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 susceptible and resistant buffalograsses in response to chinch bug feeding.*

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 buffalograsses 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 markers for use in germplasm enhancement programs, and for characterizing plant defense strategies to insect feeding.

Research by our group has identified chinch bug–resistant buffalograsses and documented the up–regulation of peroxidases as a key factor in the resistance response to chinch bugs. As a next step in further characterizing the mechanisms and genes contributing to the resistance, next generation sequencing was performed. A combination of first

Table 1. Summary of he data from the 454 and Illumina sequencing comparing Prestige and 378 plants.

Sequencing Information	378	Prestige
454 Sequencing	808,801 reads	1,695,662 reads
	217 bp/read average	209 bp/read average
	175.5 Mb yield	354.4 Mb yield
Illumina Sequencing	26 million reads	27 million reads
	55 bp/read	55 bp/read
	17,490 Mb	17,710 Mb
Assembled Transcripts	241,129 contigs	265,590 contigs
	835 bp average length	899 bp average length
Annotation	52.0% had significant hit	52.4% had significant hit

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generation (454 pyrosequencing platform) and second generation sequencing (Illumina Solexa) technologies were employed for buffalograss transcripomic analyses. cDNA libraries generated from total RNA isolated from chinch bug–infested and control plants that have been previously identified as resistant (Prestige) and susceptible (378) were submitted for 454 and Illumina analyses. Various databases of proteins (UniProKB), pathways (KEGG) and gene ontology and computational tools were used for functional annotation and mining of candidate susceptible and resistant genes in buffalograss.

Figure 1 illustrates the number of genes identified that were differentially expressed between chinch bug infested and control plants for both Prestige and 378 based upon biological processes. Green bars represent genes up–regulated and red bars signify the genes that were down–regulated. Based upon the graph, it is evident that more genes are being down–regulated in 378 plants compared to Prestige plants.

Differences were also observed in the number of genes down–regulated based upon molecular function (Figure 2). Green bars represent genes up–regulated and red bars down–regulated genes. Of interest were the two peroxidases down–regulated in 378 and the one peroxidase up–regulated in Prestige. qRT–PCR studies confirmed the differential expression of these three peroxidase in resistant and susceptible buffalograss in response to chinch bug feeding. Peroxidase expression can be regulated through ethylene dependent or independent pathways. Plants were treated with an ethylene inhibitor and transcript abundance for the specific peroxidases identified from the Illumina sequencing was measured. Of the three peroxidases found, two were confirmed to be regulated in an ethylene independent pathway and were also involved in an initial transient defense response. Based on preliminary findings, the third peroxidase is believed to be ethylene dependent and contributes to a sustained response.

Other projects currently underway include:

- Identifying additional candidate transcripts that may serve as markers for selecting buffalograsses with improved chinch bug resistance;
- Linking the next generation sequencing resources with other aspects of the buffalograss breeding program; and
- Genomic comparisons among turfgrasses to identify gene networks common across species for specific traits of interest.

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.
- Contribute genomic resources for studies with other members of the grass family.
- Provide a model for assembling and analyzing next generation sequencing data sets.

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Figure 1. GO analysis of differentially expressed genes based on biological processes.

