

# NBS-LRR GENE EXPRESSION OF BUFFALOGRASS CHALLENGED WITH LEAF SPOT PATHOGEN

B. Sajeewa Amaradasa and Keenan Amundsen

Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Nebraska 68583 USA

(bamaradasa2@unl.edu)



## Background/introduction

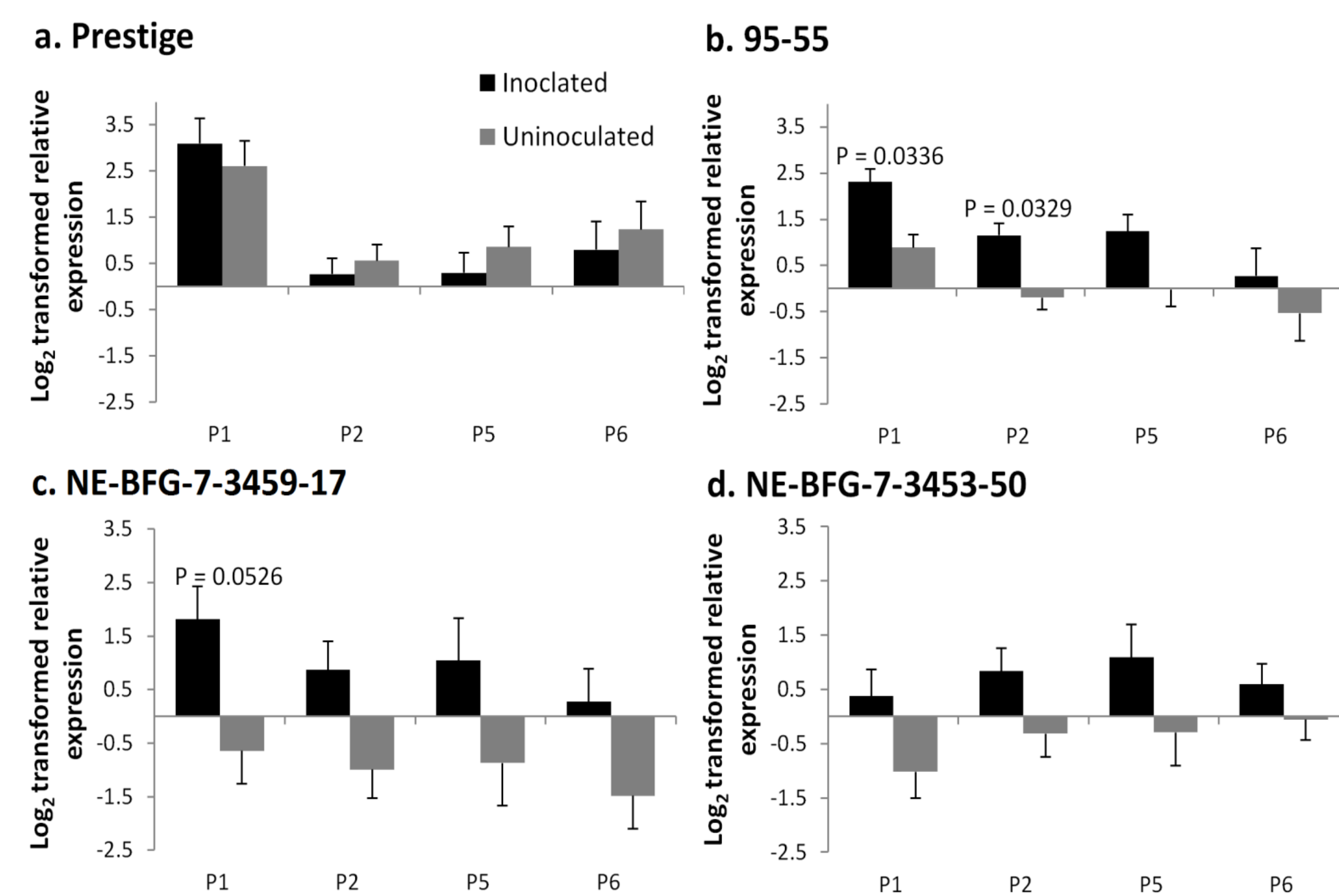
Buffalograss [*Buchloë dactyloides*] is a warm-season turfgrass native to the Great Plains of North America. It has exceptional drought, heat, and cold tolerance. Buffalograss is widely used as a turfgrass in arid and semi-arid areas of the USA. Leaf spot caused by *Curvularia inaequalis* is an important disease of buffalograss reducing visual quality. Conventional breeding for disease resistance based on inoculation, rating, and selection is difficult and time consuming. Molecular assisted screening is a viable alternative to the traditional method, and molecular strategies enhance the efficiency of a breeding program. Plant disease resistance is often conferred by *R*-genes. The interaction between the host and the disease often results in a hypersensitive reaction or localized cell death in the plant. Candidate *R*-genes have been previously identified and cloned from buffalograss, but the expression of these candidate genes in response to leaf spot disease was not tested.

