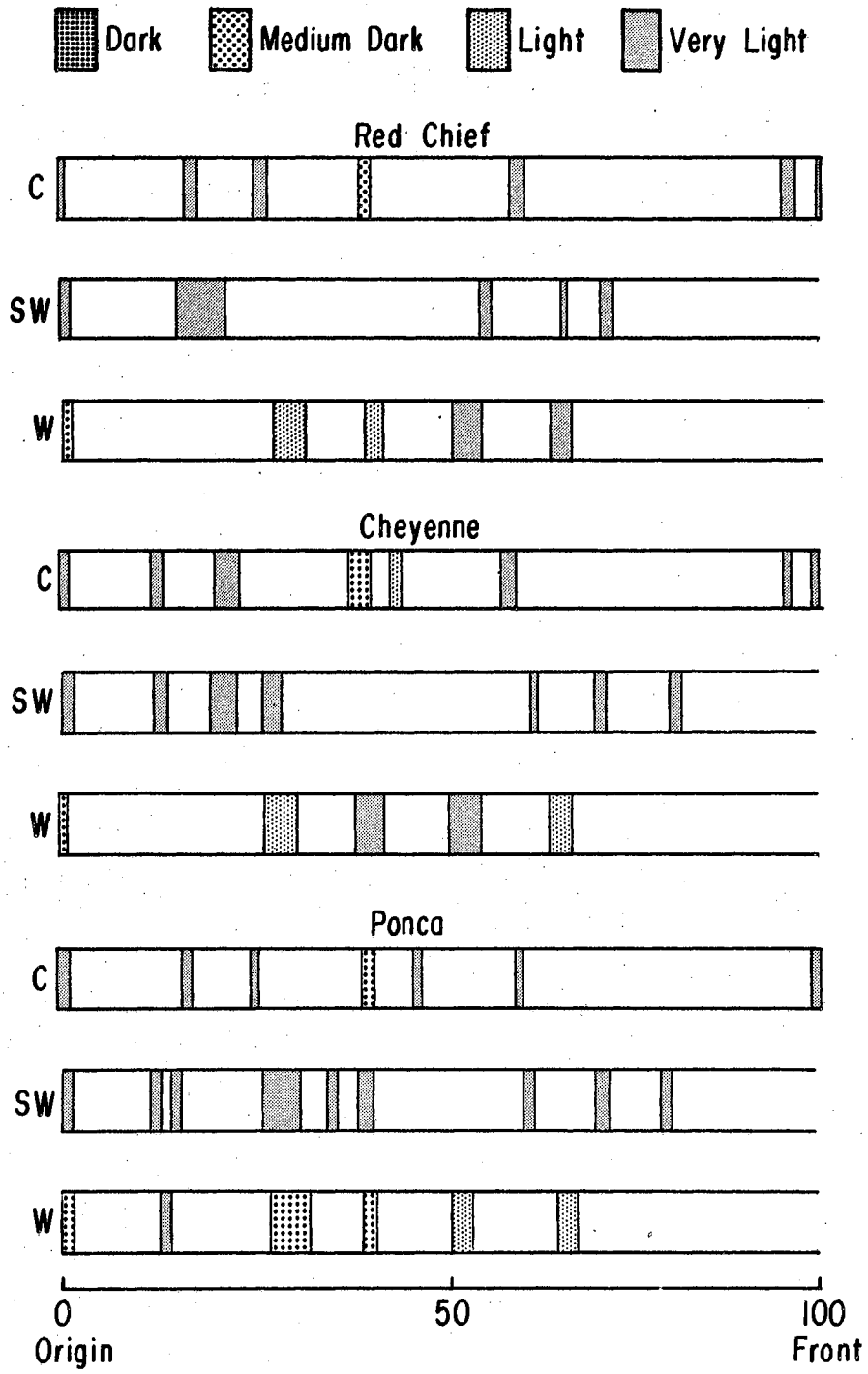


Figure 18. Diagrammatic interpretation of the electrophoretic separations on 7.5% Acrylamide gel (pH 4.5) of the soluble proteins from water stressed wheat leaves grown under controlled environment. (C=control; SW=slightly wilted; W=wilted)

the 30 to 60 Rf range, whereas Cheyenne and Ponca had an increase in the number of bands throughout the smaller molecular weight range of the gel. By comparing the diagrams of figure 17 with figure 16, diagram of egg albumin, it could be assumed that the albumin type protein is increased in the wilted plant leaves.

Figure 18 also shows a change in the type of basic proteins from the same sample as shown in figure 17. Protein separation in the Ponca variety shows a greater increase in the basic type protein than the Red Chief or Cheyenne varieties.

When the leaves were ground with Cleland's sulfhydryl reagent at 25 mg/100 ml concentration (Cleland, 1964) in the tris-glycine buffer, no additional protein bands were observed using either the pH 8.3 or 4.5 system for separation.

Wheat Plant Grown In Field Plots

Leaf samples were taken at random from wheat plants growing in field plots at different time during the growing season to compare the protein from plant leaves grown in controlled environment and that from field grown plants.

Seven different wheat varieties[2] were used which included the three varieties investigated under controlled environment. Figures 19 and 20 show the protein band profile of wheat leaf samples from 10-weeks old plants, with the separation systems at pH 8.3 and 4.5 respectively. The 10-weeks old plants were subjected to moisture stress

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[2]Red Chief CI 12109; Cheyenne CI 8885; Ponca CI 12128; Concho CI 12517; Kaw 61; Improved Triumph CI 13667; Kan King CI 12719.

