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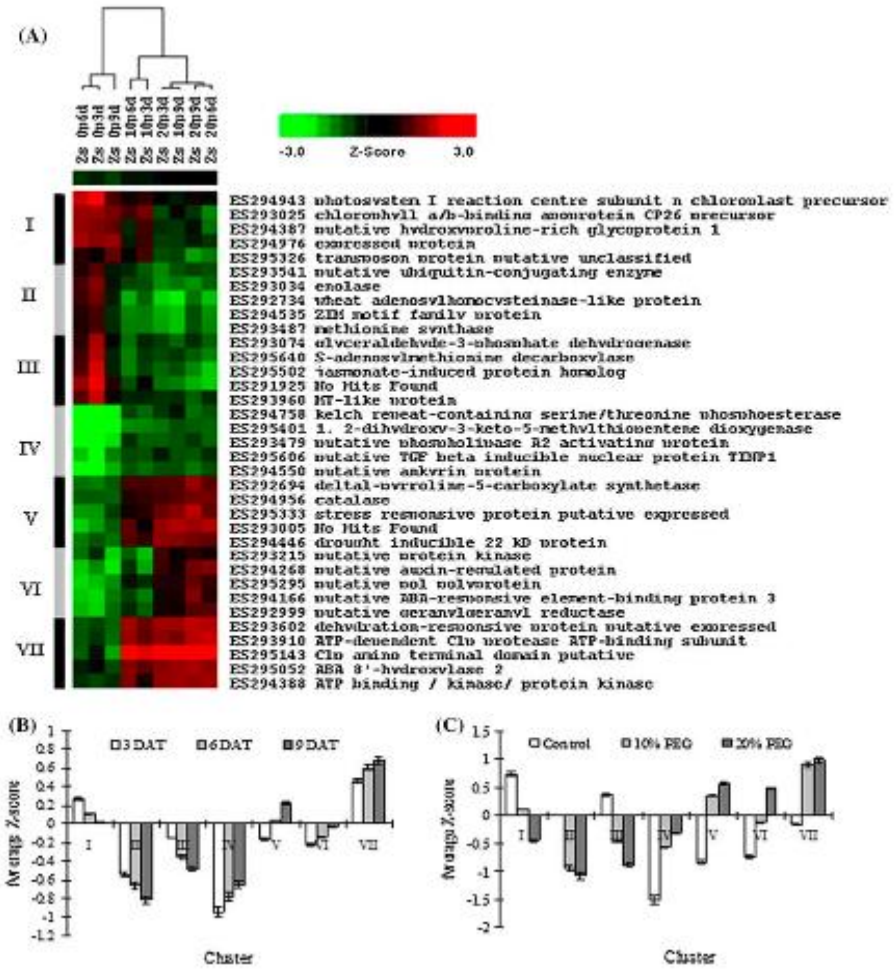

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of $30 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Drought-responsive Gene Expression in Bermudagrass

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SUMMARY

Research at the University of Georgia has begun to reveal the repertoire and organization of genes that respond to drought stress in bermudagrass and their relationships to those of well-studied models such as rice. In particular:

- A total of 120 up- and 69 down-regulated genes from two different drought-mimicking treatments (10% and 20% polyethylene glycol) and three different time points (3, 6, and 9 days after treatment) were identified.
- The 189 drought candidate genes grouped into seven different clusters according to degree of similarity in gene expression patterns.
- The three clusters of down-regulated genes included genes functioning in photosynthesis, glycolysis, sexual reproduction, and organic and electron transport activities.
- The four up-regulated clusters include some drought-responsive genes implicated in two-component sensor molecule activity and epigenetic regulation of gene expression, response to external stimulus and abscisic acid stimulus, responses to pests, pathogens, or parasites, transposase activity, and tetrapyrrole binding.
- The seven clusters of genes showing distinctive drought-responsive expression patterns mingle a few drought candidate genes with many additional genes, suggesting a diverse spectrum of responses to drought stress in this species.
- Engineering and reintroduction into the plant of modified copies of these genes might alter phenotype, perhaps in some cases conferring improved drought tolerance.
- The 'promoters' (on-off switches) of these genes might be useful to introduce other genes into the plant, and confer expression at just the right time to protect the plant from drought.
- As DNA sequences for additional bermudagrass genotypes accumulate, it may be possible to associate specific nucleotide changes with differences in drought response to better take advantage of naturally occurring variation in the development of new bermudagrass genotypes with improved drought tolerance.

