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The Biology of Plant Proteins

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I. INTRODUCTION

This article summarizes some of the salient features of plant proteins and provides a basic foundation on which subsequent articles in this issue can expound. It discusses the source of proteins in plants, explains the role and function of proteins in food plants, their natural variation, and tells how microorganisms and pathogens can sometimes introduce other substances, including proteins, into food and produce. This is not a comprehensive treatment of plant protein chemistry. For more in-depth information, readers are referred to textbooks on plant and protein biochemistry.^{1,2}

Proteins are naturally occurring polymers made up of constituent amino acids. Every cell of every organism, including those in humans, typically contains tens of thousands of proteins, each of which is structurally complex and distinct. These constituent proteins are encoded by the organism's genome. From estimates of genome size we can make rough estimates of the total numbers of proteins likely to occur in individual species. There are between 15,000 and 60,000 genes³ in the small weed plant *Arabidopsis thaliana*, and just as many proteins.

There are 20 different amino acids in plant proteins. Of prime importance to a nutritionist is the balance of constituent amino acids in food plant proteins, and their availability during digestion in the alimentary tract. The human body efficiently breaks down food proteins into constituent amino acids during digestion. It then uses the amino acids as building blocks to synthesize other proteins needed for growth, development, and maintenance of normal functions. During diges-

tion, proteins undergo partial disassembly into smaller molecules called peptides, which lack the functional activity of the original protein. These result from the enzymatic cleavage of the bonds linking specific pairs of amino acids.

In addition to their nutritional function, proteins contribute to food texture, flavor, aroma, feelings of satiety, ease of processing, and food quality. However, in sensitive people, proteins interact with immunoglobulin E (IgE), becoming allergenic or toxic to those who ingest them (see "Introduction to Allergic Diseases" by Yoseph A. Mekori and "Allergic Reactions to Food" by John A. Anderson in this issue). The process of dietary elimination and controlled food challenge reveals which, if any, food proteins individuals must avoid to remain healthy and free from food-induced problems. Catalogs of food allergies, food intolerances, and even poisonings have been assembled from such information.^{4,5} They guide physicians in advising patients about problems that may be caused by their dietary intake of certain proteins.

II. PLANT PROTEINS: BIOLOGY OF OCCURRENCE

As a result of differentiation during development, plant cells may take a variety of forms which adapt them to their primary function in the plant. However, all plant cells have a number of features in common. Each has a cellulose wall enclosing a protoplast bounded by a protein-lipid membrane that is selectively permeable to ions in solution. The protoplast consists predominantly of a skein of protoplasm lining the wall which surrounds a fluid-filled central vacuole that con-

tains various solutes. For example, the familiar leafy tissue of lettuce consists predominantly of the cell walls. The fluid in the cell vacuoles and the physical constraint imposed by the wall create turgid cells and the crisp texture that the consumer expects. The protein content of a lettuce leaf is low, about 1 to 3% of the dry weight of the leaf tissue, or less than 0.5% of the wet weight. The nucleus of the cell, located in the protoplasm, consists of various nucleoproteins and nucleic acids that, at cell division, resolve into the structures called chromosomes. The genes, carried on the chromosomes of the nucleus, direct the synthesis of proteins, many of which are enzymes. A gene is a unit of inherited information. It consists of a number of nucleotides arranged in a linear order like the words in a sentence. However, in the sentence represented by a gene there are only four letters (the nucleotides adenine, thymidine, guanine, and cytosine), and each word has only three letters. Each triplet represents an amino acid, so that the sequence of triplets encodes the linear sequence of amino acids that make up the polypeptide chain, or protein product of the gene. Gene expression is coordinated and regulated by elements within the nucleus that respond to environmental and developmental signals. It is the sum of the proteins produced in each type of cell that give that cell its properties. Major changes may be caused by the expression of groups of genes, resulting in the peculiarities of form and chemical composition that differentiate cell types from each other.