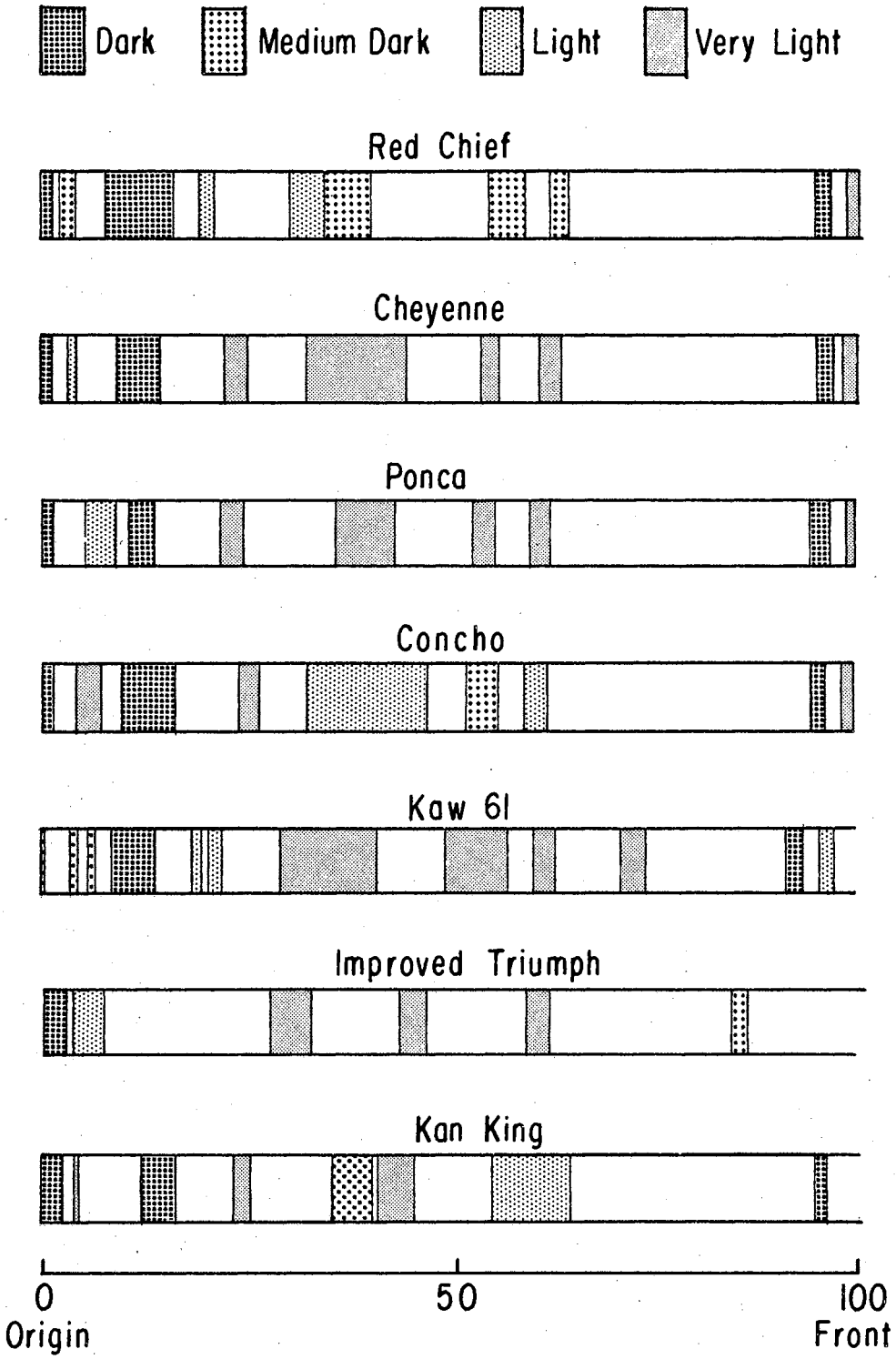

Figure 19. Diagrammatic interpretations of the electrophoretic separations of the soluble wheat leaf proteins from 10-weeks old plants grown in field plots. (pH 8.3)

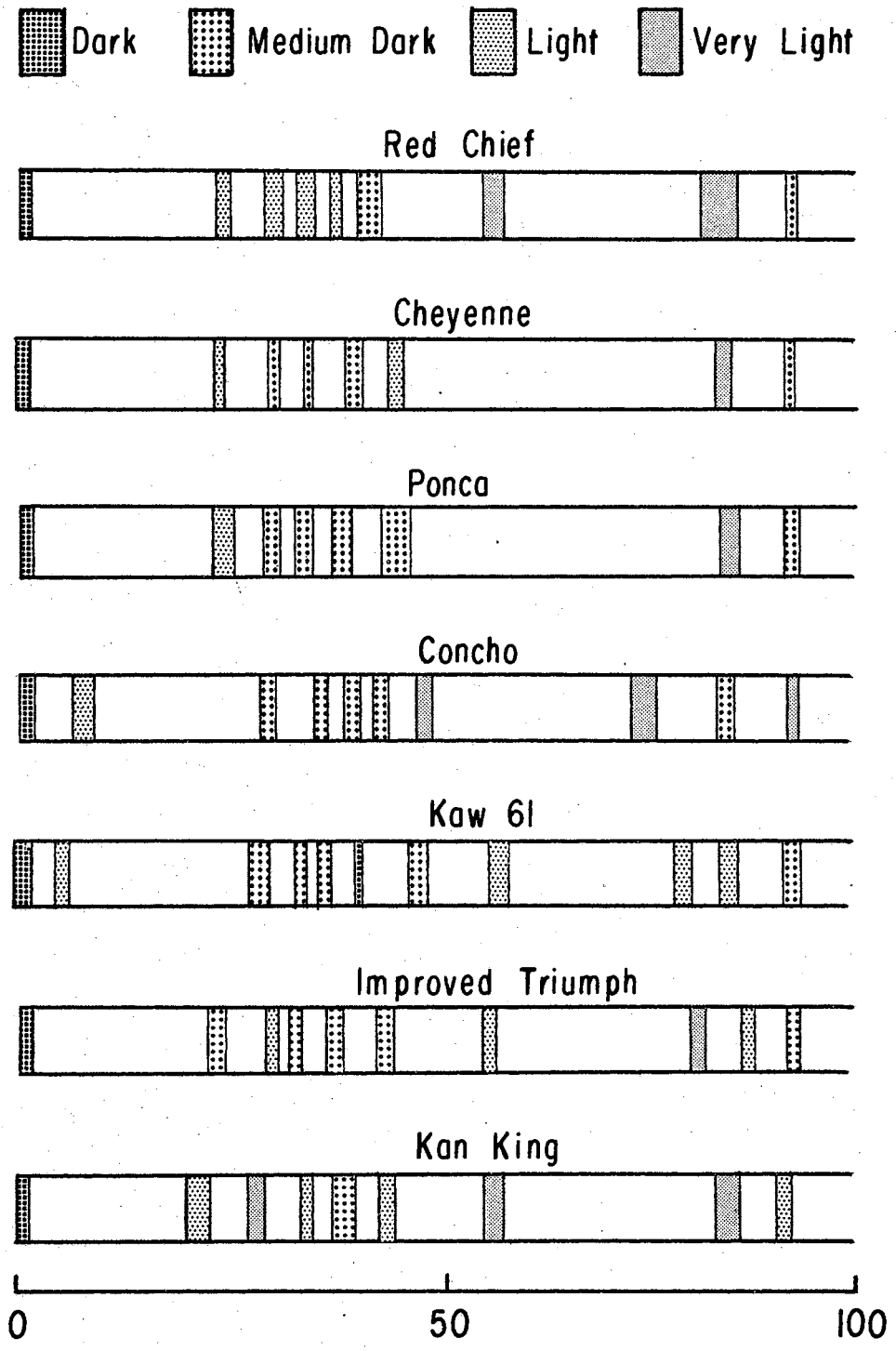

Figure 20. Diagrammatic interpretations of the electrophoretic separations of the soluble wheat leaf proteins from 10-weeks old plants grown in field plots (pH 4.5)

