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Heat Shock Protein Synthesis in Turfgrass

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HIGH TEMPERATURE stress is a physiological disruption of turfgrass growth. Whether it is acute (direct temperature kill), which rarely occurs in the northern United States, or chronic (long-term exposure to higher than optimum temperatures), the metabolic disruption results in a weakening of the plant. Turfgrasses have various mechanisms of protecting themselves during high temperature stress. Our research involves studying the role of heat shock proteins in thermal tolerance and how the identification of these proteins may aid turfgrass breeding programs.

Eukaryotic organisms, including plants, respond to superoptimal temperatures by synthesis of a unique group of proteins called heat shock proteins. Several studies involving mammals, insects, bacteria, and yeast have shown a positive correlation between heat shock protein synthesis and the development of thermal protection. Patterns of protein synthesis change when environmental temperatures are increased from about 82° to 104° F. During a temperature stress period, the synthesis of normal cellular proteins may be reduced or even stopped completely while an increase in the number of heat shock proteins occurs. Organisms responding to high temperature synthesize an assortment of these heat shock proteins, which can roughly be divided into two classifications. These are the high molecular weight (ranging in size from 60 to 110 kiloDaltons*) and the low molecular weight (ranging in size from 15 to 30 kiloDaltons). Although the exact function of these proteins is not fully understood, their presence has been associated with acquired thermal tolerance. Some research suggests that these heat shock proteins may act as transient matrices to stabilize various cell organelles and compartments during elevated temperatures, and then disassociating when temperatures return to normal.

By definition, heat shock proteins are a new set of proteins that are rapidly and abundantly produced in response to elevated temperatures. These temperature levels are variable, depending upon the organism, but are best described as a temperature shift that is 14° to 20° F above optimal growing temperatures.

To understand the role heat shock proteins (and proteins in general) can play in heat tolerance and breeding, a brief description of proteins is needed. Proteins are linear sequences of amino acids that as a group are very diverse. Proteins make up cell structure and control cell function as 1) enzymes that act as reaction catalysts, 2) structural products such as cell walls and membranes, 3) hormones, and 4) storage and transport molecules.

Linear protein sequences orient themselves into coils, sheets, folds, or globular shapes. It is these shapes that give proteins their biological roles. High temperatures can cause protein denaturation, which is the process whereby proteins lose their structure and consequently their biological activities.