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Drought stress has been a central topic of plant physiology because it significantly limits plant productivity. For example, the loss to drought in the tropics alone is thought to exceed 20 million tons of grain per year, or approximately 17% of well-watered production, with losses reaching up to 60% in severely affected regions such as southern Africa from 1991 to 1992 (6). To improve plant growth and performance under water-limited conditions, drought tolerant crops must be developed. However, drought tolerance is a particularly challenging trait to improve, due in large part to the unpredictable nature of drought.

Like food crops, many turfgrasses require appreciable water to maintain high quality and growth. One strategy to reduce irrigation requirements and water stress is to use drought resistant species and cultivars; however, the genetics and physiology of many grasses are not yet as well understood as those of major crops such as corn, wheat, and rice. More knowledge of physiological responses to water stress at the molecular level may play an important role in identifying key genes and developing new cultivars. In addition, advances in relatives such as the major cereals may be leveraged to accelerate understanding of perennial grass molecular and physiological biology.

The Case for Bermudagrass

In addition to its importance as a forage and turfgrass, bermudagrass (*Cynodon dactylon*) has been evaluated favorably as a feedstock for biofuel production due to its chemical composition (3) and is a promising plant for vegetation of dry-stack fly ash disposal areas associated with coal-burning plants (1). To maximize its utility, sustainable biomass production against various environmental stresses, particularly drought, must be a major breeding target.