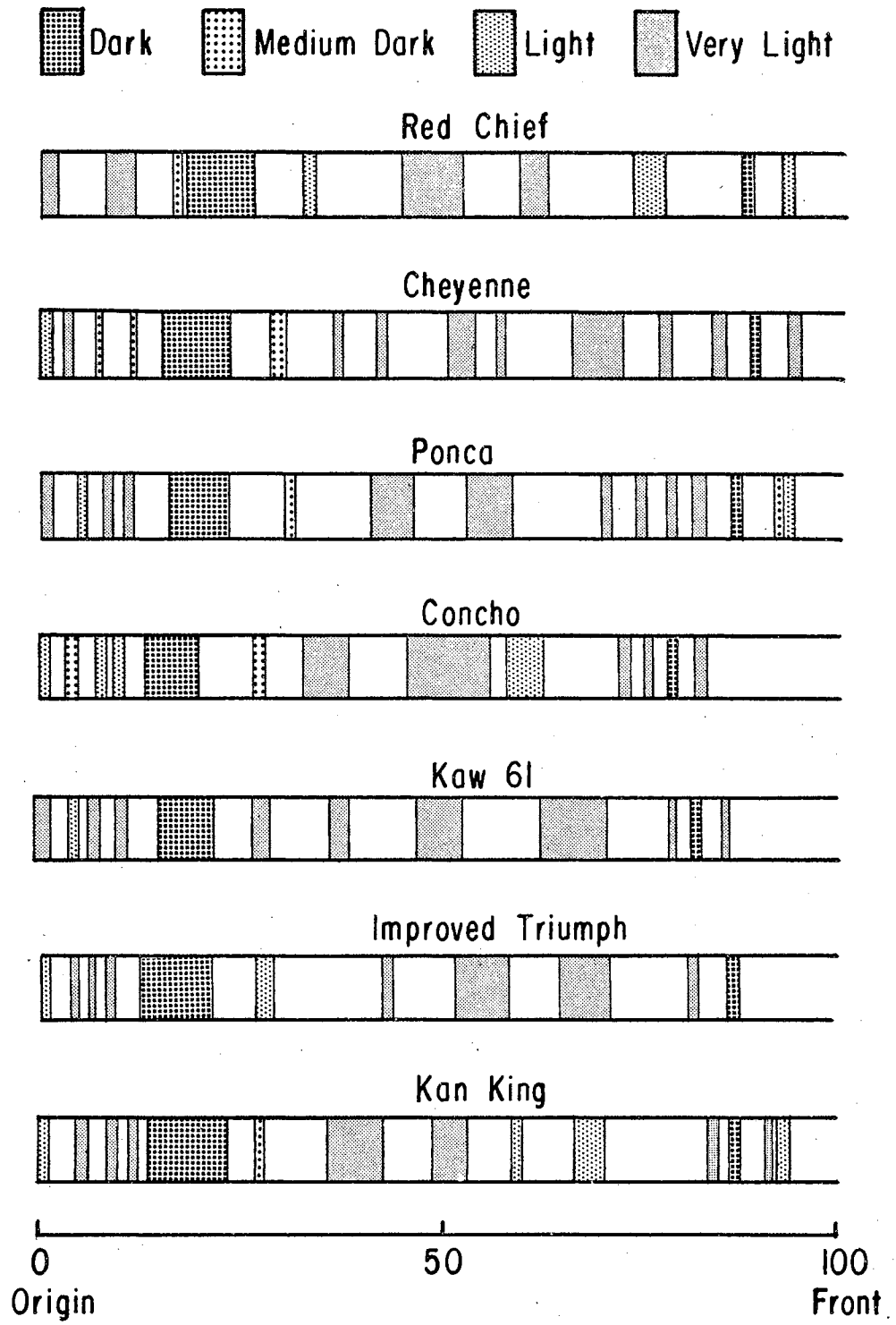

Figure 21. Diagrammatic interpretations of the electrophoretic separations of the soluble wheat leaf proteins from 20-weeks old plants grown in field plots. (pH 8.3)

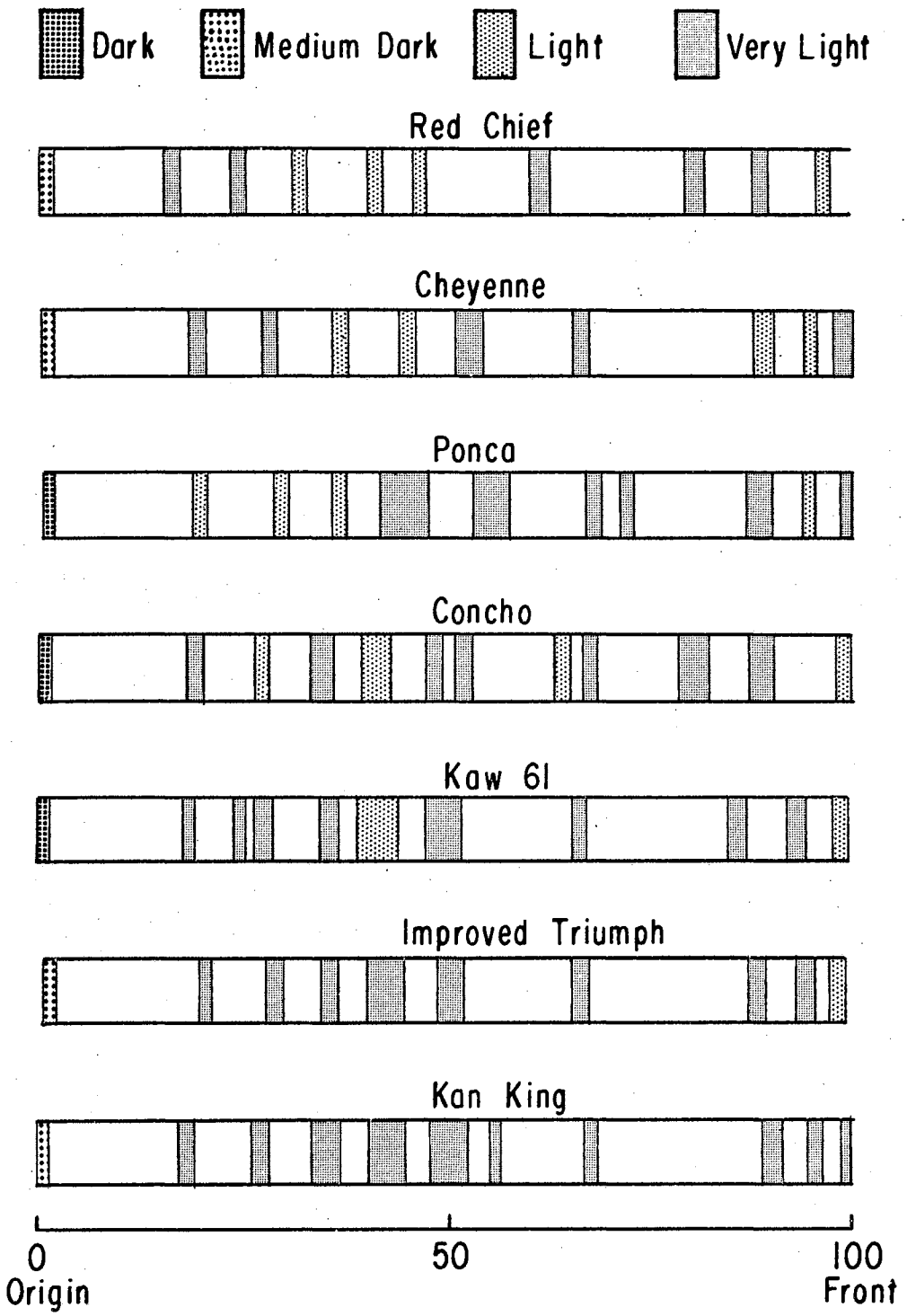

Figure 22. Diagrammatic interpretations of the electrophoretic separations of the soluble wheat leaf proteins from 20-weeks old plants grown in field plots. (pH 4.5)

during the period from October 12, to mid December, 1965, due to lack of rainfall. When comparing the 10-weeks old field grown plant with the controlled environment grown plant one could readily see a greater similarity in the wilted plant samples to the field grown samples. Representative samples of the 20-weeks old plants are shown in figures 21 and 22 which also show a progressive change in the protein types. The bands show differences among varieties with greater difference being found in the acidic proteins (pH 8.3) (Figure 21) than with basic proteins (pH 4.5) (Figure 22).

