Development and Application of Molecular Markers Linked to Heat Tolerance in Agrostis *Species*

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Objectives:

1. To identify molecular markers linked to heat tolerance for heat tolerance genes from a thermal *Agrostis* species.
2. To develop a marker-assisted selection system for improving heat tolerance in creeping bentgrass.

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To gain a better insight into the molecular mechanisms of grass tolerance to heat stress and identify molecular markers for heat tolerance, we constructed a suppression subtractive hybridization (SSH) cDNA library for thermal *A. scabra.* Plants were exposed to 20^{o} C (control) or 35^{o}C for 12 days. Leaf and root samples were collected for the SSH construction, with control samples as the driver and heat-stressed samples as the tester. After subtraction, the cDNA fragments were cloned and two differential screening steps were used to screen the heat stress up-regulated library, resulting in 121 non-redundant putative heat stress responsive cDNAs out of a pool of 1,180 clones.

The 77 genes with homology to known or unknown proteins were categorized into six functional groups, including signaling/ transcription, stress/defense, protein metabolism, carbon metabolism, other metabolism, and unknown, indicating that complex gene alterations occurred during heat stress in *A. scabra*. Of the identified clones, 21 (27.3 %) had homology to genes with unknown functions. Northern blot analysis confirmed that the transcripts of nine selected genes were strongly increased under heat stress, but with different spatial expression patterns. Transcripts of seven and eight genes were strongly enhanced or induced in shoots and roots, respectively, exposed to heat stress, while two genes were only induced in roots under heat stress.

Three most interesting and important up-regulated genes, including two heat shock proteins (Hsp) 70 and 16, and one cysteine protease 1 (CP1), were isolated and their full length cDNAs were isolated using rapid amplification cDNA end method. Expression patterns of the three genes as related to heat stress in different genotypes varying in heat tolerance were further characterized.

A full-length cDNA of AsHsp16 (450bp) was isolated, which encodes a 149-amino acid protein. Northern blot and RT-PCR confirmed that AsHsp16 was a heat-inducible gene in leaves and roots. Two ecotypes of thermal *A. scabra* and 10 genotypes of creeping bentgrass varying in the level of heat tolerance were exposed to 35°C for 14 days to examine the level of AsHsp16 expression in relation to heat tolerance. Northern blot and RT-PCR analyses revealed that the level of AsHsp16 in different genotypes was positively correlated with the level of heat tolerance in both grass species.

We identified several unique gene fragments or target DNA fragment (TDFs) that are present only in heat tolerant *Agrostis scabra* under heat stress (40^{o}C), but not present in plants exposed to normal temperatures. One of the most interesting and important up-regulated TDFs is AsEXP1 that encodes a gene controlling synthesis of expansin proteins in cell walls.

Cells exposed to stresses develop rigid cell walls that restrict cell expansion and elongation. Expansin proteins act as loosening and extension agents to keep cell walls elastic and flexible. Expansin enables cell wall extension and stress relaxation. Wall stress relaxation reduces cell turgor and thereby creates the driving force for water uptake by growing cells.

Agrostis scabra *(NTAS) and creeping bentgrass (Cv. Penncross) exposed to 35^{o}C for 12 days in a growth chamber.*

Summary Points

- Suppression subtraction hybridization (SSH) cDNA libraries from the heat-tolerant *A. scabra* were constructed and identified several important 'heat tolerance' genes or molecular markers from thermal *A. scabra*.
- Four full-length cDNAs, AsEXP1, AsCP1, AsHsp16 and AsHsp70, were isolated from *A. scabra* exposed to heat stress. Enhanced expressions of four genes were observed in leaves or roots of two ecotypes of *A. scabra* and 10 genotypes of creeping bentgrass exposure to high temperature.
- The expression of AsEXP1, AsHsp70, and AsHsp16 were more strongly up-regulated in A. *scabra* in heat-sensitive creeping bentgrass genotypes while the expression of AsCP1 was enhanced to a lower level in *A. scabra* than in creeping bentgrass, suggesting the positive roles of the up-regulation of expansin and heat shock proteins and the down-regulation of protease in heat adaptation of bentgrass.
- Expression of AsEXP1 and AsHsp16 were highly correlated to the level of heat tolerance in *A. scabra* and creeping bentgrass cultivars. These genes would be used as a molecular marker to select for heat-tolerant germplasm in bentgrass and other cool-season grass species.