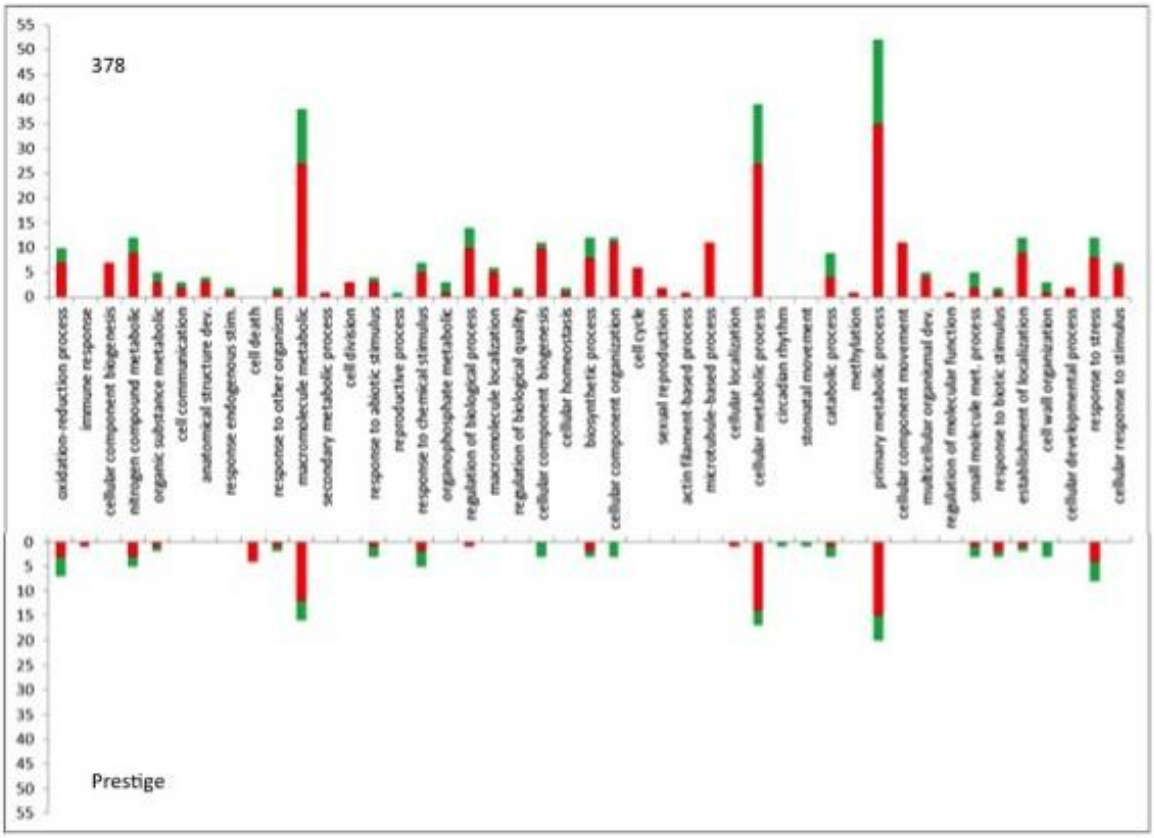
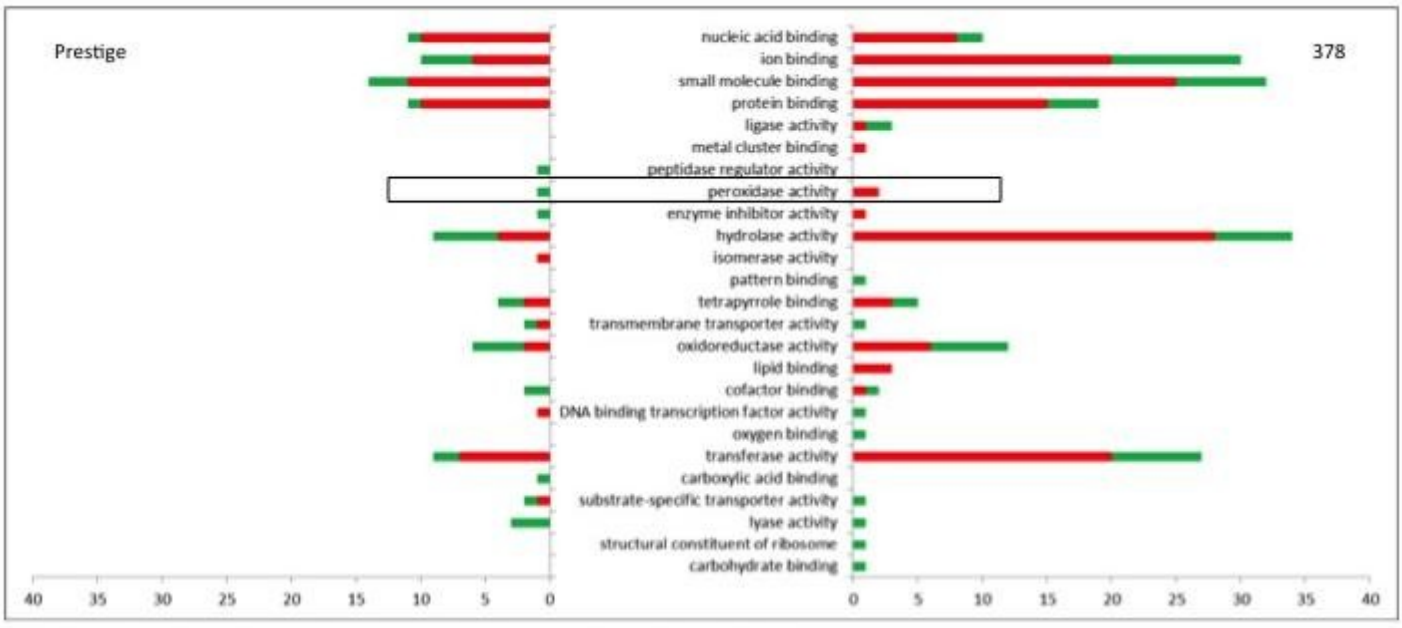


Figure 2. GO analysis of differentially expressed genes based on molecular function.



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