Although the principal location of the genetic determinants of each cell is in the nucleus, green plant cells have two other kinds of bodies, mitochondria and chloroplasts, that also carry genetic information. Chloroplasts are the site of photosynthesis, a process that uses the energy of sunlight to make carbohydrates like sugar and starch from carbon dioxide and water. During this process oxygen is formed and released. The products of photosynthesis are used as sources of energy by the mitochondria, which are the site for oxidative enzymes which create the energy currency the cell uses, and as raw materials for the synthesis of many other compounds. The enzyme ribulose biphosphate carboxylase (rubisco), which occurs in the chloroplast and plays an important

role in photosynthesis, is by far the most abundant protein on Earth. Its structure is encoded by the chloroplast DNA and by the DNA of the cell nucleus.⁶ The cells of plant parts that are green may contain from 1 to as many as 50 or more chloroplasts. Plant parts that are not green (e.g., roots and tubers) normally do not contain chloroplasts. Mitochondria are smaller than chloroplasts and are found in all plant cells in numbers ranging into the hundreds. Because they are the site of oxidative enzymes, they are involved in the process of respiration. In this process, oxygen is used to accept electrons from donor molecules, which, as a result, carry charges that enable them to drive a series of other reactions in cell metabolism.

There are two centers of active cell division in a plant seedling, called meristems, that are responsible for the growth and development of a mature plant. The shoot apical meristem gives rise to leaves, stem, and eventually to flowers. The root apical meristem gives rise to root tissue. This may be simple and unbranched like that of a carrot, or highly branched and fibrous, like the roots of lawn grass. As the characteristic form of the mature plant develops, the immature cells undergo changes in shape, chemical composition, and their complement of proteins that are usually irreversible. Once they have assumed their mature form, these cells can no longer divide and many may die. For example, the xylem, or water-conducting tissue, in the roots and stems of plants consists of tubular elements made up of empty cells. The internal diameter of these cells ranges from 0.01 to 0.10 mm and they can be from 1 to 8 mm or more in length. The individual xylem cells (xylem elements) have reinforced walls. They are strung together to create a tubular network that carries water, and materials dissolved in it, from the roots to the leaves and other plant parts above the soil. As the xylem elements reach their maximal size and mature form, they undergo programmed cell death in preparation for their final function.⁷ Other cells assume a long and narrow form and develop very thick walls and create bands or blocks of mechanically strong fibers that reinforce stems and leaves against the mechanical stresses imposed by wind and rain. In tree stems these two kinds of cells, water conducting and fiber, give rise to the wood and timber used in

commerce. In food plants fiber cells contribute important mechanical properties to the human diet.

The parts of plants commonly used as food by humans include storage organs such as roots (carrot, radish, turnip), tubers (potato, sweet potato), and stems (kohlrabi, kale, asparagus, celery), but also include a wide range of other plant parts such as leaves (cabbage, lettuce, spinach), bulbs (onion, garlic), flowers or inflorescences (broccoli, cauliflower, artichoke), fruits (apple, pear, tomato, berries, squash, melon, cucumber, banana), and seeds (corn, rice, peas, beans, wheat flour, nuts). In all of these plant parts the principal source of calories are the carbohydrates sugar and starch. Except in seeds, where they are often present in high concentrations, plant cells contain small quantities of protein even though there may be more than 1000 different proteins present.⁸ Figure 1 shows a scanned image of cell proteins isolated from a newly germinated shoot of a corn seedling and revealed by two-dimensional gel electrophoresis. More than 1500 spots, each representing a different protein, are resolved. These likely represent only a fraction of the total proteins present in shoot tissue. Seeds contain relatively large amounts of a few special proteins and are also sources of fats and oils.⁹

It was believed for many years that some 15 major crops were largely responsible for feeding the world. These are the cereals: rice, maize, wheat, and sorghum; the legumes: beans, soybean and peas, and peanuts; the root crops: potato and cassava; and sugarcane, sugar beet, bananas, and coconuts. However, recent studies have shown the importance to individual groups and societies of more than 100 food plant species.¹⁰ These include mangoes (28% by weight of the vegetable food consumed in St. Lucia), Chinese cabbage (12% in South Korea), taro (18% in Samoa), quinoa or grain amaranth (important in Bolivia), and cowpea (important in Niger).

III. THE ROLE OF PLANT PROTEINS

Proteins are an integral part of the chemical machinery for cell growth, division, and differentiation in young developing cells. The most im-

portant of the cell proteins are enzymes. These are catalysts responsible for the individual reaction steps in the biosynthetic pathways that produce the cell components. Other proteins function as signals that either turn on or turn off the expression of genes in the nucleus by binding to regions of nucleic acid that have a regulatory function. Yet other proteins have a mechanical role and provide a structural framework, or scaffold, for nucleic acids and other cell components.