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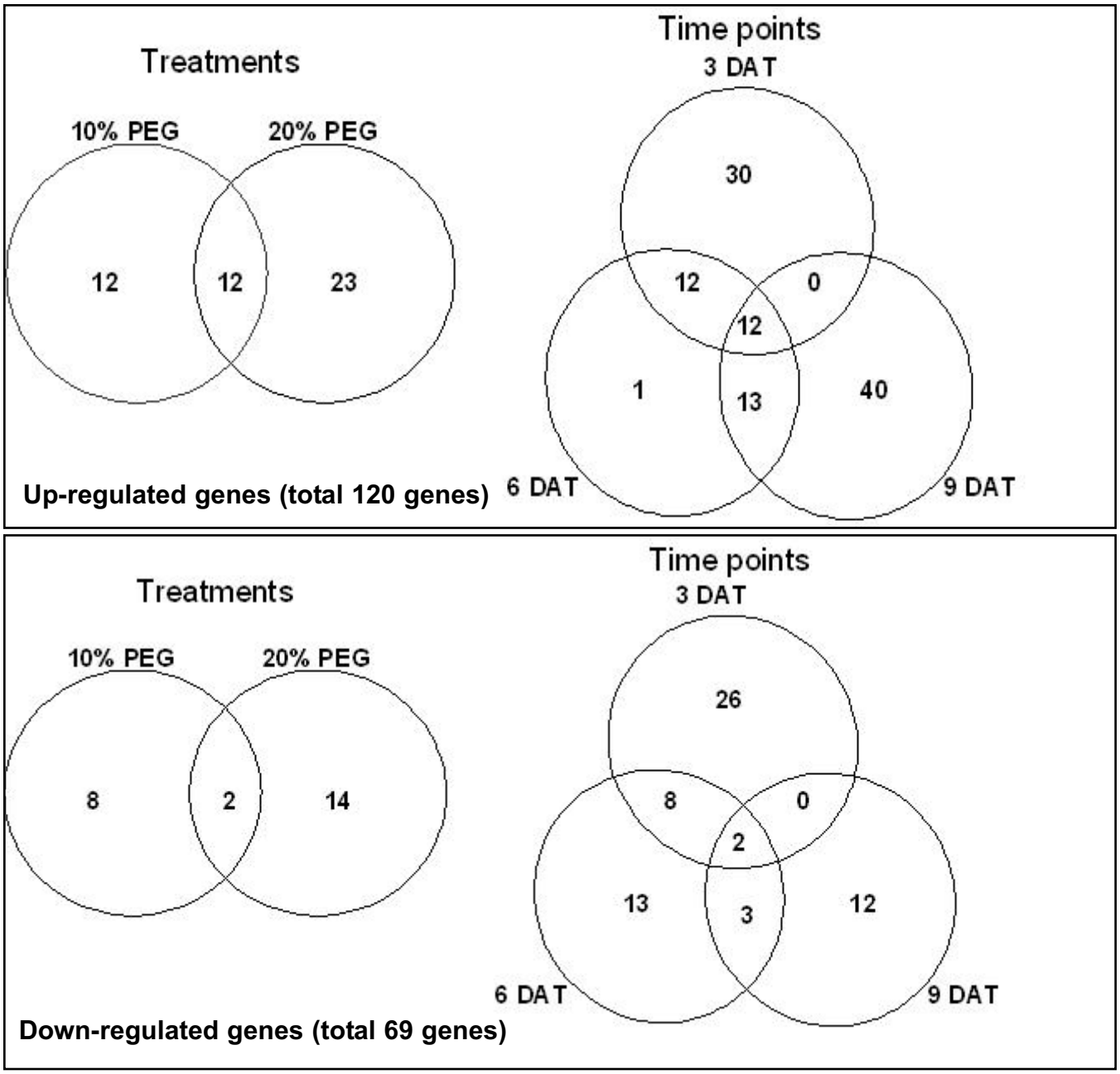

Figure 1. Venn diagrams showing the classification of genes inducible in different PEG concentrations or at different sampling time points. Note that a gene is represented in multiple groups.

The objective of this study was to identify genes in *C. dactylon* for which expression patterns are correlated with physiological responses to drought conditions. In order to induce drought-simulated conditions, we used polyethylene glycol in a nutrient solution. Strictly speaking, the experimental condition imposed osmotic stress which is often caused by drought, cold, or salt stress. Identifying genes turned on or off in response to osmotic stress will fulfill an important step toward enhancing drought tolerance and providing genes that can be deployed for testing by many biotechnology-based approaches.

Experimental Approach

A total of 4,608 bermudagrass genes that had been previously sequenced and described (4) were fixed on a nylon membrane, denatured into single-stranded DNA, and hybridized to radioac-

