NUCLEIC ACIDS IN LOW- AND HIGH-PROTEIN LINES OF WHEAT AND OTHER GRAIN SPECIES

by 45

PETER ROBERT HOBBS

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Major Professor

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INTRODUCTION

Proteins are essential dietary constituents for humans. Proteins are also the constituents in greatest shortage in developing countries. Because cereals are the primary source of protein for human consumption, a greater understanding of the physiology and biochemistry of protein biosynthesis in crop plants and the regulating factors involved is needed to increase protein yields.

Nucleic acids were believed for many years to be associated with protein biosynthesis (Brachet, 1946). However, it was not until the early 1960's that definite proof was available. Nirenberg et al. (1961) showed that adding polyuridylic acid, synthesized by the first synthesizing enzyme to be discovered (Grunberg, 1963), to a cell-free protein synthesizing system resulted in formation of polyphenylalanine. This led rapidly to allocation of all the basic triplets in the genetic code (Crick, 1963) and further postulations of the complex mechanism of protein synthesis.

There have been several studies on the nucleic acid contents of plant material. Difficulties in extracting nucleic acids in pure and undegraded forms from plant tissue compared to animal and microbial cells has limited such research. A study by Niemand and Poulsen (1963), however, using a modified Schmidt and Thannhauser (1945) procedure, yielded reasonable results with plant tissue. Thus, a study using their procedure was initiated to determine whether nucleic acid contents were correlated with grain protein contents in various grain crops.

A 'high' and a 'low' grain protein line of several grain crop species

were analyzed for total ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents in seedling leaf blade tissue. Crosses between high and low grain protein wheat varieties were also studied to relate nucleic acid content with grain protein. A field experiment with high and low protein wheat varieties related changes in total RNA and DNA contents of various plant parts to the ultimate grain protein content.

LITERATURE SURVEY

Most seed plants accumulate storage proteins for utilization by the developing embryo during germination. Biochemical changes in the developing grain have been studied extensively, especially in wheat and corn. The developing grain consists mainly of testa and pericarp tissues during the early stages and of endosperm tissue during the later stages. The endosperm is the predominant storage tissue of the grain.

Jennings (1963), working with wheat, showed that fresh weight and dry weight of the grain increased until 35 days after anthesis. The moisture content then declined and marked the onset of maturation. A similar trend was observed in the endosperm tissue, with water loss occurring 35 days after anthesis. Synthesis of storage proteins and starch in the endosperm at the expense of soluble constituents accounted for a rapid increase in the dry weight of the developing grain after fertilization (Bressani, 1961). Between the fourteenth day after anthesis and maturity, the NaOH-soluble storage proteins increased considerably compared with the buffer-soluble proteins.

Ingle (1965) showed that the development of the corn grain endosperm also occurred in two phases. The initial phase lasted for the first 28 days after pollination and was characterized by an accumulation of soluble constituents, probably by translocation from other plant parts, and by synthesis of proteins and nucleic acids. The second phase from day 28 to 40 consisted of utilization of the soluble constituents for synthesis of storage compounds.

Ingle (1965) also noted that DNA in corn grain increased from day 8 to 19 and then remained constant. If one assumes that the DNA content per nucleus is constant, then cell division ceased after day 19 and cell expansion became important.

Starch predominates in the endosperm of the wheat soon after anthesis and, although protein synthesis commences soon after anthesis, it lags behind starch synthesis (Jennings, 1963). Protein synthesis is accompanied by a rapid decline in the free amino acids in the endosperm. The free amino acids and other N sources probably arise from other plant parts by translocation (Jennings, 1963).

Carr and Wardlaw (1965) showed by $C^{14}O_2$ exchange that most of the assimilates of the wheat grain come from the ear, the flag leaf, and the green stem immediately below the ear. Carbon^{14} has also been used to follow assimilates in other cereals. Stoy (1963) reviewed this work and came to the same conclusions as Carr and Wardlaw (1965). Some results varied for different cereals, however. Carr and Wardlaw (1965) also found that the distribution of the assimilates in wheat grain changed as the leaves senesced and the ears matured. Photosynthesis by the ear increased up to 15 days after anthesis, while that by the leaves declined after anthesis.

Johnson (1967) found the low-protein wheat 'Warrior' contained more soluble nitrogen than the high-protein variety 'Atlas 66' in the leaves. He suggested Warrior was better able to absorb available soil nitrogen, but was less able to translocate nitrogen to the grain for protein synthesis. Johnson (1967) therefore separated N uptake and N translocation into two separate and independent physiological functions in wheat plants. McNeal (1966) came to the same conclusions, but also suggested that high-protein wheat varieties

were better able to synthesize protein in the grain itself. Croy (1968) has shown protease levels were higher in wheat varieties having high grain protein, and suggested the nitrogen constantly broken down by the proteases is translocated to the ear for synthesis of grain protein.

Jennings (1963) observed that in the early stages of wheat grain development, endosperm extracts had absorption spectra typical of nucleic acids. At later stages, however, the endosperm extracts contained a higher proportion of protein. Ingle (1965) also showed that the RNA content of corn grain increased up to 19 days after anthesis and then decreased. Matsushita (1959), working on the development of rice and wheat, concluded that the RNA contents of the grain increased during the early stages of development, but decreased to a constant level during maturation.

Key (1950) showed that RNA and protein synthesis were essential for cell elongation in soybean hypocotyl tissue. His evidence suggested that auxins controlled RNA synthesis and thus protein synthesis to regulate cell elongation. Harris (1960) has since shown that protein biosynthesis is related to RNA synthesis. Studies on the development of the wheat grain showed that during the period of rapid increase in the endospermal protein, a related increase in the endospermal RNA occurred (Harris, 1960). Harris (1960) also suggested that storage proteins were produced on the ribosomes of the endoplasmic reticulum in the endospermal tissue and afterwards were secreted internally to form protein storage bodies. Jennings (1963) supported the view of protein bodies and quoted examples of investigations by Duvick (1955, 1961) with corn, Watson (1955) with sorghum, and Aetschiel (1961) with peanuts, that have also observed protein bodies.

Matsushita (1957), working on the development of rice and wheat

seedlings, indicated that RNA synthesis occurred very early during the developmental sequences, and that the RNA was then degraded by ribonucleases. He also showed ribonuclease activity in maturing wheat grain, but that the mature grain still contained nucleic acids. Ingle (1965) also found increasing amounts of ribonuclease, little RNA, and large amounts of protein present in corn grain at maturity.

Changes occurring in the nucleic acid fractions during germination of a variety of plant species have been studied by numerous investigators. Such investigations have not, however, led to a clear understanding of the nucleic acid reserves in seeds or the mechanisms by which nucleic acid reserves are used by the growing embryo. Ingle (1965) showed that the corn embryo developed in a linear manner during the 40 days of maturation. He then suggested that de novo synthesis of RNA occurred in the embryo during germination, because of low RNA content in the endosperm. That contrasted with other cereals where RNA was higher in the mature grain, and where the embryo was less well developed. Pulse crops were shown by Kondo and Morita (1957) to contain even more nucleic acids in the mature seed while possessing a less well developed embryo.

Matsushita (1957) showed that RNA in wheat endosperm was degraded prior to transport into the embryo. Ledoux (1962) reported RNA was carried as macromolecules to the embryo. Sisakyan and Odintsova (1960) suggested RNA was fixed on the structures of the cell occurred as organisms develop and was thus excluded from the kinetics of metabolism. However, research indicated nucleic acids of the mature grain probably served in protein synthesis in some way as the seed germinates.

The major event leading to the present rate of nucleic acid research

was the discovery by Avery et al. (1944) that the genetic material is DNA. Nine years later, some of the mechanisms of nucleic acids were more clearly defined as a result of the very elegant Watson-Crick-Wilkins (1953) helical structure for DNA. Since then, nucleic acids have been clearly implicated in protein biosynthesis. Most of the evidence has come from cell-free preparations that incorporate amino acids into protein. Most work has been done with preparations from rat liver (Zamecnik et al., 1956) and from microorganisms (Tissieres, 1960). However, confirmatory results in higher plant systems have reaffirmed that protein biosynthesis is universally the same (Mans, 1961).

Studies on the mechanisms of protein synthesis with plant materials were initiated by Stephenson, Thimann, and Zamecnik (1956), who applied techniques developed for mammalian tissues to tobacco leaves. Mans and Novelli (1961) found cell free preparations of maize seedlings contained all the essential components found necessary in microbial studies for protein biosynthesis.

DNA had long been known to be the chemical involved in genetic and hereditary variation. Crick, Watson and Wilkins (1953) work on the helical configuration of DNA led to the work of Kornberg (1961) who showed that DNA was the template in the directed enzymatic synthesis of sequentially specific heteropolynucleotides. The millions of nucleotides in DNA are specifically ordered to form a code for all the various ordered proteins found in living matter. The work of Nirenberg (1961) showed that adding polyuridylic acid to a cell free protein synthesizing system resulted in formation of polyphenylalanine. This led to the allocation of all the base triplets in the genetic code, and the hypothesis that the immense number of triplets present

in one molecule of DNA, each coding for a specific amino acid, were responsible for the great genetic diversity found in living systems.

