



Using Differentially Expressed Genes, NBS-LRR Genes, and Comparative Genetics to Identify Leaf Spot Resistant Buffalograss

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Introduction

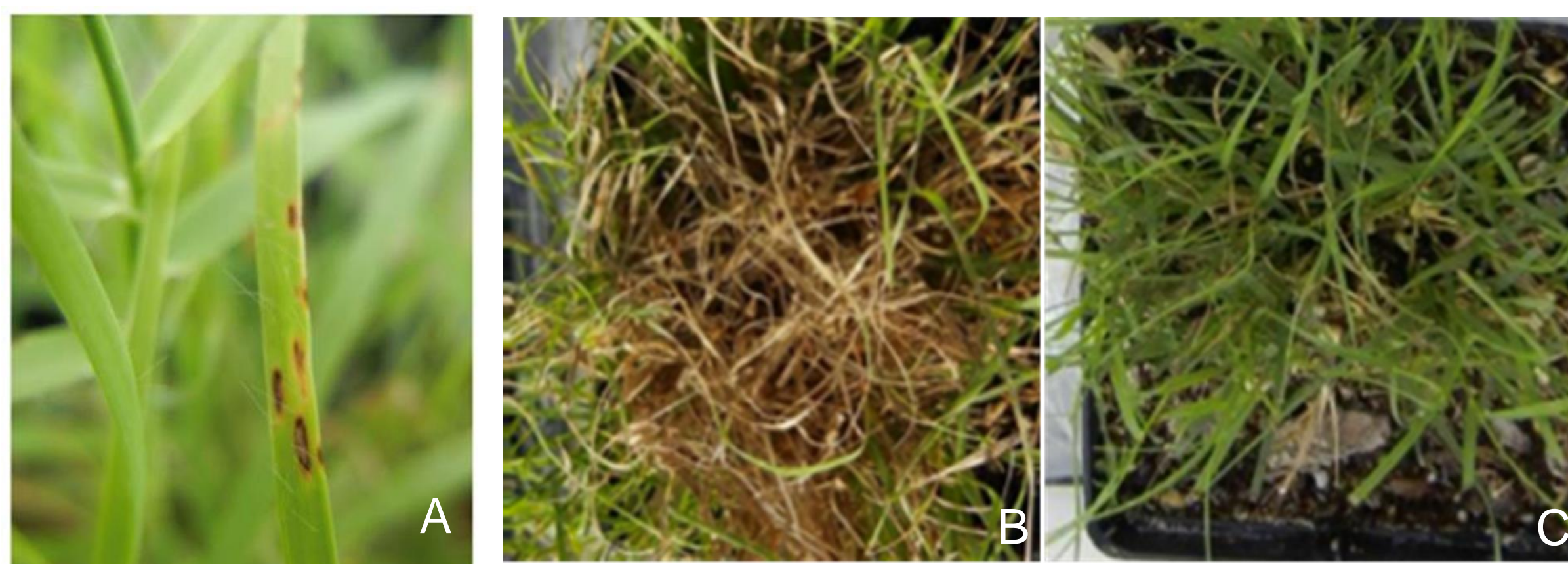
- Buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.] is an exceptionally low-input alternative turfgrass
- Leaf spot disease, caused by *Curvularia inaequalis*, negatively impacts buffalograss turf quality when conditions are conducive for disease development
- Screening buffalograss for leaf spot resistance is an important objective for buffalograss breeders, but is slow and labor intensive
- An existing high throughput sequencing study was done to identify genes differentially expressed in response to leaf spot disease in resistant and susceptible buffalograss. This data can be used as a platform for molecular marker development
- Buffalograss molecular markers associated with leaf spot can be used to identify host resistance in the absence of a grow-out test

Objective

Use existing data to develop markers based on differentially expressed genes from the high throughput sequencing study, from previously characterized defense response genes, namely NBS-LRR (nucleotide binding site – leucine rich repeat) genes, or from a comparative genetics approach.

Disease

84 buffalograss accessions were screened for leaf spot disease resistance. Resistant and susceptible accessions were used for subsequent molecular marker validation tests.