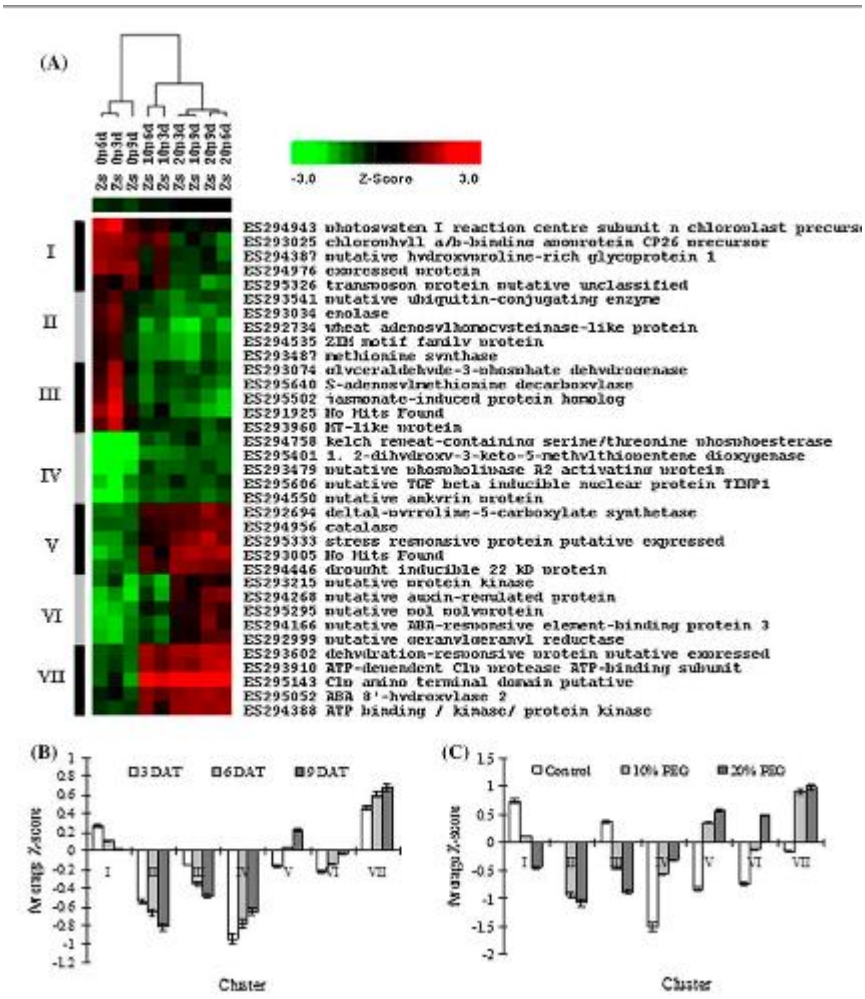

Fig. 2. Summary of cluster analysis. (a) Clustering of five drought-responsive genes from each cluster (each row corresponds to the drought candidate gene and each column corresponds to Z-score for different treatments and time points; Zs, Z-score; 0p9d, Control at 9 DAT; 0p6d, Control at 6 DAT; 0p3d, Control at 3 DAT; 10p9d, 10 % PEG at 9 DAT; 10p6d, 10 % PEG at 6 DAT; 20p6d, 20 % PEG at 6 DAT; 20p3d, 20 % PEG at 3 DAT; 20p9d, 20 % PEG at 9 DAT; 10p3d, 10 % PEG at 3 DAT). Average Z-scores of seven clusters based on either (b) sampling time points, or (c) PEG concentrations. Error bars indicate standard error of the mean (SEM).

tively-labelled messenger RNA from a series of experimental treatments. To provide the required messenger RNA samples, *C. dactylon* genotype T89 (PI 290869) was grown with half-strength Hoagland's solution in sand culture in the greenhouse at temperatures of 25°C/30°C (night/day).

Drought stress was imposed with polyethylene glycol (molecular weight 8,000, Sigma-Aldrich, St. Louis, MO) by adjusting the water potential of roots as described by (7). Three levels of water potential (ψ_w) were compared in this experiment: high ψ_w (-0.02 MPa, no PEG added), intermediate ψ_w (-0.7 Mpa, 10% PEG,) and low ψ_w (-1.6 MPa, 20% PEG), representing normal conditions, moderate drought, and severe drought, respectively. Each treatment was replicated four times in a randomized complete block design. RNA samples were obtained at 3 DAT (days after treatment), 6 DAT, and 9 DAT. Consequently, the total number of plant samples was 36 (3 different treatments × 3 different time points × 4 biological replications).