Ribonucleic acids translate the DNA code into the protein end products. Three types of RNA, soluble t-RNA, messenger m-RNA, and ribosomal r-RNA, are actively involved in protein biosynthesis.

Messenger RNA was discovered by Vinograd and Hearst (1962) and has since been shown to have a nucleotide sequence complementary to part of one strand of DNA. The m-RNA carries the information contained in a segment of the genetic material and is responsible for determining the amino acid sequence of specific proteins. It is thought that each protein has a specific m-RNA.

The transfer of information from m-RNA is accomplished through interaction of the m-RNA with t-RNA's and r-RNA. The importance and relevance of t-RNA was shown by Hoagland et al. (1958). He suggested successive triplets in the m-RNA mate by base complementation with triplets in specific aminoacyl transfer RNA's to cause successive incorporation of amino acids into protein. The 20 amino acids found in protein are first activated by enzymes in reactions involving ATP. The activated amino acids react with t-RNA to give amino-acyl t-RNA. The t-RNA's carry the amino acids to the site of protein synthesis on the ribosomes where successive t-RNA's combine according to the code of the m-RNA and produce a specifically ordered protein.

Holley (1965) demonstrated that species of t-RNA are amino acid specific. He has further determined the structure for alanine-specific t-RNA. Other workers have since shown that some of the other t-RNA's are similar in structure, but differ in certain specific areas, and that they convey the amino acid-specific characters of the various t-RNA's.

The third type of RNA is ribosomal RNA. This occurs as a nucleoprotein in ribosomes which contain 60-65% RNA and 35-40% protein. Little is known about ribosomal structure. It is assumed the m-RNA, t-RNA and other factors are brought together at the ribosomes and a specific protein is the end-product.

The role of nucleic acids in cellular growth and metabolism has been an increasingly important field in botanical research. Adequate methods, however, for extracting and estimating DNA and RNA are necessary for such research. Furthermore, the quantitative measurement of DNA has a wider interest for determining cell number in tissues. Data on other cell constituents, such as protein, can therefore be expressed conveniently in relation to DNA as a standard reference (Potter, 1960).

Most procedures for determining nucleic acids have been developed specifically for animal tissues and have been applied directly or in modified forms to a wide range of organisms. Plants have presented special problems because of large amounts of interfering substances which they contain. Of the basic procedures currently used for measuring amounts of nucleic acids in biological materials, the methods of Schmidt and Thannhauser (1945) and Schneider (1945) are the most significant.

The initial step in assaying nucleic acid contents of biological material is removal of substances that interfere with the reactions used for determining the nucleic acids. This usually involves washing the tissue briefly with cold acid to remove the acid-soluble small molecules (free nucleotides, carbohydrates, inorganic-P, and organic-P of low molecular weight). This is usually succeeded by removal of lipids and phospholipids by organic solvents. Holdgate and Goodwin (1965) suggested using 95%

ethanol saturated with sodium acetate to remove acid that may degrade nucleic acids during the hot lipid extractions. Ethanol (95% and 50%) is used to remove the other plant pigments before the acid washing.

Nucleic acids possess three chemical features that can be used for determining the amounts of RNA and DNA in tissue residues. The nucleotide units from which RNA and DNA are constructed consist of a purine or pyrimidine base, a sugar (ribose in RNA and deoxyribose in DNA), and phosphoric acid. This study utilized the ultra violet absorbing properties of the nucleotide bases to determine the amounts of DNA and RNA. This does not separate the RNA from the DNA, but differential sensitivity of RNA and DNA to alkaline hydrolysis (Schmidt and Thannhauser, 1945) can be used to do this. Steudel and Peiser (1922) laid the foundations of the modern analytical use of alkaline hydrolysis by demonstrating that yeast RNA was hydrolyzed by NaOH at 15-17C, over a period of 24 hours, whereas thymus DNA was resistant.

Figure 1 illustrates that hydrolysis of RNA by alkali is due to the proximity of the hydroxyl group on the C-2 of ribose to the phosphoric acids' 3'-5' phosphodiester linkage. This permits formation under alkaline conditions of a cyclic phospho-triester (II) involving C-2 and C-3 atoms of the ribose. The triester then undergoes hydrolysis at the C-5 linkage leading to rupture of the nucleotide chain (III) and resulting in the 2'- and 3'-mononucleotides (IV) and (V). The absence of the hydroxyl group on the C-2 atom of deoxyribose prevents formation of the cyclic triester from DNA and thus makes DNA resistant to alkaline hydrolysis.

DNA lability to alkali must be viewed in relation to conditions causing formation of apurinic acid from DNA. When DNA is treated with acid at

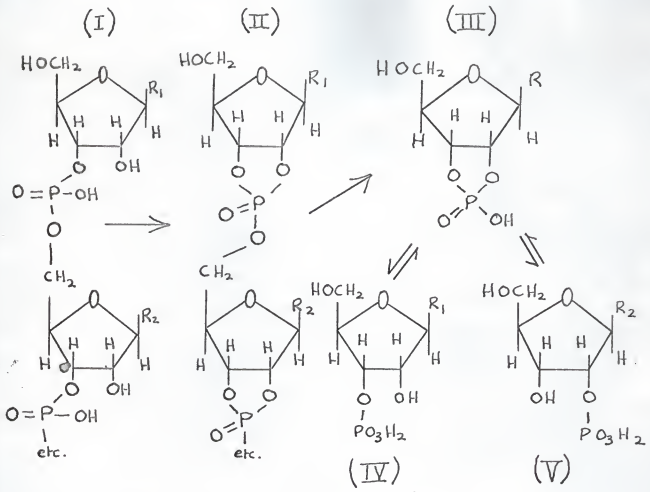

Figure 1. Alkaline hydrolysis of yeast RNA.

pH 1.0, the purine bases are released completely within 24 hours (Tamm et al., 1952) and give rise to apurinic acid. Subsequent treatment of apurinic acid with alkali exposes the OH groups as a result of the loss of the purine bases and permits the formation of cyclic triesters just as in RNA. This is followed by breakdown of tracts of the DNA chain to yield acid-soluble products that add to the ultra-violet absorption of RNA. Formation of apurinic acid can occur during the initial washing with cold acid to remove small molecules or during the extraction with lipid solvents still contaminated with acid if the temperature rises during extraction. Cold acid (4C) and washing with 95% ethanol saturated with sodium acetate prior to

lipid extraction alleviates these problems.

Alkaline hydrolysis of RNA is the basis of the Schmidt and Thannhauser (1945) procedure used in this study. It was assumed that the products of RNA hydrolysis were entirely mononucleotides, but recent evidence suggests that breakdown is often less complete. Digests of lipid-free tissue residues have shown that exposure to 0.3N alkali for one hour at 37C extracts all the RNA and destroys its precipitability by acids. Extensive work by Lane and Butler (1959), however, showed that some oligonucleotides are present. Oligonucleotides of the RNA acid soluble fraction consist chiefly of purine dinucleotides, notably diadenylic acid, which is only slowly hydrolyzed by alkali. Protein degradation also accompanies alkaline digestion of the tissue residue. Fleck and Munro (1955) demonstrated that the amount of protein solubilized by alkaline digestion increased progressively with the duration of hydrolysis and, to a smaller extent, with increasing concentration of the alkali from 0.3N to 1.0N at 37C. They concluded that one hour of digestion with 0.3N KOH at 37C solubilized all the ribonucleotides and further time merely resulted in greater contamination by degraded protein. Separation of the acid-soluble ribonucleotides from the protein and DNA residue followed by further alkaline digestion of the ribonucleotides gave a purer product.

Various alkalis have been used for hydrolyzing RNA. For analytical purposes, however, only NaOH and KOH have been used. Both appear to work in the same manner, but KOH possesses the advantage that most of the potassium can be removed as the sparingly-soluble potassium perchlorate.

De Deken-Grenson (1959) found that the alkaline hydrolysis of the RNA-containing residues of plant extracts contained interfering substances.

He also showed that ultra-violet determination of the nucleotides seldom agreed with the orcinol ribose sugar method. He purified the ribonucleotides, however, on resin columns and obtained much better agreement between the two methods. Smillie and Krotov (1960), working with higher plants, concluded that a purification step on resin columns was essential after alkaline hydrolysis of RNA to get reasonably accurate results.

MATERIALS AND METHODS

Experiment 1

Wheat (Triticum aestivum L.), corn (Zea mays L.), sorghum (Sorghum bicolor (L.) Moench.), soybeans (Glycine max (L.) Merr.), oats (Avena sterilis L.), and rice (Oryza sativa L.) were selected from a number of lines of species each for marked differences in grain protein. The high- and low-protein lines selected, their protein contents, and the authorities for the differences in protein contents are shown in Table 1. Atlas 66 was a selection derived from crosses made originally in South America. The two corn hybrids have been extensively studied at Illinois. The two soybean varieties have been studied by the USDA Regional Soybean Laboratory at Illinois. The sorghum was studied at Purdue and Nebraska. The high-protein oats are a selection from high-protein lines recently found in Israel. The two rice varieties have been studied by the USDA and the International Rice Research Institute in the Philippines.