CHAPTER V

DISCUSSION

Sephadex gel column chromatography and acrylamide gel electrophoresis were shown to be very useful in the investigation of the effects of water stress on the protein components in wheat plant tissues.

Using Sephadex gel columns, Mendiola and Akazawa (1964) found the major protein component of rice leaves had enzymatic activities associated with what has been previously reported as fraction 1 protein (Wildman and Bonner, 1947; Lyttleton and Ts'o, 1958; Dorner et al., 1957; Park and Pon, 1961; and others). The elution profile obtained for wheat leaf material indicated that fraction A is of the same general type as fraction I protein.

Estimation of the molecular weights of the fraction A and B proteins using Sephadex gel-filtration (Whitaker, 1963; Andrews, 1964) indicates that the fraction A protein is greater than 100,000 molecular weight and fraction B has a molecular weight of approximately 20,000 or less. Fraction A is mobile in a 3% acrylamide gel but not able to penetrate more than a few millimeters in 7.5% acrylamide gel at pH 8.3 which indicates also its large molecular weight.

Steward et al. (1964) applied the acrylamide gel electrophoresis technique to the separation of components of the soluble proteins from different organs of pea seedlings with high levels of sensitivity and reproducibility. Steward (1965) provided evidence that the protein complement of cells changes as they developed, based on the changing

activities of several enzymes relative to each other.

The changes in wheat leaf protein composition under water stress is shown by changes in the number and location of protein bands from acrylamide gel electrophoresis. The protein bands show changes which occurred as a result of water stress for both the acidic and basic protein groups.

Todd and Basler (1965) showed the supernatant fraction from 27,000 x g centrifugation (which contained ribosomes) decreased in nucleic acids with increasing water stress. If one considers the RNA - basic protein (histone) concept of Leslie (1961), then one would expect that the degradation of the ribosomes would increase the amount and number of free basic proteins of small molecular weights. Proteins of this type may escape detection when using acid precipitation as a means of collecting proteins from buffer extracts for analysis since the smaller basic proteins are soluble in acid media. This could explain the proteins remaining near the same total amount during the water stress, while fraction A protein was decreasing with water stress, and could very well explain the different results which have previously been reported. Some investigators have shown an increase in protein with water stress whereas others have shown a decrease. When the protein was separated and different methods of protein analysis were used in analysing wheat leaf protein, the large molecular weight proteins decreased while the smaller molecular weights proteins increased. The hypothesis that water stress causes the breakdown of the synthesizing enzyme complex and releases protein fragments which have hydrolyzing properties may be accepted if the smaller molecular weight fraction could be shown to be composed of proteins with different enzyme

activity. Such a hypothesis would also be in agreement with the conclusion of Vickery (1956) that in leaves of stressed plants the enzymes for recovery had been depleted. A change can be seen in the wheat leaf proteins as a result of the action of water stress. Such a change could be related to the loss of synthesizing enzymes and an increase in the hydrolytic enzymes.

The decrease in RNA as well as the change in the ratios of nucleotides (as was shown in Part A) could very well be the reason for the lack of recovery of some plants from severe water stress. Recovery would be greatly hampered if the synthesizing systems of the plant tissue is badly damaged as a result of the breakdown of the RNA components and the synthesizing enzyme system. Hydrolysis may be an important means of mobilizing materials for translocation to a growing point where renewed growth would be possible when the plant was rewatered (Todd and Yoo, 1964). The lower limit of water stress which must not be exceeded by the plant in order for the plant to maintain itself without excessive hydrolysis could be a critical factor for the plant. The minimum level of water content at which a plant is able to maintain its structures is therefore not only related to the total amount of protein but also to the amino acid composition of the protein (Fisher, 1965).

The more resistant wheat varieties show a greater amount of large molecular weight proteins than the non-resistant varieties and therefore should have more bound water and, consequently, the more resistant varieties would contain a higher water content under comparable levels of desiccation (Todd et al., 1962).

GENERAL SUMMARY

The method used for the determination of nucleotide composition of RNA in plant tissue was a rapid method of determination, which had good repeatibility when using test samples from the same RNA source.

The effects of water stress on the RNA components indicates not only a loss in total RNA but also a change in the nucleotide composition, exemplified by an increase in the G + C type RNA. A consistent difference in nucleotide composition was not detected which could be applied in screening wheat varieties for resistance to water stress.

The total amount of RNA was generally found to be higher for the drought resistant variety (Cheyenne) than other less tolerant varieties when subject to comparable water stresses. The percentage losss of the nucleotides adenylic and uridylic acid was much greater than for guanylic and cytidylic acid. Such losses were noted with only a very small amount of water stress (75% water content). The results of the effect of water stress on the ribonucleic acid components in the wheat tissues were interrelated with the temperature and the light intensity.

Sephadex gel filtration and acrylamide gel electrophoresis were very useful as techniques for investigating the effects of water stress on protein components in the wheat plant. From the elution profile of proteins from Sephadex G-100, two fractions were separated. When the plants were subjected to water stress fraction A decreased in amount and fraction B increased, which corresponds to a decrease in the large molecular weight and an increase in the smaller molecular weight

proteins. The same type of response was also found when the proteins were separated by acrylamide gel electrophoresis. Water stress caused an increase in the number and quantities of protein of smaller molecular weight while the larger molecular weight proteins were decreasing in quantity. A similar response was noted for both the basic and acidic proteins. The level of water stress at which a plant is able to maintain sufficient synthesizing structural components, therefore, would determine its limits for water stress. The results of the nucleic acid and the protein studies would indicate that the more drought resistant wheat varieties are better able to maintain their structural components when they are subjected to wilting. These studies have opened up many areas which might be investigated in the overall response of water stress on plants. Investigation into the enzymatic nature of the various proteins which can be separated by acrylamide gel electrophoresis would probably be one of the more useful areas of investigation.

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APPENDIX

Procedure For Preparation of Dowex-1 Cl^- Form,

Anion Exchange Column

1. Prepare 1 x 8 cm column of resin.
2. Wash with 100 ml deionized H_2O.
3. 10 ml Hydrolysate (from alkaline hydrolysis of RNA)
4. 50 ml water.
5. 50 ml 0.1 N Ammonium Chloride.
6. 50 ml water.

Fraction 1 (F_1) - 100 ml 0.01 N Hydrochloric acid.

Read absorbance - 268.7 mu and 284 mu.

Fraction 2 (F_2) - 100 ml 0.1 N Hydrochloric acid.

Read absorbance - 268 mu and 290 mu.