Bermudagrass Genes in Response to Drought

We identified a total of 120 up- and 69 down-regulated genes from two different drought-mimicking treatments (10 % and 20% PEG) and three different time points (3, 6, and 9 DAT) (5). A Venn diagram (Figure 1) indicates the number of genes differentially expressed in each treatment and time point. During water stress treatment, 12 genes and 2 genes were always up- and down-regulated, respectively. Of the 12 up-regulated genes, a putative delta-pyrroline-5-carboxylate synthetase (ES292694) is an essential enzyme to synthesize proline, which is a stress-responsive

amino acid. A putative MYB17 protein (ES295217) is a transcription factor involved in the MYB protein family which is able to induce a variety of stress-responsive genes (8).

To determine significance within or between PEG concentrations and sampling time points, the candidate genes were subjected to factorial analysis of variance and F-tests. The analysis suggested that Z-ratios in the two different PEG concentrations were not significantly different ($P = 0.2040$), whereas Z-ratios at three different sampling points were significantly different ($P < 0.0001$). Non-linear interactions between PEG and sampling time were also significant ($P = 0.0177$).

The 189 drought candidate genes were subjected to a hierarchical clustering analysis using the Cluster program (2), yielding seven different clusters according to degree of similarity in gene expression patterns. Clusters I-III include the 69 down-regulated genes, whereas clusters IV-VII include the 120 up-regulated genes. In order to exemplify the expression patterns of drought-responsive genes, five genes from each cluster were randomly chosen and shown in Figure 2a.

Of the three clusters including down-regulated genes, cluster I differs from the other two clusters in that genes involved in cluster I maintained their expression levels when mild drought was treated (3 and 6 DAT in 10 % PEG) but quickly down-regulated when severe drought was imposed. Only one gene in cluster I is of known function, ES294943, putatively encoding the photosystem I reaction center subunit. Cluster II includes genes functioning in phosphopyruvate hydratase complex and sexual reproduction. The phosphopyruvate hydratase complex is directly connected to glycolysis. Cluster III mostly includes organic and electron transporter activities, which are further annotated as sodium symporter activity.

Of the four up-regulated clusters, cluster VI showed an exactly opposite expression pattern to that of cluster I. Genes involved in cluster IV were not markedly down-regulated until severe drought was treated. Cluster IV includes some drought-responsive genes implicated in two-component sensor molecule activity and epigenetic regulation of gene expression, which are related to signal transducer activity and DNA methylation. Cluster V specifically includes genes implicated in response to external stimulus and response to abscisic acid stimulus. Cluster VI includes pattern binding genes, connected to responses to pests, pathogens, or parasites. Cluster VII includes transposase activity and tetrapyrrole binding genes, which correspond to genes ES293251 and ES295052, respectively. These two genes have significant similarity to abscisic acid-induced-like protein (ABA93823) and ABA 8-hydroxylase 2 (ABB71586) from other organisms.

Next Steps

The seven clusters of genes showing distinctive drought-responsive expression patterns mingle a few drought candidate genes with many additional genes, suggesting a diverse spectrum of responses to drought stress in bermudagrass. These low-cost macroarray analyses provided for the systematic analysis of quantitative gene expression and also represents an effective tool with which to find novel genes that are expressed in certain conditions or in certain tissues. The experimental procedures used in our study can also be applied to various abiotic or biotic stress conditions other than drought stress. By accumulating data on gene expression by tissue type, developmental stage, hormone and herbicide treatment, genetic background, and environmental conditions, it should be possible to identify genes involved in many important processes of development and responses to environmental conditions in bermudagrass.

The drought-responsive genes that we have identified might be applied in several ways. First, engineering and reintroduction of modified copies of these genes into the plant might alter phenotype, perhaps in some cases conferring improved drought tolerance. Second, the 'promoters' (on-off switches) of these genes might be useful to introduce other genes into the plant, and confer expression at just the right time to protect the plant from drought. Third, as DNA sequences

for additional bermudagrass genotypes accumulate, we may be able to associate specific nucleotide changes with differences in drought response, to better take advantage of naturally occurring variation in the development of new genotypes of bermudagrass with improved drought tolerance.

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