Seedlings of each line were germinated in moist vermiculite and transplanted to nutrient solutions 10 days after planting the rice and seven days after planting the other species. The nutrient solutions (Hoagland and Arnon, 1950) were in two liter containers and provided 1mM KH_2PO_4, 10mM KNO_3, 5mM $Ca(NO_3)_2$, 2mM $MgSO_4$, and 21uM Fe as $FeNa_2EDTA$. Micronutrients were present at levels suggested by Johnson et al. (1957). Rice received half-strength concentrations of the above nutrients plus 50uM $MnSO_4$ and 200uM SiO_2 (Okuda, 1964; Yamasaki, 1964). The nutrient solutions were

Table 1. High- and low- grain protein lines of wheat, corn, sorghum, soybeans, oats, and rice studied for factors associated with protein content.

Species	Line	Protein content	Reference
		%	
Wheat	Atlas 66	18.8	Heyne (Personal Comm.)
	Pawnee	15.7	Middleton et al. (1954)
Corn	WF9 x C103	11.3	Lang et al. (1956)
	Hy2 x Oh7	8.8	Lang et al. (1956)
Sorghum	C.I. 3071	17.8	Maranville (Personal Comm.)
	Plainsman	10.2	Maranville (Personal Comm.)
Soybeans	Wayne	41.1	Bernard et al. (1967)
	Amsoy	38.5	Bernard et al. (1967)
Oats	C.I. 8330	25.1	Murphy (Personal Comm.)
	Animated oats	11.3	
Rice	Tiachung native #1	11.1	Scott (Personal Comm.)
	Bluebelle	7.1	Juliano et al. (1964)

maintained at pH 5.0 with HCl. Six plants of corn, soybeans, or sorghum and 12 plants of wheat, oats, or rice were held in each container. Eight replications of each line were randomized in complete blocks.

The seedlings were grown in environmental chambers maintained at 25C-15C day-night temperatures with a 16-hour light period and an 8-hour dark period. Lighting of about 1200 ft-c at plant height was provided by 16 160-watt fluorescent lamps and 6 300-watt incandescent lamps. Relative humidity in the chambers was about 40%. Forced aeration was provided to each pot through 2-mm ID glass tubing inserted through the container covers and extended to 1 cm of the container bottoms.

After two weeks growth the seedling leaf blades were sampled for assays described below.

Experiment 2

Parents and high- and low-protein F_5- derived progeny between crosses of high- and low- grain protein wheat varieties were studied to determine inheritance of nucleic acid factors associated with grain protein contents. The parents, progeny, and their respective grain protein contents were: 'Kaw' (13.7%), 'Triumph' (12.3%), 'Atlas 50' (18.4%), Atlas 66 (18.8%), Kaw x Atlas 50 (13.0 and 18.0%), Kaw x Atlas 66 (13.8 and 18.0%), Triumph x Atlas 50 (13.7 and 18.7%), and Triumph x Atlas 66 (14.0 and 18.4%) (Heyne, personal communication). Four replications of the seedlings were germinated and grown as described above for the wheat and sampled two weeks later for the assays described below.

Experiment 3

'Pawnee' and 'Atlas 66' wheats were seeded at the rate of 101 kg per hectare in 3-meter x 8-meter plots October 18, 1967, on the Kansas State University Agronomy Farm, Manhattan, Kansas. Each plot was replicated four times in a completely randomized design. The plots were top dressed with 200 kg/ha of N as NH_4NO_3 on March 30, 1968. Plants were sampled randomly from each plot at weekly or biweekly intervals from April 2 to July 9, 1968. Four subsamples of flag leaves, leaf blades, heads, and grain were assayed, when available, as described below. The head tissue consisted of the developing grain, rachis, and the rest of the spikelet. The stages of maturity of the wheat when sampled were also noted. Pawnee was earlier in maturity than Atlas 66, but some of this variation was removed by adjusting the dates of maturity so similar growth stages could be compared. Differences in population, however, occurred because of winterkilling in Atlas 66.

Sample Preparation

Preparation of the plant material and determination of the nucleic acids was similar to the method used by Niemand and Poulsen (1963). The morphological plant parts assayed were harvested, cut into 1-cm sections, and mixed for sub-sampling. One gram sub-samples were frozen in liquid nitrogen and stored at -20C until required.

Dry matter percentages were determined by weighing the plant samples fresh and reweighing them after drying at 60C to a constant weight.

The frozen samples were homogenized with 20 ml of cold (2-4C) 95% ethanol for one minute using a Servall Omnimixer at 16,000 rpm with the cup immersed in an ice bath. Unmacerated tissue adhering to the side of the

cup was reimmersed and the samples were blended for another minute. The homogenates were thoroughly washed into centrifuge tubes with cold 95% ethanol and centrifuged for 10 minutes at 23,500 x g at 2C. The supernatants were discarded and the residues were resuspended and washed once with cold 95% ethanol and twice with 50% ethanol adjusted to pH 4.5 with glacial acetic acid. Between washings, the samples were centrifuged for 10 minutes at 20,000 x g and the supernatants were discarded. The residues were resuspended and washed twice in 0.2N PCA, held at 4C for 15 minutes, and centrifuged twice at 25,000 x g. The residues were washed once in cold 95% ethanol saturated with sodium acetate, twice with boiling 3:1 ethanol:ether for 3 minutes, and once with anhydrous ethyl ether at room temperature. The residue after each washing was centrifuged at 20,000 x g and the supernatant was discarded. The final white residue was free of interfering pigments and small nucleotides.

Extraction of Nucleic Acids

A modified Schmidt and Thannhauser (1945) procedure based on differential hydrolysis of RNA to acid-soluble ribonucleotides under conditions that leave DNA insoluble was used. The white residues containing nucleic acids were hydrolyzed with 5 ml of 0.3N KOH for 18 hours at 30C. The hydrolyzed residues were centrifuged for 10 minutes at 20,000 x g. The supernatants were filtered through Whatman number 4 filter paper into 15-ml calibrated centrifuge tubes. The residues were washed once with 5 ml of 0.3N KOH and then combined with the previous extract and diluted to 10 ml with 0.3N KOH. The 10 ml of extract was neutralized slowly with 15% PCA to precipitate potassium as $KClO_4$. Two ml of cold 15% PCA was added to the

extract and the extract was held at 4C for 40 minutes and centrifuged for 10 minutes at 20,000 x g. The supernatants containing the ribonucleotides were set aside and the residues were resuspended in 2 ml of distilled water. Two ml of cold 1 N PCA was added and the suspension was held at 4C for 20 minutes and centrifuged at 20,000 x g. The wash was combined with the previous extraction and diluted to 20 ml with 0.5N PCA. One-ml samples were diluted to 10 ml and their optical densities were determined at 260 nm. The residues containing the DNA fraction were resuspended in 3 ml of 0.5N PCA, held at 70C for 15 minutes, and centrifuged for 10 minutes at 20,000 x g. The supernatants were poured into 15-ml calibrated centrifuge tubes and the residues were washed once with cold 0.5N PCA. The washes were combined with the original extracts and diluted to 5 ml with 0.5N PCA. Absorbancies were determined on 1:10 diluted samples as for ribonucleotides.

A standard curve of yeast RNA and sperm DNA was used to quantitate the nucleotide absorbancies.

Purification of the Nucleotides

Alkaline hydrolysis caused some degradation of protein and, as a result, nucleotide extracts were invariably contaminated by peptides that caused overestimation of nucleic acid absorbancies. Thus, the method of Smillie and Krotov (1960) was used to purify the nucleotides of the flag leaf and grain in the field experiment.

Dowex-1-Cl anionic resin was washed successively with 1N HCl, 1N NaOH, 1N HCl, and finally twice with double distilled water. A 5- x 0.9-cm column of washed resin was rinsed with 20 ml of 0.01N NaCl. A 1-ml sample of neutralized nucleotides was applied to the column and washed onto the resin

and the column was washed with 10 ml of 0.01N HCl. The nucleotides were eluted with a NaCl-HCl solution (20 ml 10N HCl + 5.6 g NaCl + 240 ml H_2O). The first 10 ml of eluent contained all the nucleotides. A 1-ml sample of neutralized nucleotides was diluted to 10 ml without purification. Both samples, plus a hydrolyzed sample of pure RNA passed through the column as above, were read at 260 nm. A RNA sample was also prepared to determine percent retention by the column.

Determination of the Base Composition of the RNA

The base composition of ribonucleic acid in wheat grain in the field study was determined by a rapid chromatographic method based on the work of Katz and Comb (1963). Dowex 50 cationic resin (200-400 mesh, 4x cross-linked) was washed with 1N solutions of HCl, NaOH, HCl, and finally with 3N HCl. A 5-cm x 0.9-cm column of resin was washed once with 3N HCl, H_2O until neutral, and finally with 20 ml of 0.05N HCl. The hydrolyzed sample was neutralized (pH 5-8) with 15% PCA, the $KClO_4$ precipitate was removed by centrifuging at 15,000 x g, and an equal volume of 0.1N HCl was added. Two ml of the solution was applied to the column. The sample was drained into the resin and the column was washed with 1 ml of 0.05N HCl and again drained into the resin. Five ml of 0.05N HCl was added and the first 6 ml of effluent was collected in a 10-ml graduated centrifuge tube. The remainder of the HCl effluent was discarded. Under these conditions, the uridylic acid was quantitatively eluted through the column while the other nucleotides remained on the column. The concentration of uridylic acid was calculated from the extinction coefficient in 0.05N HCl and the purity was determined by the $A_{250:260}$ and $A_{280:260}$ ratios (Table 2).