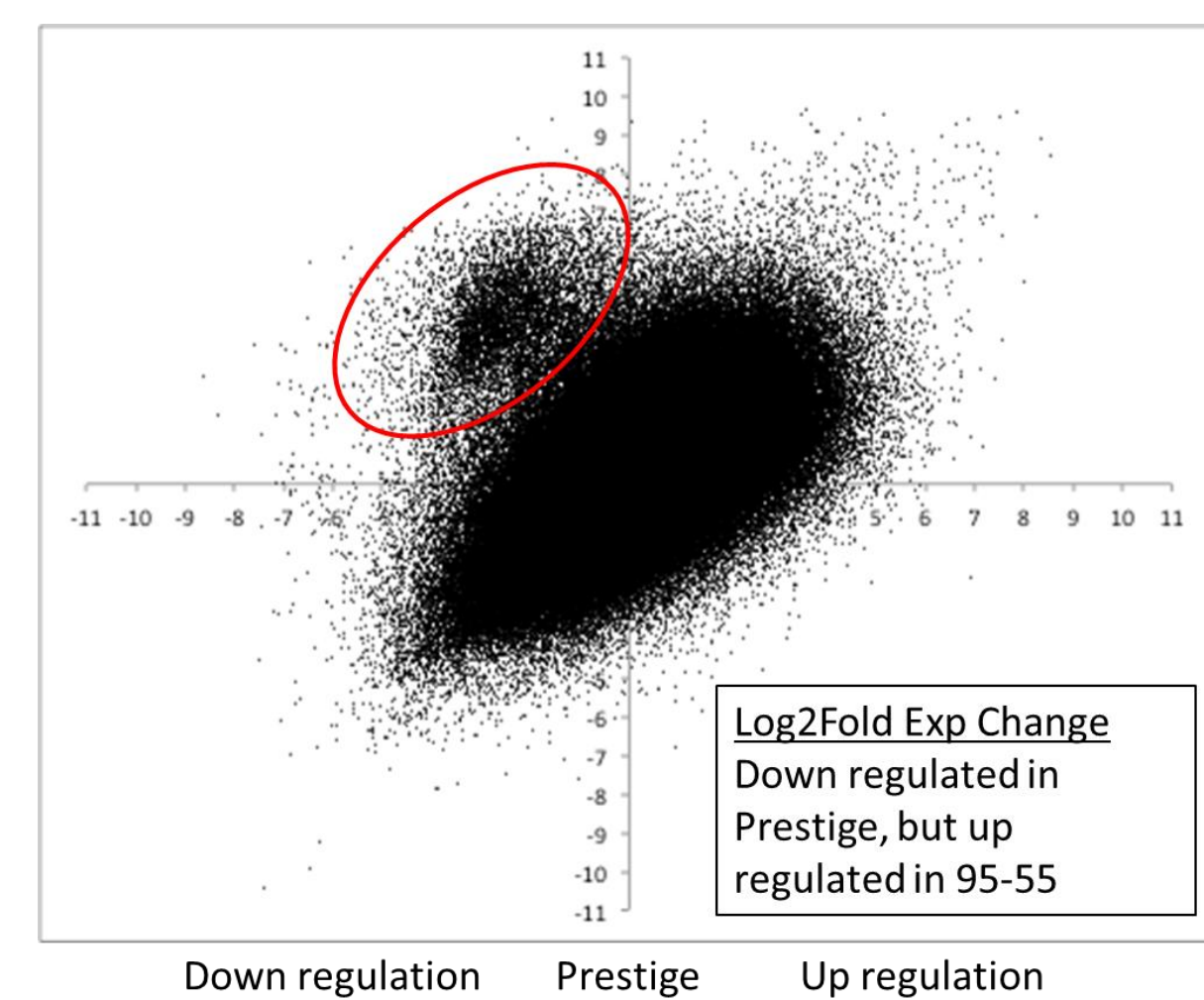


Early symptoms of leaf spot on buffalograss (A.) grown in the field. Susceptible (B.) and resistant (C.) buffalograss challenged with leaf spot disease in the greenhouse

Marker Development

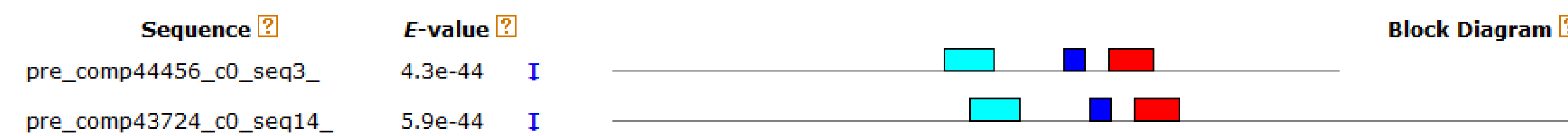
Three different methods were used to develop markers to see which of the different methods accurately identified leaf spot resistant buffalograss