## Objective

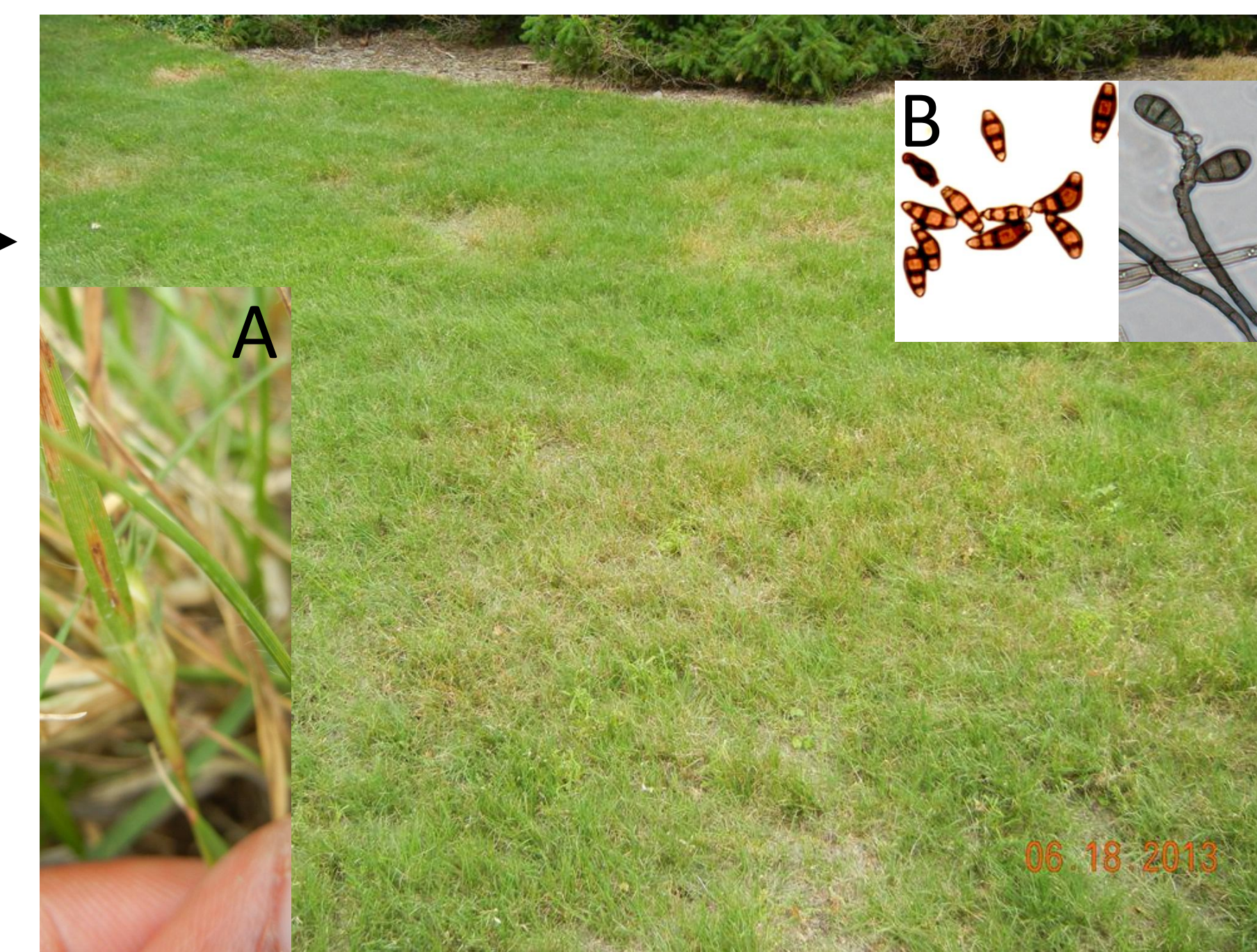
The present study was conducted with leaf spot resistant and susceptible buffalograss lines challenged with *C. inaequalis* to quantify the activity of seven previously described *R*-genes (Budak et al., 2006).