The HCl from the previous step was allowed to drain into the resin and water was passed through the column at a flow rate of not more than 1 ml per minute. The first 7.5 ml of effluent, which quantitatively eluted guanylic acid, was collected (Figure 10). The solution was acidified to 0.05N HCl with 1N HCl before absorbancies were read at 257, 260, 250, and 280 nm.

The next 25 ml of H_2O passed through the column eluted cytidylic and adenylic acids together. The final HCl concentrations were adjusted to 0.05N HCl with 1N HCl. The absorbancies were measured at 257 and 279 nm. The $A_{279:257}$ ratio in 0.05N HCl for adenylic acid is 0.238 and for cytidylic acid is 2.32. The absorbancy due to each of the nucleotides in the mixture was calculated from the following equations (Loring et al., 1955):

$$X = \frac{2.32\ (A_{257}) - A_{279}}{2.08} = \text{absorbancy at 257 nm of adenylic acid alone}$$

$$Y = A_{279} - 0.238\ X = \text{absorbancy at 279 nm of cytidylic acid alone.}$$

Table 2. Extinction coefficients and absorbancy ratios of standard 2',3' ribonucleotides.

Nucleotide	Wavelength	Extinction Coefficient	Solvent	$A_{250:260}$	$A_{280:260}$
	nm	M/cm			
Uridylic acid	260	9600	0.05N HCl	0.800	0.364
Guanylic acid	257	11800	0.05N HCl	0.949	0.655
Cytidylic acid	279	13000	0.05N HCl	-	-
Adenylic acid	257	14900	0.05N HCl	-	-

Total Nitrogen and Protein Determination

Kjeldahl nitrogen was determined in the grain of the crop species used and compared with the literature values. A 1-gram sample of dried grain, ground to a 40-mesh size with a Wiley mill, was assayed for Kjeldahl nitrogen by the method of Hiller, Plazin, and Van Slyke (1948).

Source of Chemicals

Dowex 50-H^+ and Dowex 1-Cl (200-400 mesh, 4x cross linked) resins and the nucleic acid standards were obtained from Sigma Chemicals Company, St. Louis, Missouri.

EXPERIMENTAL RESULTS

Experiment 1

The nucleic acid contents of high- and low-protein lines of wheat, soybeans, sorghum, corn, rice, and oats are shown in Figure 2. The RNA contents varied greatly among species. The two sorghum varieties had the highest RNA contents (4.62 and 4.35 mg/g fresh wt), and the two oat varieties had the lowest RNA contents (2.72 and 2.46 mg/g fresh wt).

Differences in the RNA contents between varieties varied less than the differences among species. Oats, sorghum, and soybeans contained significantly more RNA in the high-protein varieties than in the low-protein varieties. Rice and corn, however, contained more RNA in the low-protein varieties but the differences were not significant. In wheat, the high-protein variety had the higher RNA content but the mean differences were not significant.

The DNA contents also varied among species. Wheat, with 0.60 mg/g, and rice with 0.25 mg/g, contained the highest and lowest amounts, respectively. Wheat, sorghum, and corn contained similar amounts of DNA in the high- and low-protein varieties. The high-protein lines of oats, rice, and soybeans contained significantly more DNA than the low-protein lines.

The ratio of RNA to DNA also varied among species and ranged from about 5.0 in wheat to more than 13.0 in sorghum. Only the rice, however, showed large differences between varieties, with the low-protein variety having the higher ratio of RNA to DNA. In the other species, only corn and oats had a

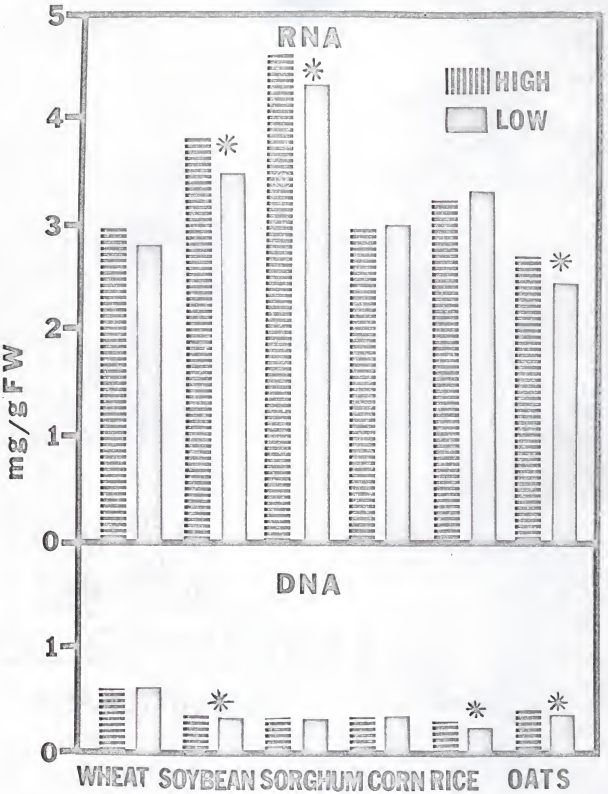

Figure 2. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of the seedling leaf blades of high- and low-protein lines of wheat, corn, sorghum, soybeans, oats, and rice. LSD (0.05) for differences between lines was 0.21 for RNA and 0.03 for DNA.

higher RNA-to-DNA ratio in the low-protein variety.

Experiment 2

The nucleic acid contents of low- and high-protein wheat varieties and their F_5-derived progeny are shown in Figure 3. Ribonucleic acid (RNA) did not differ significantly in any of the parents or progeny tested. Atlas 50 contained the most RNA (3.84 mg/g fresh wt) and Atlas 66 contained the least RNA (3.49 mg/g fresh wt) of the parents tested. The two low-protein parents had similar RNA contents. In the progeny, three of the four high-protein selections contained more RNA than their low-protein counterparts. The differences, however, were not significant. The cross Kaw x Atlas 50 had more RNA in the low-protein selection. All the progeny selections, except Kaw x Atlas 66, contained more RNA than the two low-protein parents, Kaw and Triumph.

DNA varied little. Atlas 66 and its progeny had the lowest DNA contents. Kaw and Triumph contained similar amounts of DNA, and Atlas 50 and its progeny had the highest DNA contents. No relationships were evident between the DNA contents of the leaves and the protein contents of the grain. Half of the high-protein progeny contained more DNA than their low-protein counterparts, and half contained less DNA. Atlas 66 and Kaw x Atlas 66 contained significantly more DNA than Kaw x Atlas 50.

Atlas 66 and Atlas 50 had the highest (8.73) and the lowest (8.00) RNA/DNA ratios, respectively, of the parents. Kaw (8.45) was higher than Triumph (8.26), and Kaw x Atlas 66 progeny had the highest RNA/DNA ratios of the progeny.

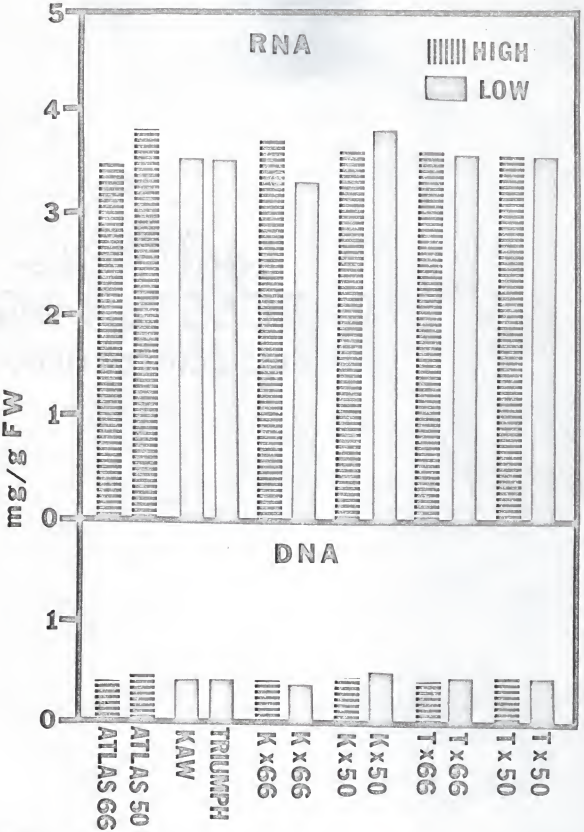

Figure 3. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of seedling leaf blades of high- and low-protein wheat varieties and their F_5-derived progeny. LSD (0.05) for differences between varieties and progeny was 0.83 for RNA and 0.09 for DNA.

Experiment 3

The nucleic acid contents of various morphological plant parts of low- and high-protein wheat varieties assayed in the field experiment are shown in Figures 4-7. The high protein variety, Atlas 66, was about two weeks later in maturity than the low-protein variety, Pawnee. The two varieties were compared at similar growth stages, however. Table 3 shows the date each growth stage was attained by Pawnee and Atlas 66.

Table 3. The dates Pawnee and Atlas 66 attained the various growth stages.