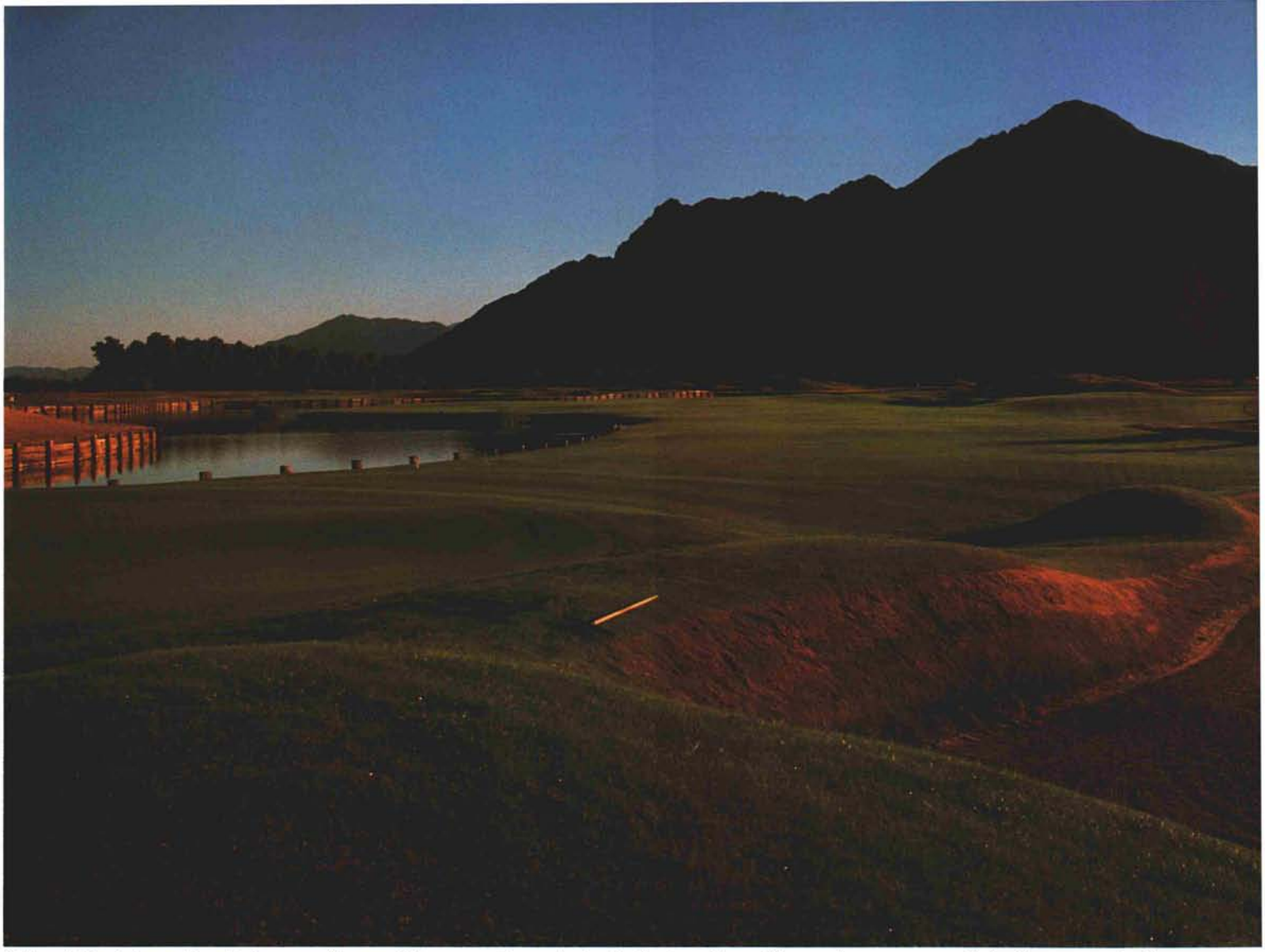
The synthesis of proteins, including heat shock proteins, begins with deoxyribonucleic acid or DNA. DNA contains the genetic instructions for protein synthesis. DNA is composed of four nucleotides (five carbon sugar containing a nitrogenous base and a phosphoryl group) called cytosine, thymine, adenine, and guanine. The matching of nucleotides (cytosine to guanine and thymine to adenine) is called base pairing. Two strands of nucleotides face each other, forming base pairs and creating a double helix.

If we look at DNA as the book of life, then the nucleotides form the coded words and sentences. Within these "sentences" (genes) are the information of protein synthesis and structure. Genes are the smallest portion of DNA that contain the hereditary information for proteins. Looking for the "sentences" of specific proteins is no trivial matter. Plant genomes or "books of life" can consist of from 10^7 to 10^{10} nucleotide base pairs.

The induction of heat shock proteins has been shown to be a universal response to thermal stress in a wide range of organisms. One of these heat shock proteins, hsp 70, is synthesized in all systems examined to date. This gene is highly conserved among these organisms, meaning that little change has occurred through evolutionary time. We have found that this gene is contained within the Kentucky bluegrass genome (Figure 1). Just because this or other heat shock genes are contained within a genome, however, does not mean the protein it codes for will be produced.

Our research focuses on the role of heat shock proteins in heritable thermal tolerance and the potential use of these genes as a molecular selection criterion for improved breeding of turfgrass cultivars for heat tolerance. In breeding, current practices involve analysis of phenotypic responses as a guide to genetic characterization of a cultivar.

*NOTE: A kiloDalton is a molecular weight measurement. A Dalton is the unit of mass equivalent to the mass of a hydrogen atom (1.66×10^{-24} gram). Kilo- is the metric prefix meaning 10^3.