## Results

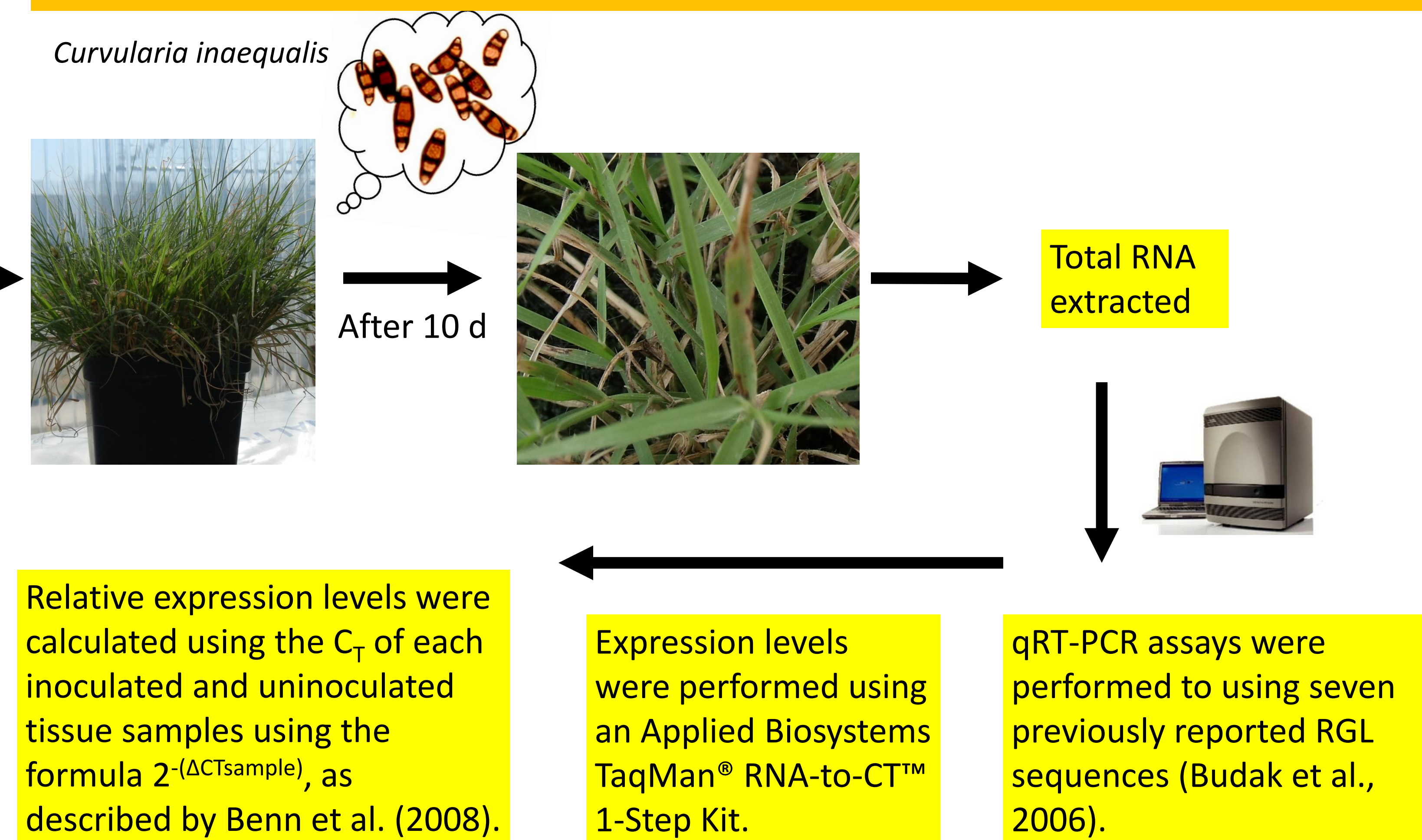
**Fig. 2** Relative expression of defense related genes P1, P2, P5, and P6 following infection by *C. inaequalis*. Prestige (a), and NE-BFG-7-3453-50 (d) are susceptible to leaf spot. 95-55 (b), and NE-BFG-7-3459-17 (c) are leaf spot resistant lines.