Stage	Date	
	Pawnee	Atlas 66
Tillering	April 2	April 2
Jointing	April 30	May 14
Heading	May 14	May 28
Anthesis	May 21	June 4
Dough	June 4	June 18
Mature	June 25	July 9

The leaf blades gradually increased in RNA content on a fresh weight basis throughout the sampling period. The lowest content (3.70 mg/g fresh wt) occurred during the early tillering stage, and the highest content (11.10 mg/g fresh wt) occurred during senescence in Pawnee. RNA contents of the blades of Pawnee and Atlas 66 followed similar trends throughout the sampling period. Atlas 66, however, always had the higher RNA content (Figure 4).

Nucleic acids in the flag leaves were purified through resin columns and are expressed on a dry weight basis (Figure 5). The data show different trends than in the leaf blades. In Atlas 66, the RNA content declined until just before heading, remained constant until anthesis, and decreased again during maturation. Pawnee RNA content followed a similar trend except that, after decreasing till anthesis, the RNA remained constant until the early dough stage. RNA then decreased as the leaf matured. The RNA values for Atlas 66 were higher than the values for Pawnee before heading and during anthesis, but decreased below the Pawnee values after anthesis.

DNA contents of the flag leaves were nearly constant throughout spring growth and were similar in Pawnee and Atlas 66. The DNA values, however, tended to decrease slightly after anthesis.

The grain nucleic acid contents were expressed similarly to the flag leaf nucleic acid contents (Figure 6). The RNA contents of Atlas 66 and Pawnee varied similarly throughout the sampling period. The RNA contents declined rapidly from the dough stage to maturity. At maturity, the RNA contents leveled off. At maturity, the grain of Atlas 66 contained slightly more RNA than the grain of Pawnee.

DNA concentrations in the grain of both Pawnee and Atlas 66 varied more than the RNA concentrations. DNA concentrations in both Pawnee and Atlas 66 decreased from the early dough stage and then leveled off at maturity. The DNA content of Pawnee grain, however, was higher than the DNA content of Atlas 66 grain except when they were similar at maturity.

The nucleic acid contents of the heads were expressed on a dry weight basis, but the nucleic acids were not purified through resin columns (Figure 7). The RNA values decreased linearly from heading through the late

dough stages and followed similar trends in both Pawnee and Atlas 66. The RNA values of Atlas 66, however, were higher than those for Pawnee. At the late dough stage the RNA contents of Atlas 66 decreased more rapidly than those of Pawnee and the two varieties had similar RNA contents at maturity.

DNA concentrations in the heads followed similar trends in Pawnee and Atlas 66. Atlas 66 contained more DNA during the interval from heading until anthesis, but contained amounts similar to Pawnee while maturing. After the dough stage, the DNA contents of Pawnee and Atlas 66 heads declined slightly.

The grain and the flag leaf RNA contents before and after purification are shown in Figure 8. Similar trends for the RNA occurred in the grain and flag leaves. Before purification, maximum absorption occurred at 270 to 280 nm and a minimum absorption occurred at 250 to 260 nm. After purification, maximum absorption was at 260 nm and the curve fell regularly from both sides of the maximum. A pure sample of yeast RNA had the same characteristics of maximum absorption at 260 nm as the purified RNA from the grain and flag leaves.

Experiment 4

The base composition and characteristic spectral curves of the RNA nucleotides of Atlas 66 and Pawnee grain throughout the season are shown in Table 4. The spectral ratios ($A_{250/260}$ and $A_{280/260}$) were similar for guanylic acid and uridylic acid in both Pawnee and Atlas 66. The purine-to-pyrimidine ratios follow similar trends in Pawnee and Atlas 66. At the dough stage, the purine:pyrimidine ratio was 1.0 and fell to 0.9 at maturity. The RNA nucleotide data differed slightly between Pawnee and Atlass 66. The

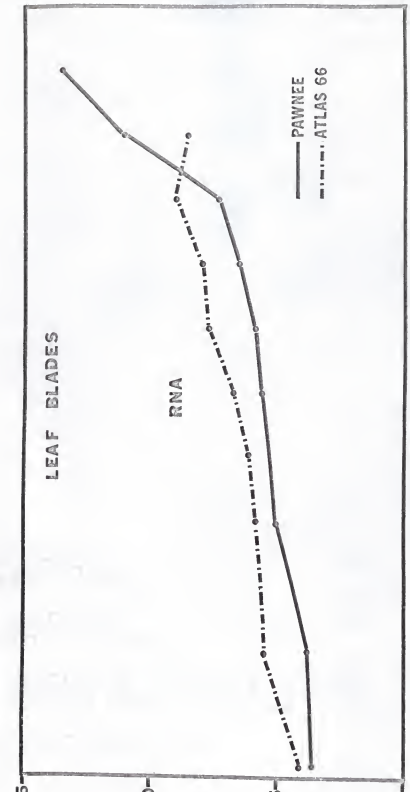

Figure 4. Ribonucleic acid (RNA) contents of the leaf blades of Pawnee and Atlas 66 wheat during spring growth.

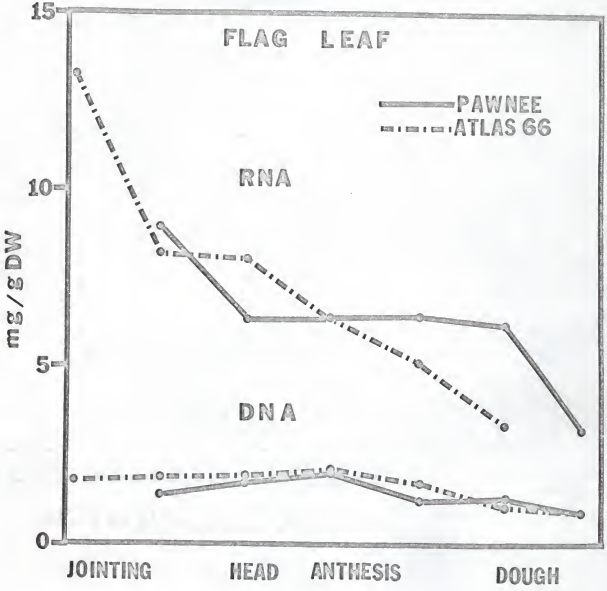

Figure 5. Purified ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of the flag leaves of Pawnee and Atlas 66 wheat during spring growth.

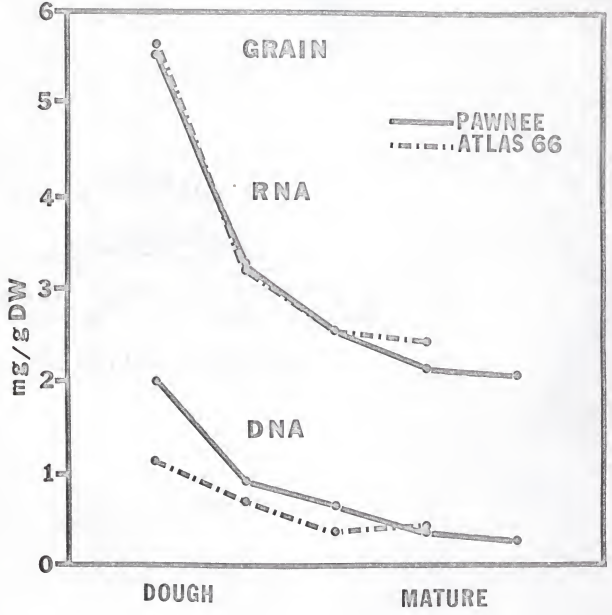

Figure 6. Purified ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of the grain of Pawnee and Atlas 66 wheat during spring growth.

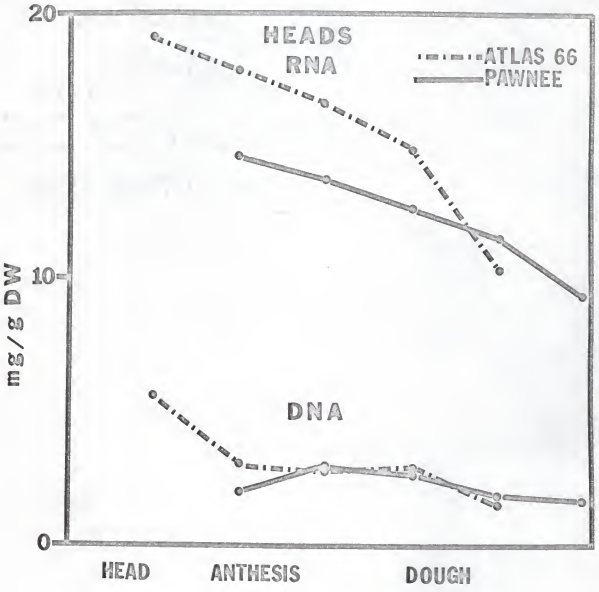

Figure 7. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of the head tissue of Pawnee and Atlas 66 wheat during spring growth.