Calculations For Nucleotide Composition:

F_1 Adenylic acid molarity

$(11.07 \times A_{268.7}) - (9.90 \times A_{284}) \times 10^{-5}$

Cytidylic acid molarity

$(9.901 \times A_{284}) - (1\ 631 \times A_{268.7}) \times 10^{-5}$

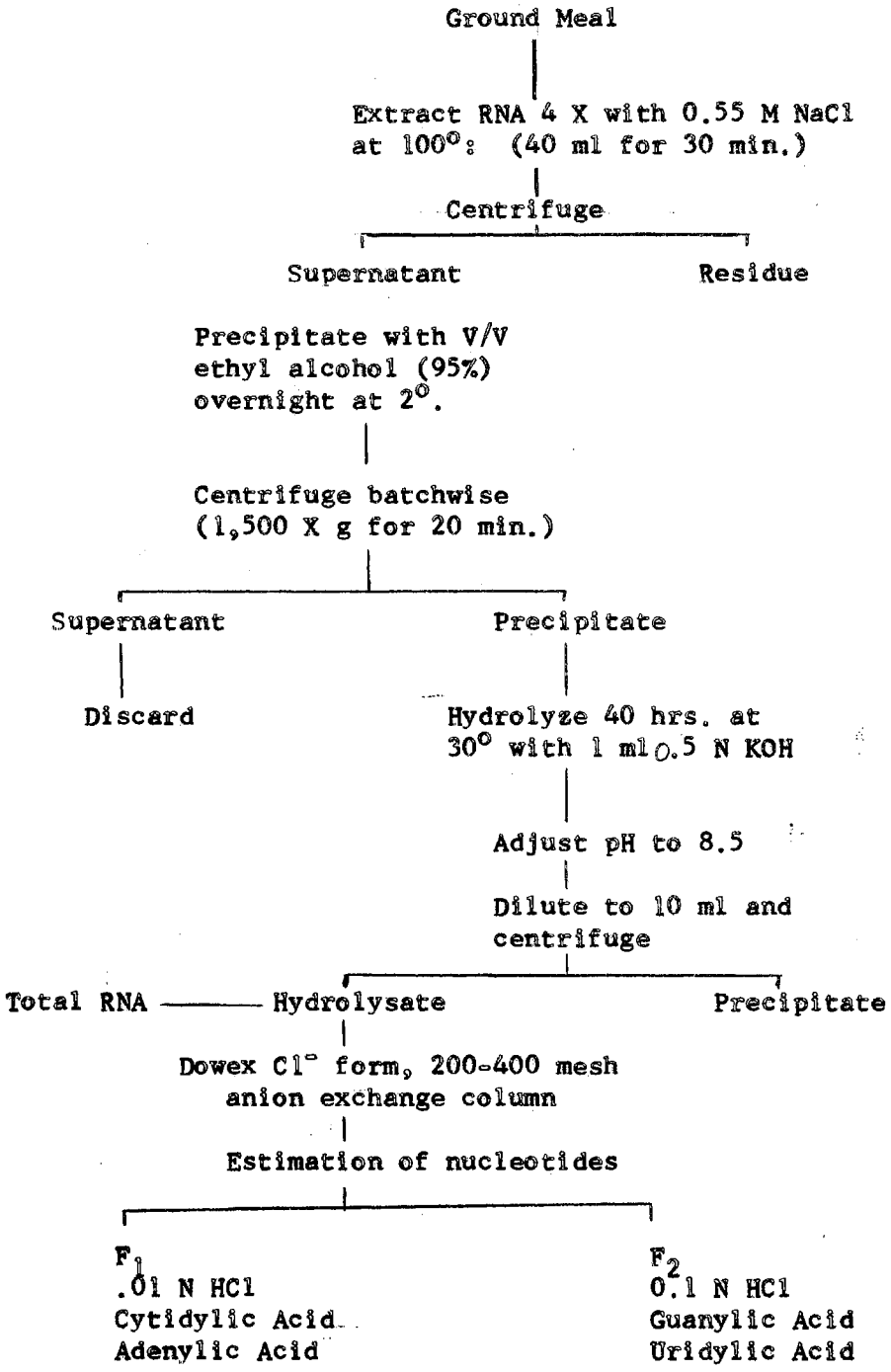
F_2 Guanylic acid molarity

$(20.329 \times A_{290}) - (0.681 \times A_{268}) \times 10^{-5}$

Uridylic acid molarity

$(12.674 \times A_{268}) - (21.111 \times A_{290}) \times 10^{-5}$

PROCEDURES FOR DETERMINING TOTAL RNA AND NUCLEOTIDE CONTENT



Preparation of Acrylamide Gel Columns

1. Stopper clean dry tubes with specially shaped silicone rubber stoppers.

2. Place tube in vertical position.

3. Mix equal parts of solutions # 3 and # 4 in aspirator flask. At room temperature remove air by evacuation. (small-pore solution)

4. Using a plastic syringe connected to a long tygon tube, the solution is then added into the tubes until the lowest mark on tube is reached. Any air bubbles should be removed.

5. Overlay the solution in the tube with a few millimeters of distilled water. A Pasteur capillary pipette with tip curvature turn up, is useful in getting a sharp interface between acrylamide liquid and water layer.

6. Leave at room temperature for approximately one hour for polymerization of the acrylamide.

7. Remove the water layer.

8. Mix equal parts of solution # 5 and water. Rinse the tubes above the gel and remove the solution immediately.

9. Mix equal parts of solution # 5 and water in an aspirator flask and remove air by evacuation. (large-pore solution)

10. Pipette the mixed solution into the tube to the desired amount for spacer gel (3-4 cm) and overlay with a small amount of water.

11. Expose the liquids to a strong fluorescent lamp at a distance of a few inches for gelification which is generally complete in approximately 30 minutes or less.

12. Remove the excess water layer and replace with the buffer system used in the compartments.

13. Allow gels to cool to operating temperature (approximately 2° - 4°) and apply sample (1-5 mg protein) in surcrose solution to the top of the gel.

14. Put the buffer for the system in the compartments.

15. Make connection to the power supply and turn on power supply. (Note: The power supply should not be turned on when the circuit is not complete.)

16. Adjust power supply settings:
pH 8.3 system - 5 mA/tube for 1 hour then 8.5 mA/tube for 4 hours.
pH 4.5 system - 8.5 mA/tube for 1 hour then 10 mA/tube for 3.5 hours.

17. Turn off power supply and remove gel from tubes and stain in 1% amido black in 7% acetic acid for 1 hour.

18. Leach out excess dye for about 4 hours in 7% acetic acid.

19. Place the gel in tubes with one end slightly constricted to retain the gel in the tubes and completely fill the tubes with 7% acetic acid. Remove all air bubbles and fill the compartments of the electrophoresis apparatus with 7% acetic acid.

20. Connect electrode to power supply; turn on power and adjust volt setting to about 600 to 800 volts. Destaining is usually accomplished in 6 to 8 hours. (Note: Avoid excess heating at high voltage by carrying out process under refrigeration.)

