Confirmation and Utilization of Candidate Gene Markers for the Selection of Heat Tolerant Bentgrass

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Objectives:

1. *Develop PCR–based markers from heat–responsive genes.*
2. *Map heat responsive candidate genes on the present bentgrass genetic linkage maps.*
3. *To test for co–localization of candidate genes with mapped heat tolerance QTLs.*
4. *Confirm candidate gene markers for use in marker assisted breeding of creeping bent–grass for improved heat tolerance*

Summer bentgrass decline is a major issue affecting many turf areas during the warmer summer months. Insufficient heat tolerance in current bentgrass lines leads to major declines in quality during these periods of prolonged heat. Work including proteomic profiling and suppressive subtractive hybridization has identified candidate genes which may play important roles in conveying tolerance during periods of high temperatures. Development of markers for these candidate genes will allow not only the further confirmation of the importance of these genes in stress tolerance but allow for the creation of more tolerant lines through marker assisted selection.

Figure 1. Plants exposed to 20 days of heat stress (38 degrees C), showing genetic variation in heat tolerance.

Two bentgrass mapping populations have been used for the initial creation and screening of markers. A creeping bentgrass and a creeping X colonial bentgrass hybrid population have been screened and shown to have variation in heat tolerance by examining overall quality under heat stress, as well as several other important parameters related to heat tolerance such as membrane stability and the maintenance of photosynthetic machinery. This information is being used to expand QTL maps which will allow for the co–localization of markers with regions of the genome shown to play a role in heat tolerance.

Methods to create potential markers of candidate genes include using existing EST databases for allele specific amplification, the use of restriction enzymes for cleavage amplified polymorphisms (CAPs) markers and well as employing single strand specific (SSS) endonucleases to find exploitable polymorphisms. To date markers for over 20 candidate genes have been screened, of which eight have successfully shown polymorphism in the hybrid mapping population consisting of catalase, cysteine protease, expansin,glyceraldehyde–3–phosphate dehydrogenase, glutathione–s–transferase, heat shock proteins 26 70 and 101, of these 6 have been successfully added to existing linkage maps.

In the creeping bentgrass mapping population five markers have shown polymorphism including chlorophyll A/B binding protein, phenylalanine ammonia–lyase and heat shock proteins 26, 70 and 90. Several markers, such as heat shock protein 26 and 70, were detected in two mapping populations of bentgrass with polymorphism demonstrated that these makers are not species specific, and could be potentially

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used for selecting heat–tolerant germplasm in other cool–season turfgrass species.

The expression analysis of candidate genes using real –time PCR were performed to further confirm the importance of candidate genes by comparing transcript levels in heat tolerant versus heat sensitive plants. Up to date, three genes that with the markers showing polymorphism and presence in the linkage maps exhibited differential level of expression. These include catalase, glutathione–s–transferase, and cysteine protease. Catalase and glutathione–s–transferase are antioxidant enzymes for scavenging reaction oxygen species that interrupt cellular functions through oxidation of lipids, proteins, and DNA. Both genes were maintained at a significantly higher expression levels in a heat–tolerant hybrid than a heat–sensitive hybrid of creeping bentgrass x colonial bentgrass. Cystein protease catalyzes protein degradation, causing leaf senescence. The expression level of cysteine protease gene was significantly lower in the heat–tolerant hybrid than the heat–sensitive one under heat stress, which could contribute to the maintenance of greener leaves and higher quality under heat stress.

Summary Points

- Markers for six candidate genes have been successfully developed and added to the linkage map, with several more currently been screened for the addition to linkage maps
- Several markers, such as heat shock protein 26 and 70, were detected with polymorphism in two mapping populations of bentgrass demonstrated that these makers are not species specific, and could be potentially used for selecting heat–tolerant germplasm in other cool–season turfgrass species.
- Real–time PCR analysis confirmed the expression of several genes linked to markers with polymorphism and was positively related to heat tolerance; these markers would be used for marker–assisted selection of heat–tolerant germplasm of bentgrass and other cool–season turfgrass species. The confirmed genes linked to heat tolerance could be utilized in genetic transformation for improving heat tolerance in turfgrass.

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