The properties of proteins are a function of their structure. Built as a linear chain of 20 different amino acids, they are large molecules that are folded precisely into three-dimensional structures. Many plant proteins have attached to them small sugar residues and are said to be glycosylated, which may affect how they react with other cell components. The surface features of proteins, which may include pockets, grooves, and projections of various kinds, and that may carry different electric charges, determine how they interact with other molecules and function as catalysts. These surface features also influence how protein molecules are perceived by the human body either through external contact with the skin or through the mucosa of the mouth or intestinal tract.

IV. PROTEIN SYNTHESIS

The amino acid sequence of each protein is encoded in the nucleotide sequence of a region of the DNA that corresponds to a gene. A universal triplet code uses a sequence of three bases to specify each of the 20 amino acids commonly found in plant proteins. The DNA sequence of the gene is first transcribed to messenger RNA (mRNA), which is then processed by small particles called ribosomes. Protein assembly occurs as the ribosome reads the mRNA sequence and translates it into an amino acid sequence by selecting from a pool of transfer RNA molecules. Each transfer RNA molecule has bound to it a specific amino acid and functions as an adapter that presents the amino acids at the right time and place during the synthesis of the protein. As the ribosome moves along the mRNA the chain of ribonucleotides disintegrates, leaving in its place

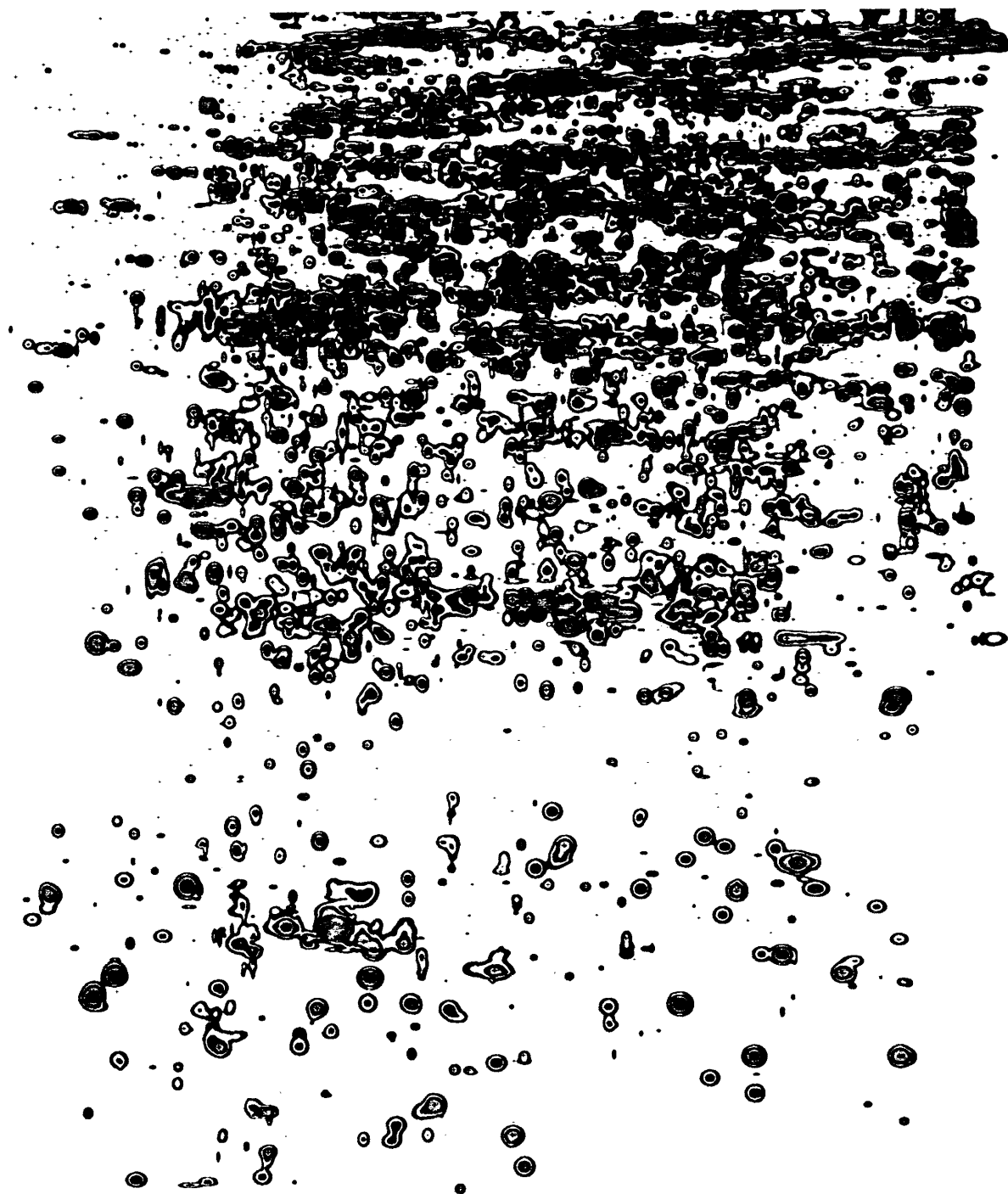


FIGURE 1. Scanned image of a two-dimensional gel electrophoretic separation of proteins from the newly germinated shoot of a corn seedling separated by isoelectric focusing in the first dimension (horizontal) and by molecular weight in the second dimension (vertical). (Reproduced, with permission, from Pioneer Hi-Bred International, Inc.)

a growing chain of amino acids. The secondary and tertiary structure imposed on this chain is in part the result of self-directed folding, often in-

duced by the establishment of chemical cross-linkages, but also influenced by chaperone proteins whose function is to adjust the final form of

the protein molecule to the most stable functional configuration possible.

The processes of transcription to mRNA and translation of the nucleic acid sequence to an amino acid sequence do not occur continuously for all genes. The process, called gene expression, is controlled by promoters, chromosomal elements located near the gene, which may be activated or repressed. Promoters are generally repressed when a small protein molecule, called a repressor, is bound to them thereby preventing them from activating gene transcription. When the gene is induced, the inducing agent releases the promoter by interfering with the binding between the repressor and the binding site of the promoter. Constitutively expressed genes are expressed all the time. The signaling controls of gene expression are critically important for normal development. They ensure that the appropriate genes are activated and expressed when and where they are needed, and that expression is shut down when no longer required.