21. Remove gels from tubes and put into tubes with 1% formalin solution and label with essential information.

Stock Solution for pH 4.5 Acrylamide Gel System

A.
- 48 ml N-potassium hydroxide
- 17.2 ml Acetic acid (glacial
- 4.0 ml Tetramethylethylenediamine
- Water to make 100 ml

B.
- 48 ml N-potassium hydroxide
- 2.87 ml Acetic acid (glacial)
- 0.46 ml Tetramethylethylenediamine
- Water to make 100 ml

C.

7.5%	15%	
30 g	60 g	Acrylamide
0.8 g	0.4 g	Methylenebisacrylamide

Water to make 100 ml

D.
- 10 g Acrylamide
- 2.5 g Methylenebisacrylamide
- Water to make 100 ml

E.
- 4.0 mg Riboflavin
- Water to make 100 ml

F. Compartment buffer
- 31.2 g β-alanine
- 8.0 ml Acetic acid (glacial)
- Water to make 1 liter

Working Solution Prepared from Stock Solutions

Small-pore solution:
- 1 part A
- 2 parts C
- 1 part water

Mix before use with an equal volume of a freshly prepared solution of ammonium persulphate (0.28 gm/100 ml).

Large-pore solution:
- 1 part B
- 2 parts D
- 1 part E
- 4 parts water

Stock Solutions for pH 8.3 Acrylamide Gel System

Solution 1:

75 g Acrylamide
2 g N-N-Methylenebisacrylamide
0.038 g Potassium ferricyanide
Make up to 250 ml with water

Solution 2:

24 ml 2 normal HCl
36 g 2-amino - 2 hydroxymethyl-1,3 propanediol (Tris buffer)
0.46 ml N,N,N^1,N^1 - Tetramethylenediamine
Make up to 250 ml with water

Solution 3:

Mix equal parts of solutions # 1 and # 2; if refrigerated, this is stable for several weeks.

Solution 4:

Catalyst for gelification of solution # 3, prepare a 0.14% ammonium persulfate in H_2O.

Solution 5:

20 g Acrylamide
5.0 g N-N-Methylenebisacrylamide
1.71 ml 86% phosphoric acid
5.7 g 2-amino - 2 hydroxymethyl - 1,3 propanediol (Tris buffer)
0.004 g Riboflavin
Make up to 400 ml with water

Solution 6:

Buffer for compartments:
12.11 g (Tris) 2-amino - 2-hydroxymethyl-1,3 propanediol
7.5 g Glycine
1000 ml Water
Adjust pH if necessary

TABLE V

NUCLEOTIDE COMPOSITION OF 4 - WEEKS OLD WHEAT PLANTS

GROWN IN GREENHOUSE

Water Stress Days	ug/gm Dry Wt. RNA	*% RT	Moles/100 Moles Nucleotide Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Cheyenne Leaves						
0	6100	89	21.0	25.6	36.4	17.0
4	4000	68	18.7	28.8	32.4	20.1
8	2600	30	19.2	30.4	30.4	20.0
Ponca Leaves						
0	6300	89	21.2	26.4	31.8	20.6
4	4600	58	22.5	25.3	29.0	23.2
8	2600	30	26.3	28.3	29.4	18.7

* RT - Relative Turgidity

TABLE VI

NUCLEOTIDE RATIOS OF 4 - WEEKS OLD WHEAT LEAVES

GROWN IN GREENHOUSE

Water Stress Days	Moles/gm Dry Wt. 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Leaves														
Cheyenne														
0	11.76	2.40	3.05	4.27	2.04	1.24	1.22	1.73	1.51	1.42	2.14	1.65	0.87	1.30
4	5.63	1.24	1.91	1.15	1.33	0.93	1.54	1.73	1.43	1.13	1.61	1.58	0.90	1.05
8	5.08	0.97	1.54	1.54	1.03	0.96	1.58	1.58	1.52	1.00	1.52	1.52	0.98	0.97
Ponca														
0	11.38	2.41	3.02	3.60	2.35	1.03	1.25	1.50	1.28	1.20	1.54	1.48	0.91	1.12
4	8 22	1.84	2.09	2.39	1.90	0.97	1.12	1.29	1.09	1.15	1.25	1.20	0.92	1.06
8	4.23	1.00	1.20	1 24	0.79	1.41	1.08	1.12	1.51	1.04	1.57	1.36	1 08	1.12

Average of three tests

TABLE VII

NUCLEOTIDE COMPOSITIONS OF 6 - WEEKS OLD

WHEAT PLANTS GROWN IN GREENHOUSE

Days Water Stressed	ug/gm Dry Wt. RNA	Moles/100 Moles Nucleotide			
		Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
*Cheyenne Leaves					
0	7920	20.72	25.95	30.72	22.61
4	5480	19.38	26.80	32.37	21.44
8	7000	17.84	25.31	37.12	20.09
Ponca Leaves					
0	8200	20.31	24.75	32.62	22.32
4	5920	19.19	25.72	33.01	22.07
8	5920	16.05	24.72	39.67	19.56
Cheyenne Crowns					
0	10,800	20.13	24.31	51.38	4.26
4	8,600	18.98	24.88	51.67	4.47
8	9,400	19.71	22.08	38.96	19.25
Ponca Crowns					
0	13,000	19 85	24.68	51.81	4.29
4	10,160	18.59	27.41	51.42	2.58
8	8,880	20.12	21.21	38.23	20.43

* Average of three tests

TABLE VIII

NUCLEOTIDE RATIOS OF 6 - WEEKS OLD WHEAT PLANTS

GROWN IN GREENHOUSE

Water Stress Days	Moles/gm Dry Wt. 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
*Leaves														
Cheyenne														
0	6.90	1.43	1 79	2.12	1 56	0.92	1.25	1.48	1.15	1.18	1.36	1.30	0.88	1.05
4	4.85	0.94	1.30	1.57	1.04	0.90	1.38	1.67	1.25	1.21	1.51	1.45	0.86	1.07
8	6.52	1.14	1.65	2.42	1.31	0.89	1.42	2.08	1.26	1.47	1.85	1.66	0.75	0.92
Ponca														
0	6.99	1.42	1.73	2.28	1.56	0.91	1.22	1.61	1.11	1.32	1.46	1.35	0.82	1.12
4	5.21	1.00	1.34	1.72	1.15	0.87	1.34	1.72	1.17	1.28	1.50	1.42	0.81	1.09
8	5.42	0.87	1.34	2.15	1.06	0.82	1.54	2.47	1.26	1.60	2.03	1.80	0.69	1.25
Crowns														
Cheyenne														
0	15.96	3.20	3.88	8.20	0.68	4.73	1.21	2.55	5.70	2.11	12.06	3.12	0.79	2.52
4	12.54	2.38	3.12	6.48	0.56	4.25	1.31	2.72	5.57	2.08	11.56	3.24	0.78	2.40
8	13.09	2.58	2.89	5.10	2.52	1.02	1.12	1.98	1.15	1.76	2.02	1.56	0.72	1.41
Ponca														
0	18.64	3.70	4.60	9.54	0.80	4.63	1.24	2.58	5.75	2.07	11.93	3.12	0.80	2.46
4	14.74	2.74	4.04	7.58	0.38	7.20	1.47	2.77	10.66	1.88	19.93	3.72	0.84	2.34
8	12.92	2.60	2.74	4.94	2.64	.98	0.95	1.90	1.04	1.80	1.87	1.47	0.71	1.40

*Average of three tests; 1 gm. Dry Wt./sample.