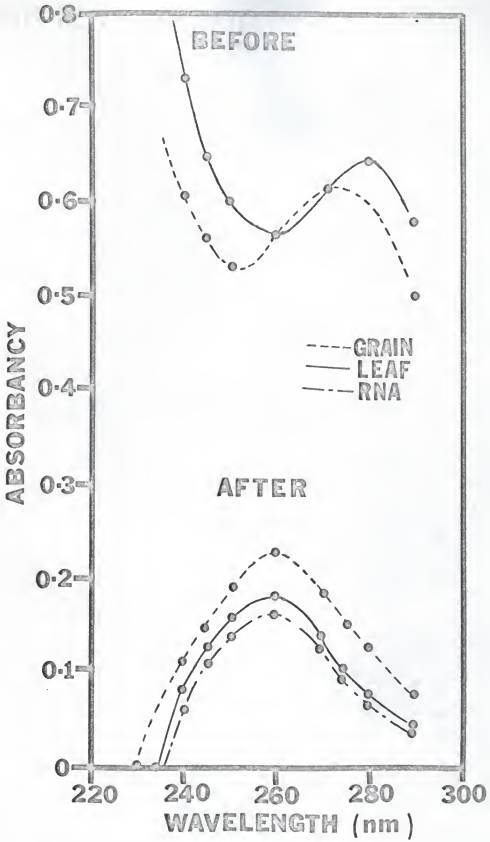

Figure 8. Spectral absorbancies of leaf, grain, and yeast RNA before and after purification through Dowex-1 resin.

nucleotides, however, had similar spectral ratios. Cytidylic acid was highest (around 30 mole%) and uridylic acid was lowest (around 20 mole%) in both Pawnee and Atlas 66 at all stages of growth. Adenylic acid and guanylic acid were about equal in quantity in Pawnee and Atlas 66.

The separation of the ribonucleotides on Dowex 50 cationic resin is shown in Figure 9. The aliquots taken were free of contamination by other nucleotides, except adenylic acid and cytidylic acid, which were mixed. Relative concentrations of the latter were calculated mathematically, however. The adenylic acid and cytidylic acid were eluted completely in the 25 ml collected.

Table 4. Nucleotide composition of grain.

	Pawnee					Atlas 66			
	June 4	June 11	June 18	June 25	July 2	June 18	June 25	July 2	July 9
Uridine	19.8	21.4	20.6	19.4	22.6	22.6	20.8	20.7	--
Guanine	25.9	26.8	27.2	23.7	23.1	26.7	23.3	23.4	--
Adenine	24.8	23.4	24.3	24.3	24.8	23.2	23.6	24.3	--
Cytosine	29.6	28.4	28.0	32.6	29.5	27.5	32.2	31.6	--
Purine/ Pyrimidine	1.03	1.01	1.06	0.92	0.92	1.00	0.88	0.92	--

Characteristic spectral readings of RNA nucleotides obtained from wheat.

Pawnee				Atlas 66			
		$A_{250/260}$	$A_{280/260}$			$A_{250/260}$	$A_{250/260}$
4th	Ur	0.95	0.42	18th	Ur	0.96	0.46
	G	1.01	0.71		G	1.01	0.71
11th	Ur	0.97	0.48	25th	Ur	0.95	0.47
	G	1.02	0.72		G	0.99	0.72
18th	Ur	0.99	0.49	2nd	Ur	1.00	0.51
	G	1.02	0.74		G	1.01	0.74
25th	Ur	1.03	0.53				
	G	1.04	0.73				
2nd	Ur	0.97	0.48				
	G	1.00	0.72				

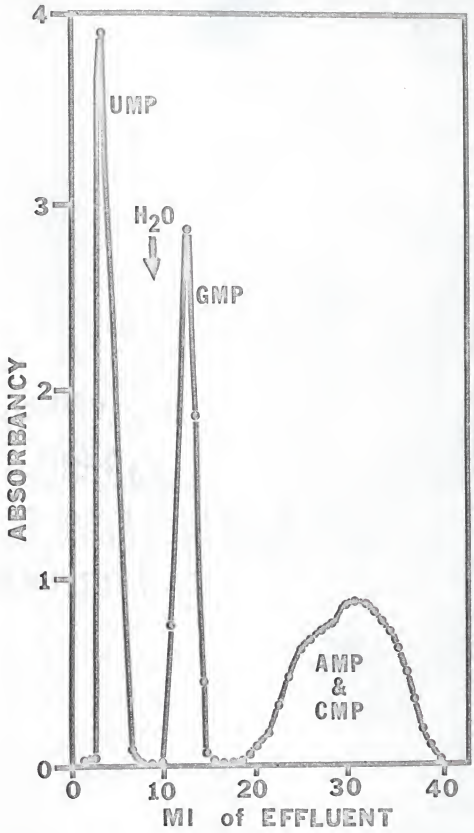

Figure 9. Absorbance spectrum of the nucleotides from yeast RNA.

DISCUSSION

Nucleic acids are only one of many factors involved in protein biosynthesis, and correlations between RNA content and protein content should only be tentative. There have been few studies in the literature on actual total RNA and DNA contents in plant tissues. Nucleic acid contents of seedling leaf blade tissue of high- and low-protein varieties of several crop species, however, were determined in these growth chamber studies.

Differences in RNA content among species was greater than within species. Sorghum for instance, had the highest RNA content, but not the highest grain protein content. Differences in RNA content in the high- and low-protein varieties of sorghum, soybeans, and oats leaves, however, were significant and correlated with grain protein content. In wheat, corn, and rice these differences were not significant. The second growth chamber experiment with high- and low-protein wheat varieties and their F_5-derived progeny also showed no statistical variation in RNA content between varieties. There was, however, a residual carryover from the parent to their derived progeny in some cases. Atlas 50 had the highest RNA content of the parents and some of the Atlas 50-derived progeny had the highest RNA contents of the progeny.

DNA was also more variable among species than within species. Wheat had the highest DNA content, but not the highest protein content. The DNA contents of soybeans, oats, and rice showed a significant difference between varieties that was correlated with grain protein content. DNA differences in sorghum could also be correlated with high grain protein content, but as

with wheat and corn, the differences were not significant. DNA contents of the wheat varieties and their F_5-derived progeny in the second experiment did not differ significantly. The results, however, like the RNA contents, did show a parental effect on the progeny. Atlas 50 had the highest DNA content as also did its progeny.

The greater variation in the nucleic acid content among species than between varieties and the indicated parental effect on the nucleic acid content of the progeny suggest that nucleic acid content may be genetically determined. Further crosses, however, among the wheats tested were needed to test this hypothesis statistically. The hypothesis, however, is supported by the work of Yanofsky and Spiegelman (1962) who have shown biochemically that various cellular RNA species are synthesized from the nucleus, and that portions of the DNA molecule can hybridize with various RNA species. The differences in RNA content of sorghum, soybeans, and oats between the high- and low-protein varieties may be a determining factor in the final grain protein content if the differences continue throughout the growing season. The DNA contents of soybeans and oats, however, also correlated with grain protein content. If the assumption that DNA content per cell is constant, then the higher DNA content in the high-protein lines of soybeans and oats indicate a higher concentration of living cells than in the low-protein lines. Thus, differences in cell number may account for the higher RNA content in the high-protein varieties of soybeans and oats.

The grain protein content of crops is far removed morphologically and biochemically from the nucleic acid content of two-week-old seedling leaf blades. Many environmental and metabolic changes occur throughout the life cycle of the plant, and in the different morphological plant parts, that may

affect the final grain protein content. The field study measured the nucleic acid differences in various plant parts as they matured.

RNA content increased throughout the sampling period in the leaf blades and was probably due to the loss of water and gain in dry weight as the leaves matured. Increased protein contamination, however, might also have been a factor (Smillie and Krotov, 1960). Also, a brown pigment occurred in the alkaline digests in Pawnee towards the end of the sampling period. This pigment was not identified, but it appeared to add to the absorbancy readings. The differences between Pawnee and Atlas 66 in RNA content in the leaf blades correlated with final grain protein content. The nucleotides, however, were not purified, so differences should be viewed with caution. Also, differences in plant density in the field because of winterkilling may have influenced the amounts of nucleic acids in the leaf.

The results of purifying the nucleotides of the flag leaves and grain on resins indicated that most impurities were removed. The spectral curves before and after purification show maximum absorbancies at 270 to 280 nm and indicated protein contamination. After purification, however, a maximum absorbancy at 260 nm indicated pure RNA was eluted. The standard yeast RNA sample showed little loss due to adsorption on the column, which indicated that pure nucleic acids were eluted. This agreed with the work of Smillie and Krotov (1960), who showed that alkaline digestion of nucleic acids contained protein contamination that could be removed on resin columns.

The purified ribonucleotides of the flag leaves followed three trends. Nucleic acids were initially high during jointing, and fell rapidly to just before heading. At heading, the RNA contents leveled off. This leveling off persisted longer in Pawnee than in Atlas 66. The ribonucleic acid

content then declined to maturity. The initial decline may have been the result of ribonuclease activity (Matsushita, 1959). At heading, the leveling off may have been a result of rapid translocation of metabolites to the developing heads preventing an increase in dry weight (Carr and Wardlaw, 1965). Increase in nucleic acids at this stage of maturity seems doubtful. The differences in the time of leveling of nucleic acid contents between Pawnee and Atlas 66 may have been due to selection of green tissue from Pawnee for the 1-gram sample, whereas with Atlas 66, the flag leaves senesced much faster and there was less green tissue in the samples. The final decline in RNA during maturation was probably the result of senescence and water loss from the leaves. Ribonuclease activity may also have been high. There was a higher RNA content in Atlas 66 than Pawnee before anthesis. This may be a factor in grain protein content. The greater RNA content may have enabled more protein synthesis in the leaf, which was then hydrolyzed and translocated to the developing grain. The RNA contents of both species were similar at the senescent stage. Greater ribonuclease activity in Atlas 66 at the time of anthesis may also have caused breakdown of more RNA and translocation of this to the heads for protein synthesis. Matsushita (1959) studied the wheat leaf during its development and suggested that the ribonuclease activity increased as the leaf senesced.