From studies of the controls of expression in transgenic plants there is evidence that developmental controls are very similar among different plants of different genera and families. For example, Altenbach et al.¹¹ used a promoter from a French bean to express the Brazil nut 2S seed storage protein in canola and tobacco. Similarly, a gene encoding a wheat seed endosperm gluten protein introduced into tobacco was only expressed in tobacco seed.^{12,13} In each example, the cloned gene included a promoter that was able to respond to the signaling controls of entirely different plants.

Many of the larger protein molecules are composed of subunits. This presumably simplifies their assembly and synthesis, because different genes may be responsible for different subunits. For example, rubisco is composed of two kinds of subunits: a large one of ~55 kDa encoded by a chloroplast gene, and a small subunit of ~15 kDa encoded by a nuclear gene.¹⁴ The complete structure also includes a metal atom or prosthetic group that is usually critical to the catalytic activity of the enzyme and is located in a position where the primary chemical reactions take place. The three-dimensional structure of protein molecules is established by X-ray crystallography. This technique uses diffraction

patterns caused by a crystal of the protein placed in an X-ray beam to reconstruct a three-dimensional image of the molecule.

Many proteins are not used at the sites within the cell where they are synthesized. Some of these proteins may have, at one end of the amino acid chain, a so-called signal or targeting sequence that directs the protein to a specific location in the cell and is then removed.^{15,16} This may be a site on, or within, a membrane where the properties of the protein are called into play.¹⁷

V. PROTEIN VARIATION

Broadly speaking, the more closely related organisms are the more similar are their constituent proteins. Many enzymes, such as rubisco, and other so-called house-keeping enzymes that perform basic cellular functions, are common to and conserved in most plants. A single change in the base sequence of the DNA that encodes a protein may lead to the substitution of an inappropriate amino acid at one point in the molecule. Depending on the site of this change, or mutation, it may have the effect of either abolishing or altering the function of the protein. Mutational changes over evolutionary time may also result in amino acid changes that for the most part have little effect on the way the protein molecules function. A common effect of these changes is to alter the mobility of the protein in electrophoresis, a technique that separates proteins on the basis of their size and charge. The altered forms of enzyme proteins are called isozymes. Different forms may occur within the same species or vary between species. In some respects these changes are evolutionary scars and almost certainly occurred as a result of spontaneous mutations at earlier stages of evolution. Changes that abolish important or vital functions, or substantially modify them, may be lethal or may reduce the fitness of the organism. In general, these changes would be selected against in nature because they would not survive and produce seeds in competition against unimpaired forms. Occasionally, mutations may be beneficial to the organism and confer competitive advantages over other types. This is the basis of evolution.