TABLE IX

NUCLEOTIDE COMPOSITIONS OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 16° DAYS AND 10° NIGHTS

Days Water Stressed	ug/gm Dry Wt. RNA	ug.RNA / Plant	Moles/100 Moles Nucleotide Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Cheyenne Leaves						
0	5387	107.6	17.5	28.7	34.8	19.0
4	5543	138.5	19.4	28.8	31.7	20.1
8	4875	130.0	18.5	28.7	32.2	20.6
C	5440	94.5	19.4	29.0	32.6	19.0
Ponca Leaves						
0	5387	101.0	20 8	27.8	29.7	21.7
4	5337	115.0	19.5	27.6	33.8	19.1
8	4003	94.0	20.0	27.5	34.0	18.5
C	5850	110.0	17.7	28.8	35.4	18.1
Cheyenne Crowns						
0	3438	26.2	12.8	27.2	40.7	19.3
4	5697	49.4	14.0	27.2	39.4	19.4
8	5543	48.5	14.6	28.0	39.4	18.0
Ponca Crowns						
0	2566	17.5	13.6	27.4	43.8	15.2
4	5337	35.5	14.8	28.2	38.2	18.8
8	5286	37.0	16.9	27.5	37.3	18.3

TABLE X

NUCLEOTIDE RATIOS OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 16° DAYS AND 10° NIGHTS

Water Stress Days	Moles/gm Dry Wt. 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Leaves														
Cheyenne														
0	9.14	1.62	2.62	3.17	1.73	0.92	1.64	1.99	1.51	1.21	1.83	1.72	1.07	1.10
4	8.81	1.71	2.54	2.79	1.77	0.97	1.48	1.63	1.43	1.10	1.58	1.53	0.94	1.04
8	8.61	1.59	2.47	2.78	1.77	0.90	1.55	1.74	1.39	1.12	1.56	1.47	0.89	1.03
C	9.57	1.86	2.77	3.12	1.82	1.02	1.49	1.68	1.53	1.12	1.72	1.60	0.94	1.08
Ponca														
0	9.33	1.94	2.59	2.77	2.03	0.96	1.34	1.43	1.28	1.07	1.37	1.35	0.94	1.02
4	9.40	1.83	2.60	3.17	1.80	1.02	1.42	1.73	1.45	1.22	1.77	1.59	0.89	1.14
8	7.14	1.43	1.97	2.42	1.32	1.08	1.38	1.70	1.49	1.24	1.84	1.59	0.91	1.17
C	9.92	1.76	2.86	3.51	1.78	0.98	1.63	2.00	1.59	1.23	1.96	1.80	0.87	1.13
Crowns														
Cheyenne														
0	8.72	1.12	2.37	3.55	1.68	0.67	2.13	3.18	2.13	1.50	2.11	2.11	0.69	1.15
4	13.29	1.86	3.61	5.24	2.58	0.72	1.94	2.81	1.94	1.45	2.03	2.00	0.71	1.15
8	13.78	2.00	3.85	4.43	2.50	0.80	1.92	2.70	1.92	1.41	2.19	2.04	0.74	1.01
Ponca														
0	6.91	0.94	1.89	3.02	1.06	0.89	2.01	3.22	2.01	1.60	2.88	2.46	0.70	1.34
4	12.62	1.86	3.53	4.85	2.38	0.79	1.90	2.58	1.91	1.35	2.03	1.97	0.71	1.14
8	13.01	2.20	3.57	4.86	2.38	0.92	1.63	2.21	1.63	1.36	2.04	1.84	0.80	1.19

TABLE XI

NUCLEOTIDE COMPOSITION OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 21° DAYS AND 16° NIGHTS

Days Water Stressed	ug/gm Dry Wt. RNA	ug.RNA Plant	Moles/100 Moles Nucleotide Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Cheyenne Leaves						
0	3772	93.8	19.8	27.4	33 6	19 2
4	2874	66.3	18.3	27.8	35.6	18.3
8	2514	51.8	15.6	30.8	38.6	15.0
C	5646	102.5	18.6	27.3	34.6	19.5
Ponca Leaves						
0	4875	89.7	20.3	28.8	33.7	17.2
4	4824	80.0	20.2	28.6	34.6	16.6
8	0616	10.9	09.9	32 6	41.0	16.5
C	7288	88 5	18.7	27.8	36.3	17 2
Cheyenne Crowns						
0	3284	22.8	09.1	28.7	45.5	16.7
4	4003	30.4	16 4	26 4	36.1	21 1
8	46 19	32 8	12.7	27.4	39.8	20 1
Ponca Crowns						
0	4773	26.0	16.1	29 0	36.4	18.5
4	5253	34.0	15 3	27.3	38 2	14.2
8	2208	13.4	12.4	27.5	50.2	09.9

TABLE XII

NUCLEOTIDE RATIOS OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 21° DAYS AND 16° NIGHTS

Water Stress Days	Total	Moles/gm Dry Wt. 10^{-6}				Ratios								
		A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Leaves														
Cheyenne														
0	6.93	1.26	1.75	2.14	1.22	1.03	1.38	1.70	1.43	1.23	1.75	1.56	0.90	1.14
4	5.12	0.96	1.43	1.87	0.96	1.00	1.52	1.95	1.52	1.28	1.95	1.73	0.86	1.18
8	4.13	0.80	1.58	1.98	0.77	1.04	1.97	2.47	2.05	1.25	2.57	2.27	0.86	1.18
C	8.44	1.57	2.30	2.92	1.65	0.95	1.47	1.86	1.40	1.27	1.77	1.59	0.84	1 14
Ponca														
0	7.49	1.52	2.16	2.52	1.29	1.18	1.42	1.66	1.67	1.17	1.96	1.66	0.97	1.17
4	7.33	1.48	2.10	2.53	1.22	1.22	1.42	1.71	1.72	1.21	2.08	1.72	0.95	1.20
8	1.60	0.16	0.52	0.65	0.27	2.42	3.29	4.14	1.98	1.26	2.48	2.80	0.74	1.03
C	10.77	2.01	2.99	3.91	1.86	1.09	1.49	1.94	1.62	1.31	2.11	1.79	0.87	1.22
Crowns														
Cheyenne														
0	7.48	0.68	2.15	3.39	1.26	0.54	3.15	5.04	3.15	1.59	2.72	2.86	0.61	1 19
4	9.75	1.62	2.54	3.53	2.06	0.79	1.61	2.20	1.61	1.37	1.71	1.65	0.74	1.13
8	10.36	1.32	2.84	4 12	2.08	0.63	2.16	3.13	2.16	1.45	1.98	2.05	0.67	1.10
Ponca														
0	11.30	1 82	3.28	4.12	2.08	0.88	1.80	2.26	1.81	1.26	1.97	1.90	0.82	1.11
4	10.82	1.66	2.96	4.12	2.08	0.80	1.78	2.50	1.78	1.40	2.69	1.89	0.75	1.15
8	5.66	0.70	1.55	2.85	0.56	1 25	2.22	4.05	2.22	1.83	5.07	3.49	0.65	1 68