The DNA content of the flag leaves remained relatively constant in Pawnee and Atlas 66 throughout the sampling period and did not differ significantly between the two varieties. DNA contents decreased at anthesis during senescence of the flag leaves. The constant DNA content in Pawnee and Atlas 66 indicated that the living cell number was similar in both varieties. Thus, the RNA differences during early development of

Atlas 66 indicated a higher RNA content per cell than in Pawnee. This may have been a factor in grain protein content.

The purified RNA contents from the grain indicated that RNA content in the grain did not correlate with the final protein content. The rapid drop in RNA from the dough stage to maturity was probably due to translocation, synthesis, and accumulation of diluting metabolites within the grain. At maturity, the values leveled off to a constant level when further dry matter accumulation and water loss had ceased. Ribonuclease activity was also probably increased while the grain was maturing and caused a drop in nucleic acid content. At maturity, when moisture content was low, ribonuclease activity probably ceased (Matsushita, 1959).

The DNA content of the grain of Atlas 66 and Pawnee also declined from the dough stage to maturity and a constant level was attained at maturity. This decline was probably the result of dilution of the DNA by dry matter accumulation. The DNA content of Pawnee was higher than the DNA content of Atlas 66 during early development. At maturity, however, the DNA contents of both varieties were equal. This indicated that the grain of Atlas 66 had less living cells than Pawnee and, because the RNA contents of Pawnee and Atlas 66 were equal, Atlas 66 had a higher RNA content per cell than Pawnee. This may be a factor in grain protein content if the RNA content is proportional to protein synthesis.

The RNA content of the head tissue followed the same trends in both Pawnee and Atlas 66. An initial slow decrease in RNA concentration was followed by a rapid decline between the dough stage and maturity. The initial slow decrease in RNA content was probably caused by an increase in dry matter, resulting from an increase in structural compounds, while nucleic

acid synthesis was still active (Jennings, 1963). At the dough stage the ear tissue began to mature and the grain increased rapidly in size by translocation from other plant parts and synthesis of starch and protein for storage. Ribonuclease activity may have also been active, and all these factors accounted for the rapid decline to maturity. Significant differences between Atlas 66 and Pawnee RNA contents were positively correlated with grain protein content. This difference may be a factor in grain protein content. At maturity, both species had similar RNA contents, indicating a greater ribonuclease activity in Atlas 66 than Pawnee. The ribonucleotides hydrolyzed by the ribonuclease may then have been available for the developing grain.

DNA contents in the head tissues did not differ between varieties, which indicated that the living cell number did not differ between Atlas 66 and Pawnee. However, this again indicated that Atlas 66 had more RNA per cell than Pawnee. DNA content dropped in both Pawnee and Atlas 66 from anthesis to maturity, probably due to the influence of dry matter accumulation in the developing grain and senescence of the ear tissue.

The nucleotide composition of the RNA from Atlas 66 and Pawnee grain indicated the RNA composition and perhaps the protein composition changed as the grain matured. At maturity, the purine:pyrimidine ratio changed from 1.0 to 0.9 because of an increase in cytidylic acid and a decrease in guanylic acid. Pawnee and Atlas 66 RNA had the same base composition, but at maturity Pawnee contained slightly more uridylic acid and slightly less cytidylic acid than Atlas 66. That suggested a difference in protein composition at maturity. Both Atlas 66 and Pawnee nucleotides were free of contamination, so that absorption ratios agreed with the literature values.

Differences in the types of RNA species present in the developing grain may be the reason for the change in the purine:pyrimidine ratio as the grain matured. However, the separation of the different RNA species was not attempted in this study.

Milhailovic (1964) studied the nucleotides of wheat leaves of high- and low-yielding wheat varieties. He concluded that no significant differences occurred in the nucleotide composition of the high- and low-yielding varieties tested. This agreed with the above results for the wheat grain. Hadzeyev et al. (1968) also studied RNA of wheat leaves and came to the same conclusions.

SUMMARY

Investigations were conducted with various crop species to determine whether grain protein content was related to nucleic acid content. Two-week-old seedling leaf blades of high- and low- grain protein varieties of six species were grown under constant conditions and analyzed for total RNA and DNA contents. The results showed greater variation among species than within varieties. However, soybeans, sorghum, and oats had significantly higher RNA contents in the high-protein variety than in the low-protein variety, and soybeans, oats, and rice had significantly higher DNA contents in the high-protein variety than in the low-protein variety. The other species showed no statistical difference in either RNA or DNA between the high- and the low-protein varieties tested. Several high- and low-protein wheat varieties and their F_5-derived progeny were grown and analyzed as above. No statistical differences were found in either RNA or DNA contents. Atlas 50, however, did have the highest nucleic acid content and some of its derived progeny had the highest nucleic acid contents of the progeny.

A field experiment with high- and low-protein wheat varieties indicated that some morphological plant parts showed correlation between grain protein content and RNA content. The leaf blade RNA content was positively correlated with grain protein content throughout the sampling period. The flag leaf and head RNA contents also correlated with grain protein content early in their development. The grain showed no differences in RNA content between the two species. The grain, however, did show a negative correlation

between DNA content and protein content early in its development. None of the other plant parts differed significantly in DNA content between species.

Nucleotide changes in the developing grain indicated a change in the RNA species as the grain matured. These differences may have had an effect on the final amino acid composition of the grain protein.

Purifying the flag leaf and grain nucleic acids on resin columns after alkaline hydrolysis decreased their indicated contents, but gave a product with spectral characteristics similar to pure hydrolyzed yeast RNA. The nucleotide composition of the grain during development changed in the purine:pyrimidine ratio as the grain matured, but no differences between the two species.

The results of the growth chamber experiments support the work of recent investigators who have shown that nucleic acids are synthesized in the nucleus and are thus genetically determined. The results also show that in some crop species, including soybeans, sorghum, and oats, RNA content in the leaf blades are correlated with grain protein content. These differences may be a factor in the final grain protein content if the differences persist throughout the plants development. However, differences in the DNA content in soybeans and oats may account for the RNA differences, by indicating that these species have a higher living cell number in the high-protein variety.

Differences in RNA content of some of the developing plant parts of wheat appeared to correlate with grain protein content. Also, the results indicated that Atlas 66, the high-protein variety, had more RNA per cell than Pawnee. The RNA differences occurred early in the development of these plant parts, when probably most of the protein synthesis occurred. These differences may be a factor in the final grain protein content. Other factors, however, are also involved.

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APPENDIX

Table I. Ribonucleic acid (RNA) contents of seedling leaf blades of high- and low-protein lines of wheat, corn, sorghum, soybeans, oats, and rice (mg/gm Fwt).

Reps	Wheat		Soybean		Sorghum		Corn		Rice		Oats	
	Atlas 66	Pawnee	Wayne	Amsoy	3071	Plainsman	WF9x 107	Hy2x 0h7	Tiachung #1	Bluebelle	CI8330	Animated
1	2.87	2.74	3.92	3.24	4.52	4.45	2.75	2.98	3.50	3.50	2.68	2.52
2	3.24	2.74	3.72	3.23	4.26	4.23	2.87	3.02	3.50	3.42	2.72	2.40
3	2.75	2.82	3.92	3.83	4.94	4.41	2.91	3.72	2.92	3.43	2.50	2.17
4	2.78	2.67	3.57	3.42	4.50	4.56	2.90	3.11	2.91	3.23	2.58	2.44
5	3.07	2.88	3.82	3.48	4.71	4.51	3.17	2.99	3.32	3.67	2.75	2.66
6	3.06	2.92	3.57	3.42	4.54	4.07	2.91	3.07	3.01	3.43	2.75	2.49
7	2.98	2.90	3.71	3.39	4.83	4.25	3.24	3.23	3.73	2.98	2.82	2.50
8	2.98	2.91	4.41	3.92	4.66	4.33	2.98	3.07	3.00	3.00	2.97	2.52
Total	23.73	22.58	30.64	27.93	36.96	34.81	23.73	25.19	25.89	26.66	21.77	19.70
Mean	2.97	2.82	3.83	* 3.49	4.62	* 4.35	2.97	3.09	3.23	3.33	2.72	* 2.46

* 0.05 LSD = 0.21 at 0.05 level

Table II. Deoxyribonucleic acid (DNA) contents of seedling leaf blades of high- and low-protein lines of wheat, corn, sorghum, soybeans, oats, and rice (mg/gm Fwt).