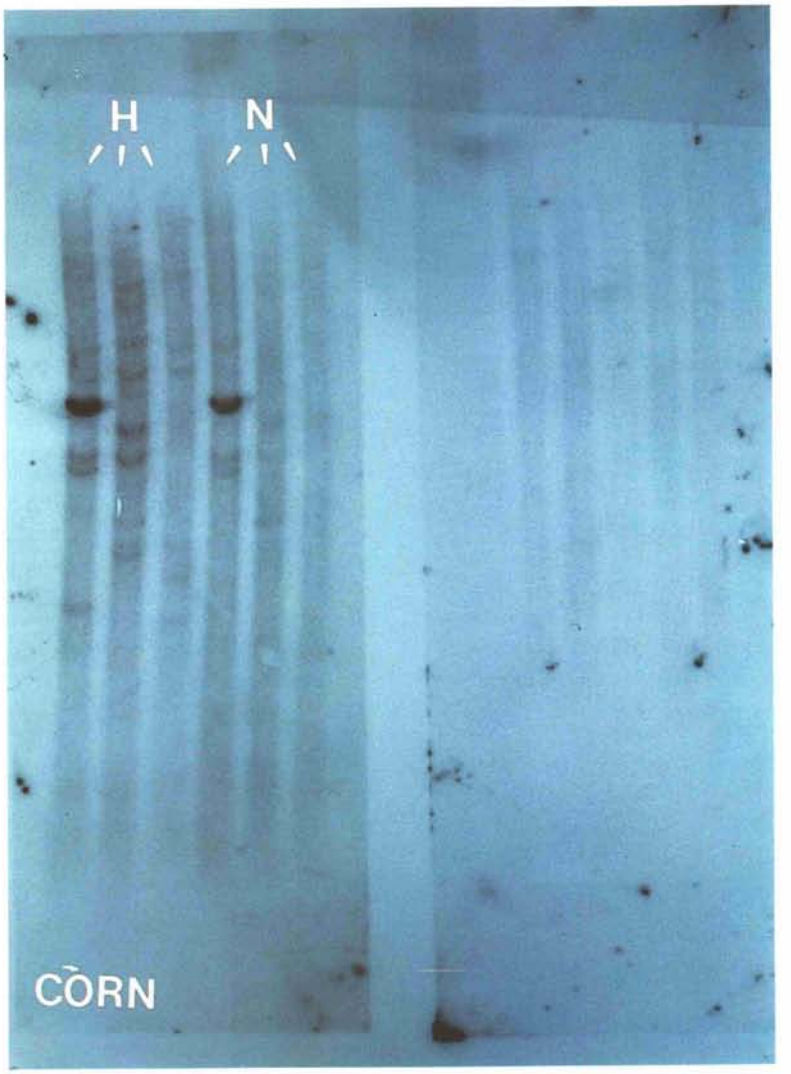
(Above) The inherent heat tolerance of perennial ryegrass is one factor in the transition of bermudagrass overseeded with ryegrass in the spring. (Opposite page, top right) Autoradiograph showing regions of homology in Kentucky bluegrass with the hsp 70 gene probe from maize (corn). The H = "Huntsville" and the N = "Nugget" Kentucky bluegrass cultivars. The homologous regions are the dark banding patterns located at various points of the 6 lanes. (Opposite page, bottom) View of restricted or "cut" genomic DNAs prior to blotting.

