

Molecular Characterization of Chinch Bug-Resistant Buffalograsses

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Objectives:

1. Assess the role of oxidative enzymes, specifically peroxidases, in the defense response of buffalograsses resistant to the western chinch bug.
2. Increase the genomic resources available for buffalograss using next generation sequencing technology.
3. Identify genes differentially expressed between chinch bug-infested and noninfested buffalograsses through the use of normalized and subtracted cDNA libraries for susceptible and resistant plants.

Start Date: 2010

Project Duration: 3 years

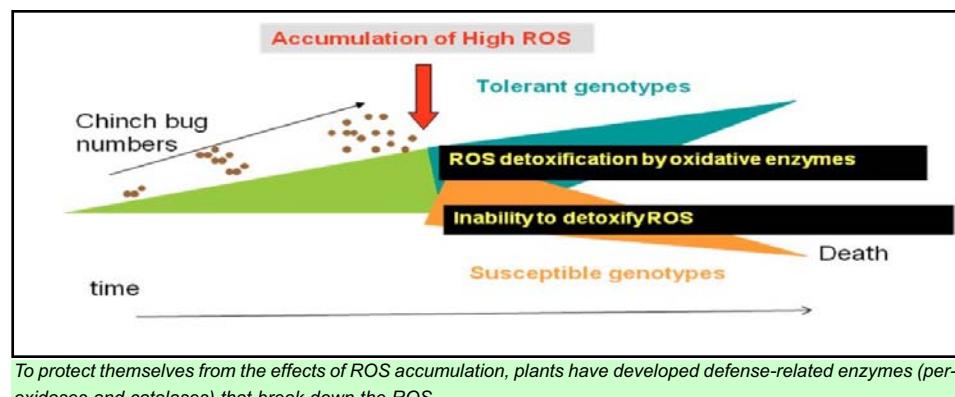
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Turfgrass resistance (specifically tolerance) to insects, when used as part of an integrated pest management (IPM) program, offers the opportunity to effectively and economically reduce chinch bug infestations while dramatically reducing pesticide inputs. Unfortunately, deployment of chinch bug-resistant turfgrasses has been seriously hampered by insufficient knowledge of plant resistance mechanisms and genes contributing to the resistance.

This information is fundamentally important for formulating plant breeding strategies, and subsequently developing chinch bug-resistant germplasm through conventional breeding and biotechnological techniques. In addition, knowledge of specific resistance mechanisms would be valuable for identifying biochemical and physiological markers for use in germplasm enhancement programs, and for characterizing plant defense strategies to insect feeding.

Plants have developed defense strategies to overcome the abiotic and biotic stresses to which they are exposed. One component of this defense system uses a wide array of stress-related proteins which can be elevated or repressed in response to specific or global stress conditions. Changes in the expression of these proteins can play direct or indirect role(s) in the plant's defense response to stress. Documenting changes in these stress-related proteins can provide important information on the extent and severity of a particular plant stress response, as well as the ability of the plant to overcome a specific stress and potential resistance mechanisms.

Reactive oxygen species (ROS), such as hydrogen peroxide, are known to



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be important early signals for altering gene expression patterns in plant cells in response to abiotic and biotic stressors. Despite the benefits gained from molecules like hydrogen peroxide as defense signals, accumulation of these ROS can be toxic to cells. To protect themselves from the effects of ROS accumulation, plants have developed defense-related enzymes (peroxidases and catalases) that break down the ROS.

Previous research by our group has documented increased levels of peroxidases following chinch bug feeding in the resistant (tolerant) buffalograss, 'Prestige', and a loss of catalase activity in the susceptible buffalograss, '378'. Our research group has also successfully identified two peroxidases that are specifically up-regulated in 'Prestige' plants in response to chinch bug feeding. These findings support our working hypothesis that an initial plant defense response to chinch bug feeding is to elevate the levels of specific oxidative enzymes, such as peroxidase, to help detoxify peroxides that accumulate as a result of plant stress.

A second component of this research is to increase the genomic resources available for buffalograss using next generation sequencing technology. To accomplish this, RNA was extracted and purified from chinch bug-infested and non-

infested '378' and 'Prestige' buffalograsses. These samples were reverse transcribed and subjected to Solexa DNA and 454 sequencing technology.

The next step will be to assign and compare expression profiles between genotypes at different time points after infestation and identify candidate transcripts that may serve as markers for selecting buffalograsses with improved chinch bug resistance. A final objective of this research is to use the next generation sequencing data to identify specific genes conferring resistance to chinch bug.

Summary Points

- This research will (1) allow comparison of gene expression between resistant and susceptible buffalograsses, and serve to identify genes differentially expressed in response to chinch bug feeding, (2) provide insights into the biological pathways impacted by chinch bug feeding, and help elucidate plant tolerance mechanisms, and (3) facilitate development of improved buffalograsses with tolerance to chinch bugs through marker-assisted selection.

- This research will also shorten the timeframe needed to identify and improve buffalograsses with superior chinch bug resistance, and because of shared genetics among members of the grass family, may contribute to similar improvements in other grass species.