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Developing and Validating a New Method to Improve Breeding for Cold-tolerant Bermudagrass

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Objectives: To develop a new technique that simplifies evaluation of bermudagrass for cold tolerance, and thereby improves breeding efficiency and facilitate the process.

In 2016, we performed a number of experiments to further evaluate the correlation and/or association of bermudagrass (*Cynodon dactylon* (L.) Pers.) in response to aryloxyphenoxypropionate (AOPP) herbicide and cold temperature stresses. The first experiment we conducted is to prove and validate the concept that responses of bermudagrass to the two stresses are truly linked somewhere in the pathway, rather than just correlated and individual events. The underlying logic is if the two pathways are truly linked, then subjecting the plant to Stress 1 at lower intensity and/or duration would enable the plant to "acclimate" so when Stress 2 occurs, subsequent plants that are susceptible will be better adapted. This concept is just like acclimating plants to chilling stress in fall before the winter freezing temperature occurs. We conducted a preliminary study in the past, and in 2016 we performed a complete experiment. We chose the bermudagrass cultivars "Riviera" and "Celebration" with known differences in cold-tolerance as the experimental materials and subjected the plants to chilling temperature at 4 ^{o}C for 0, 1, 2, 4, 8, and 11 days. Plants were then moved to the greenhouse for 2 hours before subjecting them to a fenoxaprop-ethyl (Acclaim Extra®) application at 0.2 kg ai/ha with NIS at 0.25% v/v. Bermudagrass responses to AOPP herbicide without pre-chilling temperature treatment or with pre-chilling for different lengths were objectively evaluated by digital image analysis using WinRhizo (Regent Instruments Inc., Quebec, Canada) every week up to 4 weeks for percent discoloration. The experimental design was a 2 × 6 factorial combination of bermudagrass cultivar and pre-chilling treatment arranged in a CRD with 3 replications. To be concise, we only report data collected at 2 weeks after herbicide application (WAT) when maximal herbicide injury is visible. Results indicated that as expected, without herbicide application "Riviera" showed better tolerance to chilling stress, evidenced by reduced leaf discoloration (Fig 1). Two weeks after AOPP herbicide application, without pre-chilling treatment both "Riviera" and "Celebration" showed injury symptoms and "Celebration" showed more than twice the discoloration compared to "Riviera" (Fig 2). With pre-chilling treatment, both "Riviera" and "Celebration" showed reduced amount of herbicide injury as the length under chilling temperature increased. The most interesting part is after 4 days under chilling temperature, both "Riviera" and "Celebration" showed minimal herbicide injury at 15% discoloration or less, and the cultivars showed no differences in herbicide damage (Fig 2). When days under pre-chilling temperature increased to 8 or 11 days, the two cultivars segregated again. This finding was very intriguing as it confirms that the mechanism of bermudagrass in response to the two stresses is truly linked. To confirm the difference of cold-tolerance of these two cultivars, we also conducted a freezing test and determined that the LT_{50} of "Riviera" is -10.6 ^{o}C, significantly lower than "Celebration" (Fig 3).

In 2016, we have also performed experiments to profile the lipids of the two bermudagrass cultivars at normal conditions, under chilling stress, and after AOPP herbicide application. We are in the process of analyzing the data and will likely reach a conclusion next year.

In 2016, we have also continued working on the segregating population created by co-PI Wu by crossing “A12935” and “A12936”. The entry “A12396” is a breeding line selected from the Oklahoma State University (OSU) bermudagrass germplasm known for cold hardiness. The entry “A12395” is a collection from Puerto Rico which is susceptible to low temperature stress. We performed a freezing test of the two genotypes and determined their LT_{50} to be -11.1 ^{o}C and -8.2 ^{o}C for cold-hard “A12396” and cold-sensitive “A12935”, respectively (Fig 4). We have previously established the tolerance of the two genotypes to AOPP herbicides; built upon it we continued the screening of the segregating progenies for AOPP herbicide tolerance (Fig 5). After screening 116 progenies (n=4), we were able to determine their distribution of tolerance to AOPP herbicide (Fig 6). The nature of the heritability of the tolerance trait is yet to be determined. Currently, selected progenies in each category have been propagated in the greenhouse; their tolerance to cold temperature will be determined in the laboratory and under field conditions in 2017. Additionally, our future plan also includes evaluating the expression of *ACCase*, and evaluate possible downstream genes in the fatty acid biosynthesis pathway.