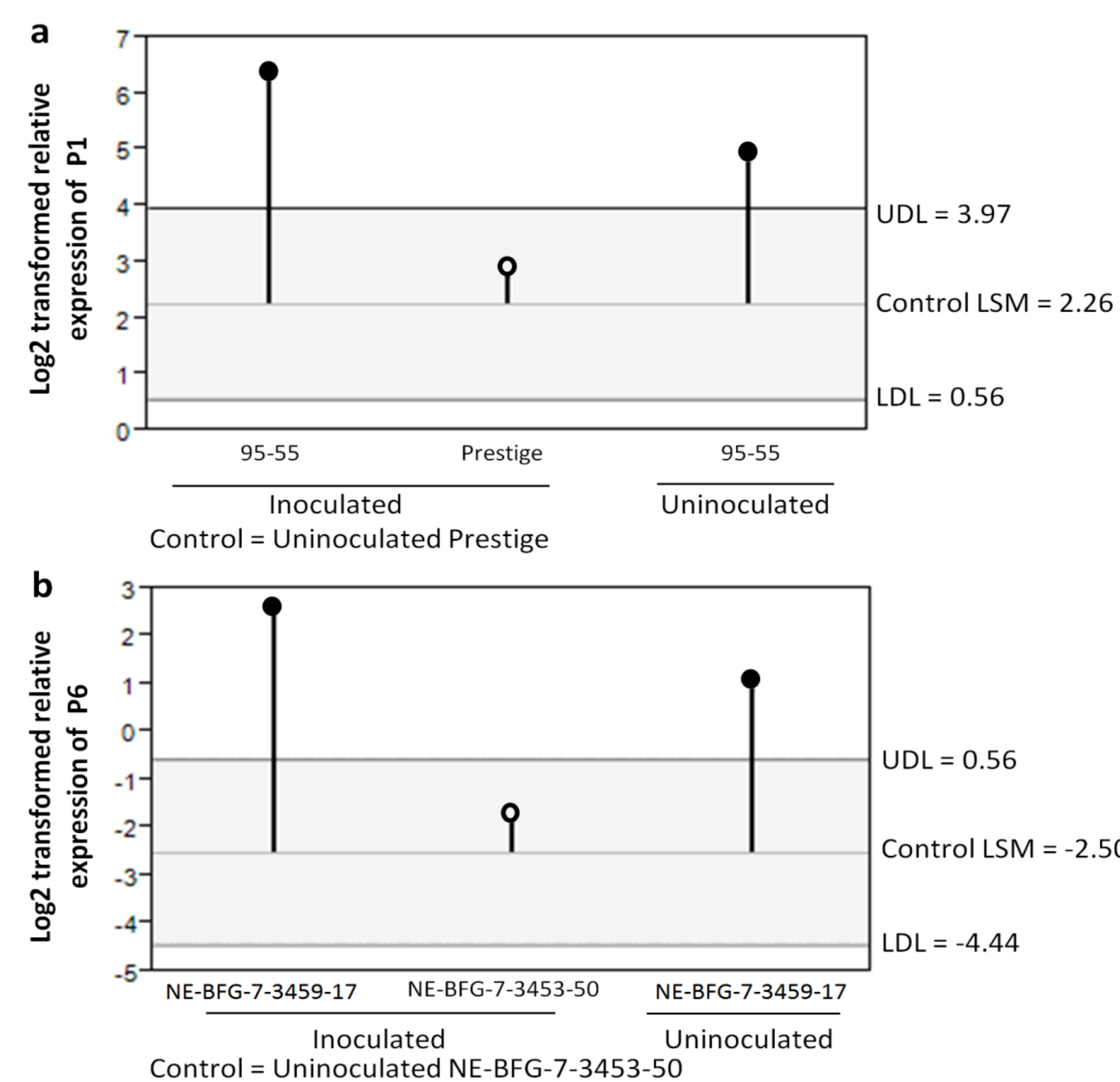
**Fig. 1** A buffalograss lawn with leaf spot symptoms. Inset A: Leaf spot symptoms on an individual leaf. Inset B: Conidia of *C. inaequalis*.