TABLE XIII

NUCLEOTIDE COMPOSITION OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 27° DAYS AND 21° NIGHTS

Days Water Stressed	ug/gmDry Wt. RNA	ug/RNA Plant	Moles/100 Moles Nucleotide			
			Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Cheyenne Leaves						
0	6004	122.0	25.2	27.6	30.1	17.1
4	3720	60.3	21.2	27.9	31.7	19.2
8	4516	67.3	21.0	29.5	30.8	18.7
C	6466	102.0	19.5	28.9	29.9	21 7
Ponca Leaves						
0	5337	84.8	23.0	29 6	30.4	17.0
4	4388	68.2	22.4	30.1	29.1	18.4
8	3387	42.0	19.0	31.0	34.6	15 4
C	6260	89.5	17.8	28.2	36.6	17.4
Cheyenne Crowns						
0	3336	20.9	15 4	17.0	43.5	22 5
4	2617	16.42	16.5	28.8	37.1	17.6
8	5645	36 6	12.8	29.8	39.5	17.9
Ponca Crowns						
0	2515	13.5	12.5	34.8	33.7	19.0
4	2874	18.3	14.8	29.2	38.6	17.4
8	2412	12 9	13.9	30.0	41.4	14 7

TABLE XIV

NUCLEOTIDE RATIOS OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT

AT 27° DAYS AND 21° NIGHTS

Water Stress Days	Moles/gm Dry Wt 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Leaves														
Cheyenne														
0	7.41	1.87	2.05	2.23	1.26	1.47	1.10	1.19	1.61	1.09	1.76	1.36	1.12	1.24
4	5 56	1.18	1.55	1.76	1.07	1.10	1.32	1.49	1.45	1.14	1.65	1.47	0.97	1.12
8	7.24	1.52	2.13	2.23	1.36	1 12	1.41	1.47	1.58	1.04	1.65	1.52	1.02	1.08
C	10.31	2.01	2.99	3 11	2.20	0.90	1.48	1.53	1.33	1.03	1.38	1.45	0.94	0.99
Ponca														
0	8.14	1.87	2.42	2.47	1.36	1.35	1.29	1.32	1.74	1.03	1.79	1.48	1.11	1.14
4	7.02	1 57	2.12	2.04	1.29	1.22	1.34	1.30	1.64	0.97	1.58	1.45	1.10	1.06
8	4.43	0.84	1.37	1.53	0.69	1.23	1.63	1.82	2.01	1.12	2.25	1.89	1.00	1.15
C	10.68	1.90	3.01	3.91	1.86	1.02	1.58	2.06	1.62	1.30	2.10	1.84	0.86	1.19
Crowns														
Cheyenne														
0	6.23	0.96	1.06	2.81	1.40	0.69	1.10	2.82	1 10	2.56	1.93	1.64	0.48	1.53
4	8.13	1.34	2.34	3.01	1.44	0.93	1.74	2.25	1.75	1.29	2.11	1.92	0.83	1.15
8	11.41	1.46	3.41	4.50	2.04	0.72	2.33	3.09	2.33	1.33	2.21	2.26	0.73	1.09
Ponca														
0	6.64	0.83	2.31	2.24	1.26	0.66	2.78	2.70	2.78	.97	1.77	2.17	0.93	0.86
4	7 31	1.08	2.13	2.82	1.28	0.84	1.97	2.61	1.97	1.32	2.22	2.10	0.78	1.14
8	6.88	0 96	2.06	2 84	1.02	0.94	1.16	2.98	2.16	1.38	2.82	2.47	0.78	1.23

TABLE XV

NUCLEOTIDE COMPOSITION OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 41° DAYS AND 21° NIGHTS

Days Water Stressed	ug/gm Dry Wt. RNA	ug.RNA / Plant	Moles/100 Moles Nucleotide			
			Adenylic Acid	Cytidylic Acid	Guanylic Acid	Uridylic Acid
Cheyenne Leaves						
0	4310	70.6	16.5	28.2	35.3	20.0
4	6882	78.0	19.2	27.1	35.8	17.9
8	8980	101.0	26.0	28.9	34.7	10.4
Ponca Leaves						
0	3613	52.0	16.6	29.6	32 3	21.5
4	8376	123.0	21.9	28.6	29.3	20.2
8	9545	99.5	28.0	28.2	32.3	11.5
Cheyenne Crowns						
0	3800		20.4	23.6	37.5	18.5
4	2770		18.6	28.0	34.8	18.6
8	4160		23.3	24.4	36.8	15.5
Ponca Crowns						
0	4620		18.6	24.1	39.5	17.8
4	4970		13.9	22.0	45.6	18.5
8	4360		16.9	25.2	41.5	16.4

TABLE XVI

NUCLEOTIDE RATIOS OF 4 - WEEKS OLD WHEAT PLANTS GROWN IN CONTROLLED ENVIRONMENT AT 41° DAYS AND 21° NIGHTS

Water Stress Days	Moles/gm Dry Wt. 10^{-6}					Ratios								
	Total	A	C	G	U	A/U	C/A	G/A	C/U	G/C	G/U	$\frac{G+C}{A+U}$	$\frac{A+C}{G+U}$	$\frac{A+G}{C+U}$
Leaves														
Cheyenne														
0	6.35	1.05	1.79	2.24	1.27	0.83	1.71	2.14	1.41	1.25	1.77	1.73	0.81	1.08
4	10.42	1.99	2.83	3.74	1.86	1.07	1.41	1.86	1.51	1.32	2.00	1.71	0.86	1.22
8	9.80	2.55	2.83	3.40	1.02	2.50	1.11	1.33	2.78	1.20	3.34	1.75	1.22	1.55
Ponca														
0	6.32	1.05	1.87	2.04	1.36	0.77	1.78	1.95	1.38	1.09	1.50	1.62	0.86	0.96
4	13.26	2.91	3.79	3.89	2.67	1.08	1.31	1.34	1.42	1.02	1.45	1.38	1.02	1.05
8	12.25	3.43	3.46	3.96	1.40	2.43	1.01	1.15	2.45	1.15	2.81	1.54	1.28	1.52
Crowns														
Cheyenne														
0	17.27	3.50	4.08	6.49	3.20	1.10	1.16	1.84	1.16	1.59	2.03	1.58	0.79	1.37
4	14.61	2.73	4.10	5.00	2.70	1.00	1.51	1.87	1.51	1.24	1.87	1.69	0.88	1.14
8	15.74	3.66	3.85	5.80	2.43	1.50	1.05	1.58	1.05	1.51	2.37	1.60	1.24	1.51
Ponca														
0	17.32	3.20	4.18	6.84	3.10	1.04	1.30	2.12	1.30	1.64	2.22	1.75	0.75	1.38
4	14.48	2.70	4.28	8.90	3.60	0.75	1.58	3.28	1.58	2.07	2.46	2.08	0.55	1.47
8	15.72	2.66	3.98	6.52	2.56	1.03	1.49	2.46	1.49	1.65	2.53	2.01	0.73	1.40

VITA

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