Naturally occurring variation in protein composition can be most easily detected by two-dimensional electrophoresis of plant extracts^{8,18,19} (see Figure 1). However, this technique probably resolves only a fraction of the total number of proteins present in a particular tissue.

Changes that are beneficial or neutral with respect to protein function could be of significance if they created molecules that are allergenic or have enhanced allergenicity. However, in view of the very low frequency of IgE-mediated allergic responses in humans to the very large numbers of proteins and naturally occurring protein variants they are normally exposed to in their diet, the probability of this occurring is almost certainly very low. It is also important to bear in mind that, except for seed storage proteins, the majority of individual plant proteins are present only in small amounts and at concentrations that may be below the threshold needed to stimulate an IgE-mediated response.

VI. PROTEINS INTRODUCED INTO THE DIET BY PLANT PRODUCTS

Some proteins have a mechanical or structural function contributing to the form of plant cells and their organelles. For example, the membrane systems within cells, which are the sites of various reactions inherent in cell metabolism, are proteinaceous. However, carbohydrate derivatives, often strengthened with polymers such as lignin, are far more important in giving rise to the gross rigid and fibrous structures we are familiar with in plants.

Enzymes are proteins that direct the many biochemical processes that accompany cell growth, cell division, and metabolism. There are large protein reserves in seeds that are used to sustain the growth and development of the seedling during and after germination. These often occur in cells as protein bodies and are broken down, or mobilized, by enzymes and transported and reassembled in regions undergoing rapid growth when the seed germinates. Unlike most other plant parts, seeds have large amounts of protein that may make up 10 to 50% or more of their dry weight.⁹ This is why seeds and nuts are important protein

sources in the human diet. Many of the stored proteins are present in relatively high concentrations. Numerous reports have suggested a correlation between the levels of protein and allergenicity (see "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Samuel B. Lehrer and "Food Allergens" by Robert K. Bush and Susan L. Hefle in this issue). The high levels of storage proteins in seeds may explain, in part, why some people become sensitive to them after consuming them (e.g., peanuts) or processed seed products (e.g., wheat flour in bread).

In addition to the diversity of proteins that are normally present in plant tissues, other proteins are introduced by microorganisms associated with the plant, or by invading pathogens such as viruses, bacteria, and fungi. All fresh food and produce have a microbial flora. Fruits commonly carry yeasts that grow on their surfaces. All plant surfaces have soil organisms carried there on dust particles and other debris, or splashed on by rain or irrigation water. Airborne fungal spores are deposited on plants.

Some of the fruits and vegetables we purchase for consumption may carry plant disease organisms. Plant viruses increase to enormous numbers in infected cells such that the viral coat proteins, or other viral proteins that form inclusion bodies, compose a significant portion of the total cell protein. Data in Table 1 show the levels of coat protein from different viruses that were measured in four different fruits (cantaloupe, honey dew melon, yellow crook neck squash, and zucchini squash) obtained from a supermarket. These data illustrate the variability and extent of the different viral coat proteins that are components of our food supply.

In most cases the presence of organisms causing plant disease is revealed by the rots and discoloration (chlorosis and necrosis) that are associated with yet more changes in composition. These symptoms may appear in the field, before harvest, or after harvest when produce is in storage prior to distribution and sale. Soft rots are caused by the release of extracellular enzymes, formed by the invading microorganisms, that break down the pectin cement between cell walls and the cell walls themselves. In some cases host cell death is caused by toxins made by the pathogen

TABLE 1

Measurements of Viral Coat Protein in Supermarket Fruit as Measured by Enzyme-Linked Immunosorbent Assay

Fruit	CMV ($\mu\text{g/kg}$ fruit)	PRV ($\mu\text{g/kg}$ fruit)	ZYMV ($\mu\text{g/kg}$ fruit)	WMV2 ($\mu\text{g/kg}$ fruit)
YC77EZW20	ND	ND	68.4	430.6
C1	355,200	18,000	14,400	10,320
C2	130,464	5,472	10,944	115,488
C3	ND	252,000	28,800	720
C4	ND	ND	864	ND
C5	>2,400,000	1,200	8,400	ND
C6	>3,216,000	ND	14,000	ND
C7	>3,216,000	ND	12,864	ND
H1	ND	7,200	9,480	ND
H2	ND	6,840	1,800	ND
H3	ND	ND	2,200	ND
H4	359	4,752	3,888	173
H5	269	3,168	3,168	260
H6	238	ND	2,592	ND
H7	ND	5,928	1,824	137
H8	664	13,272	1,896	190
H9	82	960	24	24
H10	ND	ND	250	ND
H11	ND	ND	1,560	ND
H12	ND	ND	480	ND
H13	ND	ND	2,200	ND
H14	ND	3,120	720	ND
H15	ND	10,080	1,700	ND
H16	ND	ND	3,100	ND
Y1	ND	ND	11,424	ND
Y2	ND	ND	ND	ND
Y3	ND	ND	1,152	ND
Y4	ND	ND	13,056	ND
Z1	ND	ND	140	ND
Z2	ND	ND	ND	ND
Z3	ND	ND	454	ND
Z4	ND	ND	ND	ND
Z5	ND	ND	ND	ND
Z6	ND	ND	576	ND
Z7	43	ND	2,592	ND
Z8	14	ND	2,900	ND