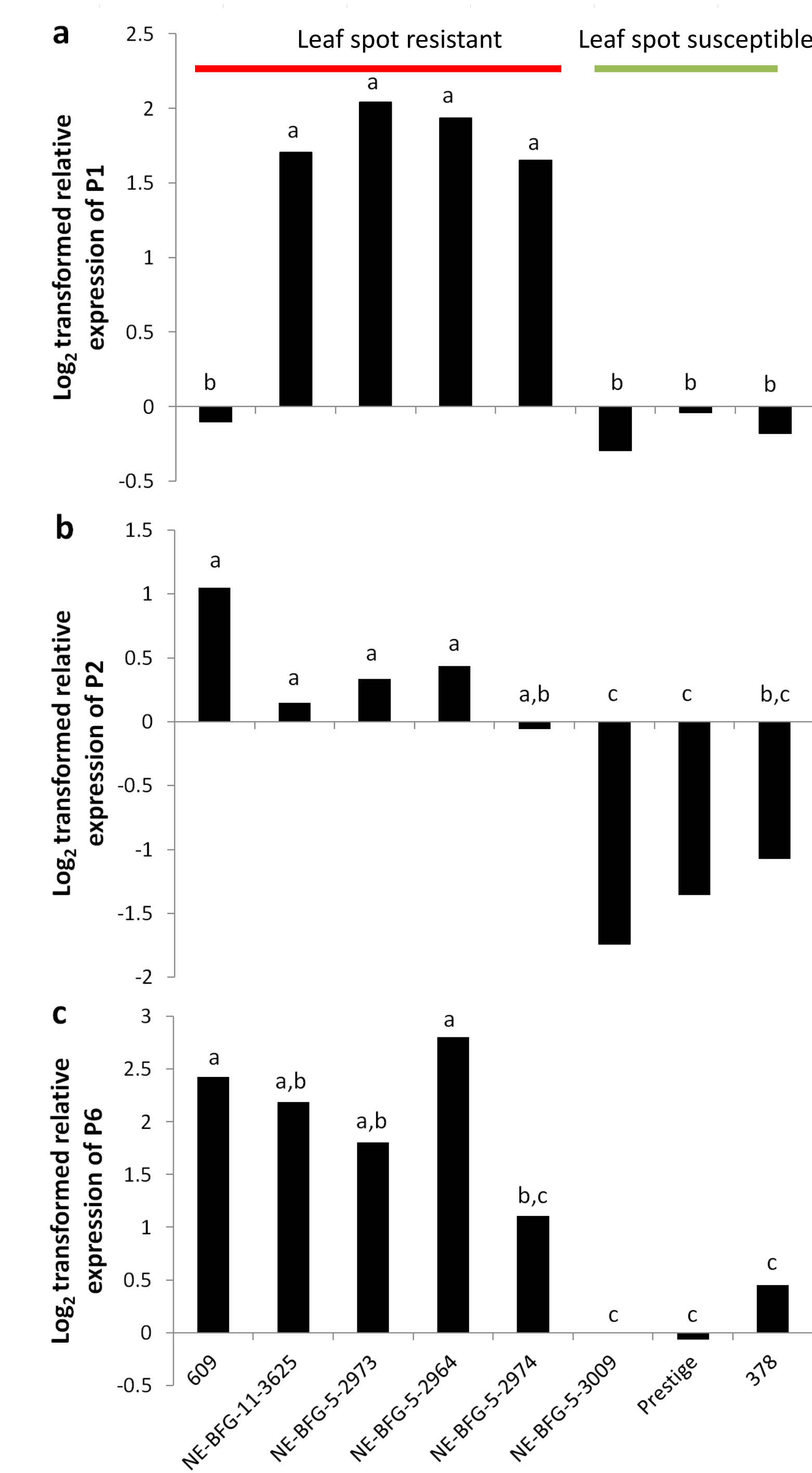
## Methods



**Fig. 3** a) Expression of P1 in inoculated and healthy 95-55 (leaf spot resistant) and Prestige (susceptible). b) Expression of P6 in inoculated and healthy NE-BFG-7-3459-17 (leaf spot resistant) and NE-BFG-7-7359-50 (susceptible).



**Fig. 4** Basal expression of defense related genes P1, P2 and P6 in eight buffalograss lines.



## Summary

- P3, P4, and P7 primers did not amplify a PCR product.
- Susceptible lines did not show difference in expression of P1, P2, P5, and P6 when influenced by *C. inaequalis* (Fig. 2a and 2d).
- The two-way ANOVA performed on cultivar x treatment showed higher expression P1, P2 or P6 in the uninoculated resistant lines compared to the uninoculated susceptible lines (Figs. 3a and 3b)
- Though P1, P2, and P6 were not capable of differentiating all resistant and susceptible lines individually, they showed differential expression accurately in most lines (Fig. 4).

## Conclusions

Analysis of basal expression of at least two amplicons (P1/P2/P6) in combination may provide a diagnostic tool for categorizing leaf spot resistance in unknown buffalograss lines.

## Acknowledgments

This study was partly funded by the United States Golf Association and the Nebraska Turfgrass Association. Authors thank Dr. Tiffany Heng-Moss and Dr. Teresa Donze for their assistance in carrying out real-time PCR work.

## References

1. Amaradasa, B.S. and K. Amundsen. 2013. First report of *Curvularia inaequalis* and *Bipolaris spicifera* causing leaf blight of buffalograss in Nebraska. Plant Dis. "First Look" paper <http://dx.doi.org/10.1094/PDIS-05-13-0487-PDN> (posted 14 Aug. 2013).
2. Budak, H., Z. Kasap, R.C. Shearman, I. Dweikat, U. Sezerman and A. Mahmood. 2006. Molecular characterization of cDNA encoding resistance gene-like sequences in *Buchloe dactyloides*. Mol. Biotechnol. 34: 293-301.
3. Benn, C.L., H. Fox and G.P. Bates. 2008. Optimisation of region-specific reference gene selection and relative gene expression analysis methods for pre-clinical trials of Huntington's disease. Mol. Neurodegener. doi:10.1186/1750-1326-3-17.