Differentially Expressed Genes



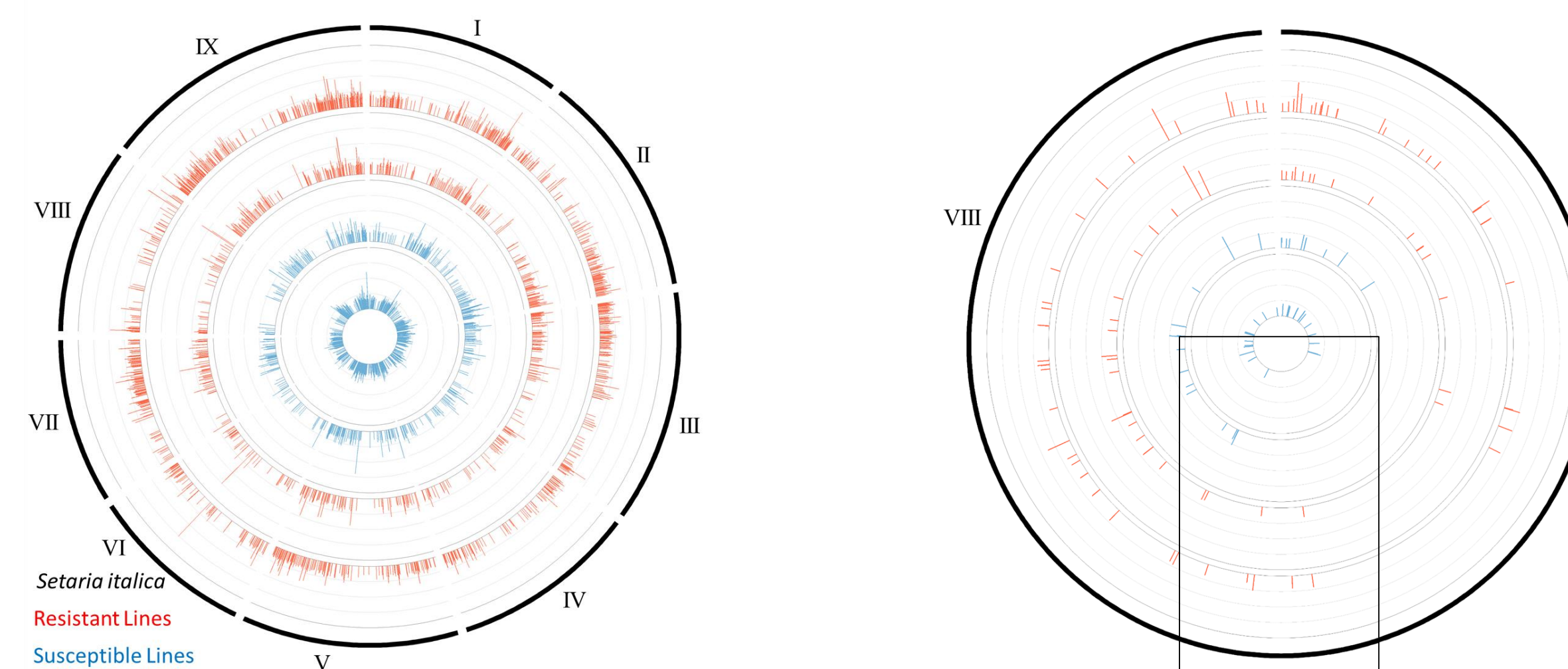
Global gene expression profiles of Prestige (susceptible) and 95-55 (resistant) were compared. Most genes were expressed similarly between the susceptible and resistant lines, however, the circled genes were upregulated in the resistant line and down regulated in the susceptible line suggesting that these genes may be important for resistance. Molecular markers were designed for several of the highlighted genes.