Note: CMV, cucumber mosaic virus coat protein; PRV, papaya ringspot virus coat protein; ZYMV, zucchini yellow mosaic virus coat protein; WMV2, watermelon mosaic virus coat protein; ND, not detected; C1–7 are cantaloupes, H1–16 are honeydew melons, Y1–4 are yellow crookneck squash, and Z1–8 are zucchini squash. The top line is a transgenic line of squash being developed for market.

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that then uses the killed host material as a substrate for further growth and sporulation.²⁰ Endogenous proteins may also be formed by plants in response to pathogen invasion or as a result of other environmental stresses.

VII. STRESS RESPONSE PROTEINS

When plants are invaded by pathogens they frequently respond by synthesizing the components (often proteins) of defense systems designed

to limit or contain the growth of the invading pathogen. In recent years, much research has focused on the so-called pathogenesis-related (PR) proteins that appear during the early stages of plant infection.²¹ The amounts of these proteins are not large, less than 0.01% of the total cell protein. Their function is not understood in terms of specific resistance mechanisms and how these are controlled by PR proteins. Two PR proteins from kidney bean (*Phaseolus vulgaris*) have been identified as possible allergens.²² There is no evidence that the proteins produced by pathogens cause such effects.

There is considerable information that consuming the chemical products formed by successful invading pathogens may have severe consequences. For example, stored peanuts may be invaded by molds (*Aspergillus* spp.) that produce nonproteinaceous compounds, aflatoxins, known to be carcinogenic.²³ There is also evidence that infected or contaminated feeding stuffs given to cattle may result in aflatoxins being present in their milk.^{24,25}

A second example, which is not a problem in human nutrition, is encountered by livestock producers who may have problems with forage grasses that contain fungal endophytes.²⁶ These endophytic fungi live in the tissues of the grass leaves and stems without producing visible signs of disease until they reproduce sexually and form spores. However, certain chemical metabolites produced by the fungi are responsible for neurologic impairment and may cause a disorder known as staggers in grazing animals. Other compounds produced by the fungus are abortifacients and lead to stillborn calves. There is evidence that these compounds confer insect resistance and drought tolerance on the grasses, both of which are selective advantages. For this reason they are of interest to turf grass breeders.²⁷

Both of these examples of potentially dangerous materials in food and fodder are produced by fungi. They are not proteins and result from somewhat complicated synthetic pathways that are controlled by the products (enzymes) of a number of genes. In the production of new crop cultivars, plant breeders typically screen much material collected from nature and from other breeders to look for certain characteristics they need. Pest and disease resistance are important features of

most such screening programs. Care must be taken to ensure that the resistant products do not contain unduly high concentrations of naturally occurring toxic compounds that may be partly responsible for their resistance.

Plant tissues that are stressed by high temperature or extreme drought form heat shock proteins.²⁸ These proteins are used by plant cells to help them recover their normal functions when the temperature falls, or more water becomes available.²⁹ Heat shock proteins play a role in ensuring that other important cell proteins have the correct three-dimensional configuration. Heat shock proteins are also called chaperones.

VIII. CONCLUSION

Proteins play important and intrinsic roles in plant structure, function, and development. Because humans cannot synthesize essential amino acids, plant and other sources of proteins are essential components of our diet. Each day we consume tens of thousands of different proteins from plants and plant-associated microorganisms. Most of these are present in the diet in small quantities, but some, such as the storage proteins in grains and seeds, may make up a substantial part of the plant proteins we consume. Although these proteins serve many different roles in the plant, from structural to enzymatic, they are typically rapidly degraded after consumption to individual amino acids, which are then used by our bodies to synthesize other proteins and nutrients.

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