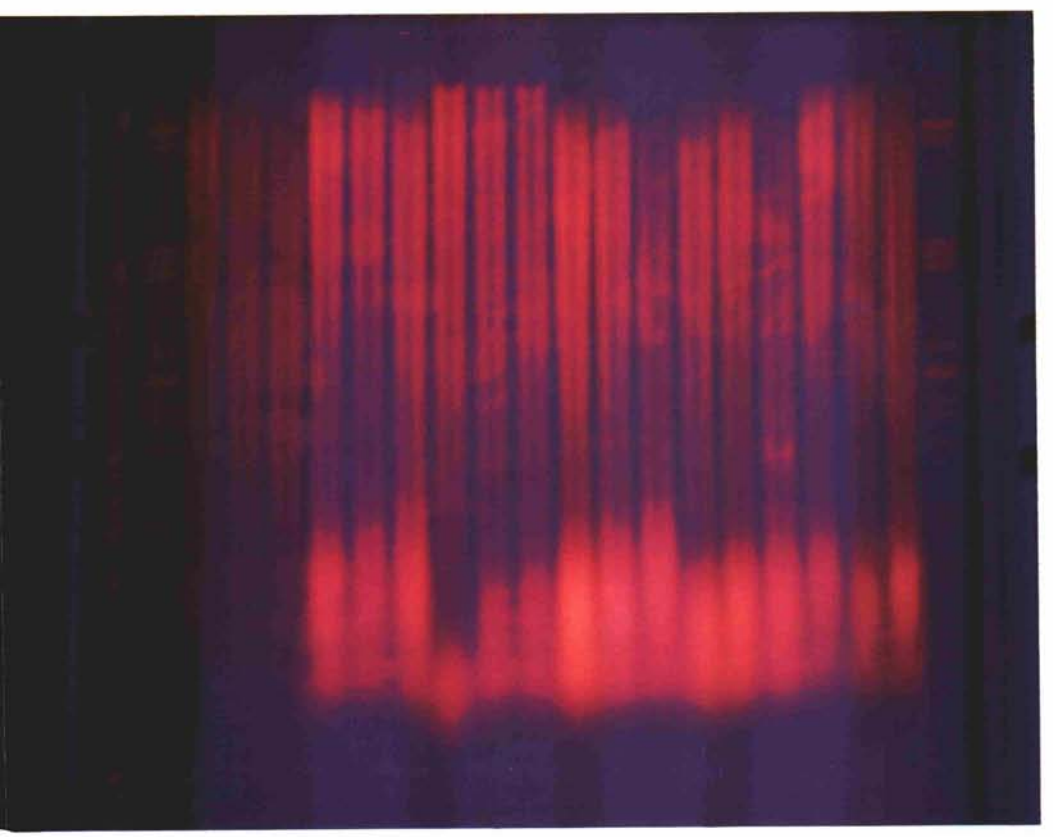
This involves much time, money, and effort to allow possible cultivars to grow and mature to express their phenotypes. These phenotypes are not an accurate picture of the plants' genetic potential, for they are expressions of genes according to environmental conditions. In many situations, outside influences mask the genotype, thus the phenotype may be providing an imperfect measure of a plant's genetic potential. Research efforts must be applied to find standards that are not variable and that can be taken from the plant at all stages. Current laboratory techniques that can expand upon field testing procedures can be used. The technology most frequently used to detect genetic polymorphisms, or differences in a genome, between cultivars is called restriction fragment length polymorphisms (RFLP).

Working with RFLPs involves the application of molecular biological techniques to the basic concepts of transmission genetics. This technique relies on the use of restriction enzymes that recognize specific DNA sequences and "cut" the double helix (Figure 2).

Theoretically, if the DNA of samples does not contain the same genes or dissimilar ones, they are "cut" at different regions within the genome. The genetic differences detected are revealed after blotting (transfer of DNA fragments to a solid support) and hybridizations with sequence-specific probes. These probes or markers are single-copy DNA segments cloned from an identified gene coding region. Probes recognize homologous regions of the genome of the samples being analyzed. Linkages (homology) between the probes and the genomes of interest permit one to infer the presence of a gene with similarities to that of the probes.

In the area of plant genetics, RFLP work could serve a function as genetic markers. Although the applied use of RFLPs in plant breeding has been examined theoretically, investigations have been more limited. RFLP applications can be incorporated into existing plant breeding procedures, enabling researchers to access desirable traits more rapidly and with greater accuracy. The use of molecular probes should allow the plant breeder to make earlier decisions about his/her selection of (in our case potential heat tolerance) cultivars while examining fewer samples. The integration of RFLP markers into plant breeding programs can lead to other applications than just the probing with markers. For instance,





this technology can also be used to make the analysis of polygenic characters into single Mendelian factors, permit the transfer of novel genes from related wild species, and even organize relationships of reproductively dissimilar plants.

The potential of molecular biology is only beginning to be realized. As techniques are developed and perfected, the ability to produce turfgrass cultivars that are stress tolerant and pest resistant will be greatly enhanced. Stay tuned.

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