Known Defense Genes



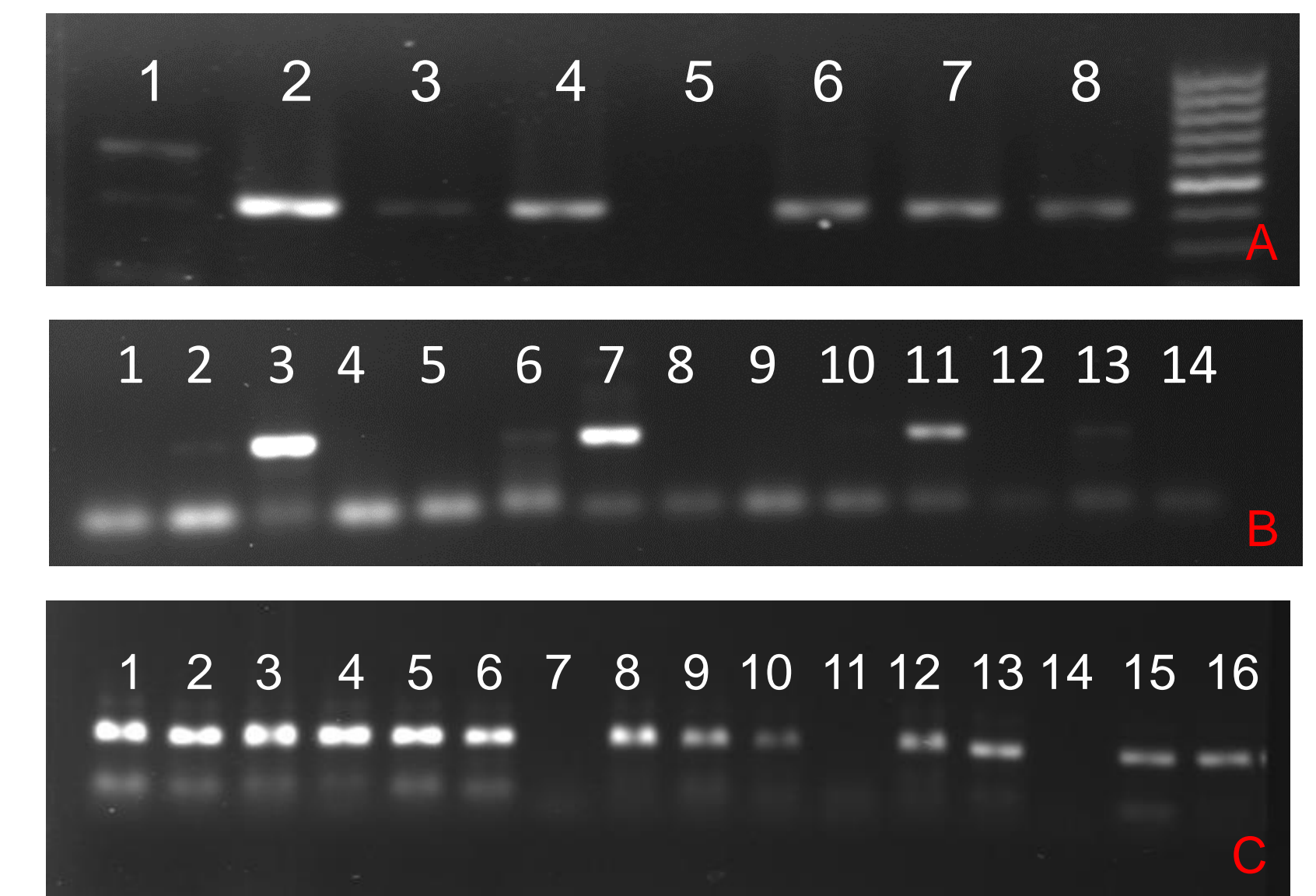
Markers were designed to a subset of the 981 NBS-LRR (nucleotide binding site-leucine rich repeat) genes identified from the 196,168 buffalograss assembled transcripts. Genes in the NBS-LRR family are known to be important for plant defense. Above, MAST 4.10.2 shows the conserved NB-ARC domain (light blue), a component of NBS-LRR genes, in two buffalograss transcripts.

Comparative Genetics



Genes differentially expressed in response to leaf spot were mapped to the *Setaria italica* genome (left). The position of each bar indicates the location of each mapped gene and the magnitude of each bar shows the relative change in gene expression. A closer look of chromosome 8 (right) reveals certain genomic locations harboring differentially expressed genes in resistant lines (red), but not in the susceptible lines (blue). Markers were developed to these important genomic regions.

Results



Agarose gel images showing markers developed using the A. differentially expressed genes, B. NBS-LRR genes, and C. comparative genetics approach. The markers were designed such that the presence of bands predict resistance. These data were compared to the greenhouse disease screen data to determine if and to what extent the markers are associated with leaf-spot disease resistance.

Summary Table

	Differentially Expressed	Comparative Genetics			NBS-LRR
	Marker 1	Marker 1	Marker 2	Marker 3	Marker 1
Resistant	100	71	29	29	29
Susceptible	0	86	0	43	14

Percent of bands that accurately predicted resistance in the tested buffalograss genotypes for each marker class

Conclusions

Genes identified from the differential gene expression studies were more informative than those designed to known defense genes or from the comparative genetics.

Acknowledgements

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