Reps	Wheat		Soybean		Sorghum		Corn		Rice		Oats	
	Atlas 66	Pawnee	Wayne	Amsoy	3071	Plainsman	WF9	Hy2	Tiachung	Bluebelle	CI8330	Animated
1	0.56	0.56	0.40	0.27	0.26	0.27	0.29	0.34	0.30	0.25	0.39	0.37
2	0.63	0.56	0.34	0.30	0.36	0.33	0.24	0.44	0.25	0.24	0.45	0.40
3	0.62	0.54	0.41	0.30	0.27	0.31	0.38	0.28	0.32	0.21	0.39	0.37
4	0.65	0.56	0.38	0.38	0.30	0.28	0.39	0.37	0.31	0.22	0.47	0.38
5	0.57	0.65	0.32	0.35	0.40	0.26	0.38	0.40	0.32	0.29	0.41	0.38
6	0.65	0.67	0.41	0.33	0.48	0.43	0.39	0.35	0.33	0.26	0.43	0.38
7	0.58	0.69	0.36	0.42	0.38	0.44	0.37	0.34	0.32	0.25	0.44	0.37
8	0.55	0.61	0.37	0.35	0.28	0.32	0.40	0.36	0.33	0.26	0.42	0.39
Total	4.81	4.85	3.00	2.71	2.73	2.66	2.84	2.87	2.47	1.98	3.40	3.04
Mean	0.60	0.61	0.37	* 0.34	0.34	0.33	0.36	0.36	0.31	* 0.25	0.43	* 0.38
* 0.05			LSD = 0.03 at 0.05 level									
RNA/DNA	4.95	4.62	10.35	10.26	13.59	13.18	8.25	8.58	10.42	13.32	6.33	6.47

Table III. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents of seedling leaf blades of high- and low-protein wheat varieties and their F_5-derived progeny (mg/g Fwt).

Reps	RNA						DNA						Ratio RNA/ DNA
	1	2	3	4	Total	Mean	1	2	3	4	Total	Mean	
Atlas 66	3.52	3.57	3.33	3.55	13.97	3.49	0.40	0.37	0.42	0.40	1.59	0.40	8.73
Atlas 50	3.87	3.65	3.97	3.88	15.37	3.84	0.46	0.48	0.52	0.45	1.90	0.48	8.00
Kaw	3.46	3.47	3.77	3.50	14.19	3.55	0.37	0.46	0.46	0.37	1.66	0.42	8.15
Triumph	3.64	3.72	3.32	3.50	14.18	3.55	0.40	0.48	0.33	0.50	1.70	0.43	8.26
Kaw x 66 (H)	3.81	3.79	3.71	3.68	14.99	3.75	0.42	0.44	0.44	0.37	1.67	0.42	8.93
Kaw x 66 (L)	3.33	3.48	3.21	3.38	13.40	3.35	0.37	0.41	0.37	0.38	1.53	0.38	8.82
Kaw x 50 (H)	3.65	3.57	3.76	3.74	14.72	3.68	0.39	0.45	0.52	0.44	1.81	0.45	8.18
Kaw x 50 (L)	3.93	3.79	3.79	3.84	15.35	3.84	0.41	0.53	0.53	0.50	1.98	0.50	7.68
Triumph x 66 (H)	3.76	3.68	3.55	3.61	14.59	3.65	0.46	0.46	0.37	0.42	1.71	0.43	8.49
Triumph x 66 (L)	3.46	3.61	3.77	3.61	14.44	3.61	0.45	0.53	0.43	0.39	1.80	0.45	8.02
Triumph x 50 (H)	3.66	3.48	3.49	3.79	14.42	3.61	0.49	0.48	0.43	0.51	1.90	0.48	7.52
Triumph x 50 (L)	3.54	3.59	3.67	3.51	14.31	3.58	0.46	0.48	0.45	0.44	1.83	0.46	7.78
	LSD = 0.826 at 0.05 level						LSD = 0.09 at 0.05 level						
	All F tests n.s.						F test n.s.						

Table IV. Ribonucleic acid (RNA) contents of leaves, heads, and grain of Pawnee and Atlas 66 wheat.

Date	Leaf blades mg/g/Fwt		Flag leaf* mg/g/Dwt		Grain* mg/g/Dwt		Heads mg/g/Dwt	
	Paw-nee	Atlas 66	Paw-nee	Atlas 66	Paw-nee	Atlas 66	Paw-nee	Atlas 66
2nd April	3.70		--	--	--	--	--	--
16th April	4.00		--	--	--	--	--	--
30th April	5.20		--	--	--	--	--	--
7th May	5.50		9.00	--	--	--	--	--
14th May	6.02		6.39	13.26	--	--	--	--
21st May	6.65		6.38	8.31	--	--	14.7	--
28th May	7.38		6.45	8.18	--	--	13.8	19.1
4th June	11.10		6.23	6.44	5.50	--	12.7	17.9
11th June	13.48		3.51	5.18	3.28	--	11.5	16.7
18th June	--		--	3.34	2.54	5.64	9.3	14.9
25th June	--		--	--	2.15	3.20	--	10.3
2nd July	--		--	--	2.11	2.55	--	--
9th July	--		--	--	--	2.46	--	--

* Purified on Dowex-1 from 200-400 mesh.

Table V. Deoxyribonucleic acid (DNA) contents of leaves, heads, and grain of Pawnee and Atlas 66 wheat.

Date	Leaf blades		Flag leaf*		Grain*		Heads	
	Paw-nee	Atlas 66	Paw-nee	Atlas 66	Paw-nee	Atlas 66	Paw-nee	Atlas 66
2nd April			--	--	--	--	--	--
16th April			--	--	--	--	--	--
30th April			--	--	--	--	--	--
7th May			1.32	--	--	--	--	--
14th May			1.84	1.80	--	--	--	--
21st May			1.94	1.85	--	--	2.1	--
28th May			1.25	1.95	--	--	2.9	5.6
4th June			1.24	2.05	2.01	--	2.5	3.0
11th June			0.97	1.74	0.95	--	1.8	2.8
18th June			--	1.00	0.68	1.15	1.6	2.9
25th June			--	--	0.38	0.72	--	1.4
2nd July			--	--	0.31	0.38	--	--
9th July			--	--	--	0.40	--	--

Table VI. Spectral absorbancies from 220 nm to 290 nm of wheat leaf, grain, and yeast RNA before and after purification through Dowex 1 resin.

Wave-length	Optical Density					
	Before			After		
	Leaf	Grain	Yeast RNA	Leaf	Grain	Yeast RNA
220	1.150	1.350	0.135	0	0	0
230	0.940	0.920	0.105	0	0.000	0
240	0.730	0.610	0.151	0.080	0.098	0.059
245	0.650	0.560	0.184	0.123	0.145	0.100
250	0.600	0.535	0.209	0.157	0.186	0.137
260	0.570	0.565	0.227	0.182	0.228	0.165
270	0.610	0.610	0.183	0.135	0.188	0.125
275	0.625	0.615	0.145	0.105	0.156	0.094
280	0.650	0.600	0.104	0.080	0.130	0.070
290	0.580	0.495	0.035	0.045	0.075	0.032

NUCLEIC ACIDS IN LOW- AND HIGH-PROTEIN LINES OF WHEAT AND OTHER GRAIN SPECIES

by

PETER ROBERT HOBBS

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Nucleic acid contents were compared for possible relationships with grain protein content in low- and high-protein lines of wheat (Triticum aestivum L.), corn (Zea mays L.), soybeans (Glycine max (L.) Merr.), sorghum (Sorghum bicolor (L.) Moench.), rice (Oryza sativa L.), and oats (Avena sterilis L.). Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were separated by differential alkaline hydrolysis and determined spectrophotometrically. The nucleic acid contents of leaf blades of two-week-old seedlings of the above species grown under controlled conditions varied more among species than between high- and low-protein lines. Differences in RNA content of soybeans, sorghum, and oats, and in the DNA content of soybeans, oats, and rice were significant and correlated positively with grain protein content. In a similar study, nucleic acids in leaf blades of two-week-old seedlings of high- and low-protein varieties of wheat and their F_5 progeny did not correlate statistically with grain protein content. Similarities in nucleic acid content between the parents and their progeny, however, suggested nucleic acid contents were genetically determined.

A third experiment determined nucleic acid changes in various morphological plant parts of high- ('Atlas 66') and low-protein ('Pawnee') wheat varieties throughout development in the field. Differences and trends in nucleic acids varied according to the method of expression and the purity of the nucleotides. On a fresh weight basis, the nucleic acid contents of the leaves increased throughout the sampling period, whereas on a dry weight basis, the nucleic acid contents decreased. Purifying the nucleotides on resins decreased the indicated nucleic acid contents of the grain and flag leaves. RNA content of the leaf blades throughout the growing season was associated positively with the final grain protein content. The purified

ribonucleotides of the flag leaves and grain were not correlated with grain protein content except before heading in the flag leaves. DNA contents were negatively correlated with grain protein content in the early stages of development of the grain. The RNA content of the head tissue was positively correlated with grain protein content at their earlier stages of development. All the other DNA and RNA contents did not differ significantly. The results indicated that the RNA contents in the early stages of the development of the flag leaves and heads may be associated with grain protein content. The results also indicated that the RNA content per cell were probably higher in the high-protein variety, Atlas 66, than in the low protein variety, Pawnee.

A method for purifying the nucleotides of the leaves and grain gave consistent and rapid results. Another method for determining nucleotide base composition of nucleic acids also gave satisfactory results. The purine:pyrimidine ratio decreased as the grain matured. The nucleotide base compositions of Atlas 66 and Pawnee grain did not differ significantly (at similar maturities) during development.