Summary:

- We generated evidence that further indicates bermudagrasses’ tolerance to AOPP herbicide and cold temperature is likely linked;
- Utilizing a segregating population, we were able to further prove the usage of AOPP herbicide as a method to differentiate bermudagrass based on their cold-tolerance;
- We are still working on the mechanism, and we expect an in-depth discovery sometime in the next year or beyond.

Fig 1. Bermudagrass “Riviera” and “Celebration” percent leaf discoloration (%) evaluated by image scanning system (WinRhizo, Regent Instruments Inc.) following 0, 1, 2, 4, 8 or 11 days at chilling temperature of 4 ^{o}C. Error bars represent standard error for each mean (n=3) at P<0.05. Means for the same pre-chilling treatment labeled with the same cap letter were not significantly different based on Fishers’ Protected LSD at P<0.05; Means for the same cultivar labeled with the same small letter were not significantly different based on Fishers’ Protected LSD at P<0.05.

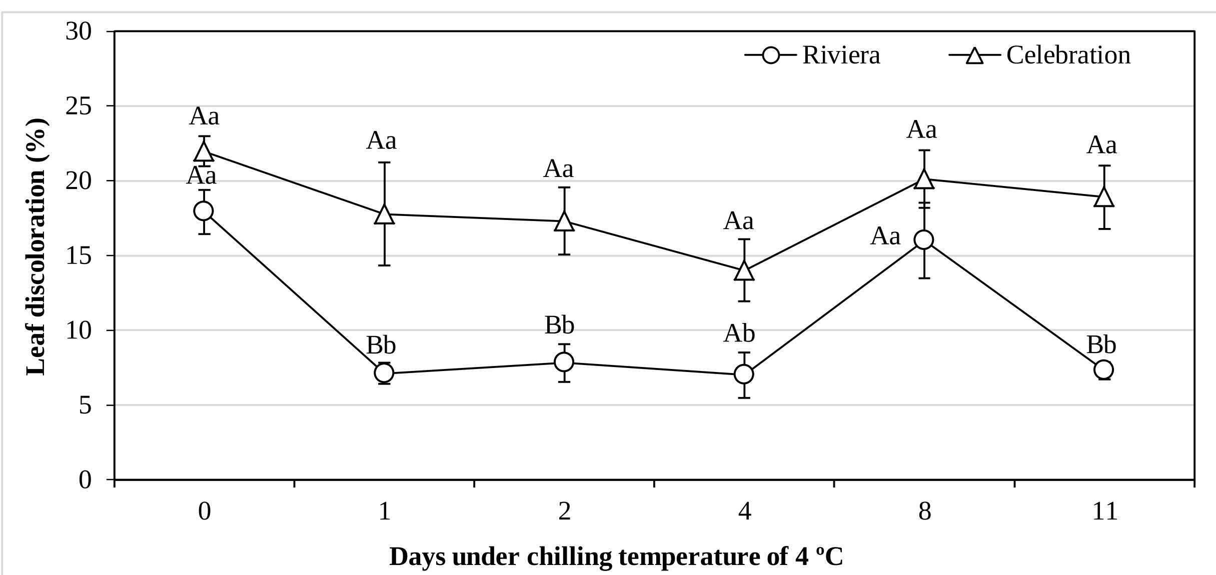

Fig 2. Bermudagrass "Riviera" and "Celebration" percent leaf discoloration (%) evaluated by image scanning system (WinRhizo, Regent Instruments Inc.) at 2 weeks after fenoxaprop-ethyl application with or without pre-chilling treatment at 4 °C for 0, 1, 2, 4, 8 or 11 days. Error bars represent standard error for each mean (n=3) at $P<0.05$. Means for the same pre-chilling treatment labeled with the same cap letter were not significantly different based on Fishers' Protected LSD at $P<0.05$; Means for the same cultivar labeled with the same small letter were not significantly different based on Fishers' Protected LSD at $P<0.05$.

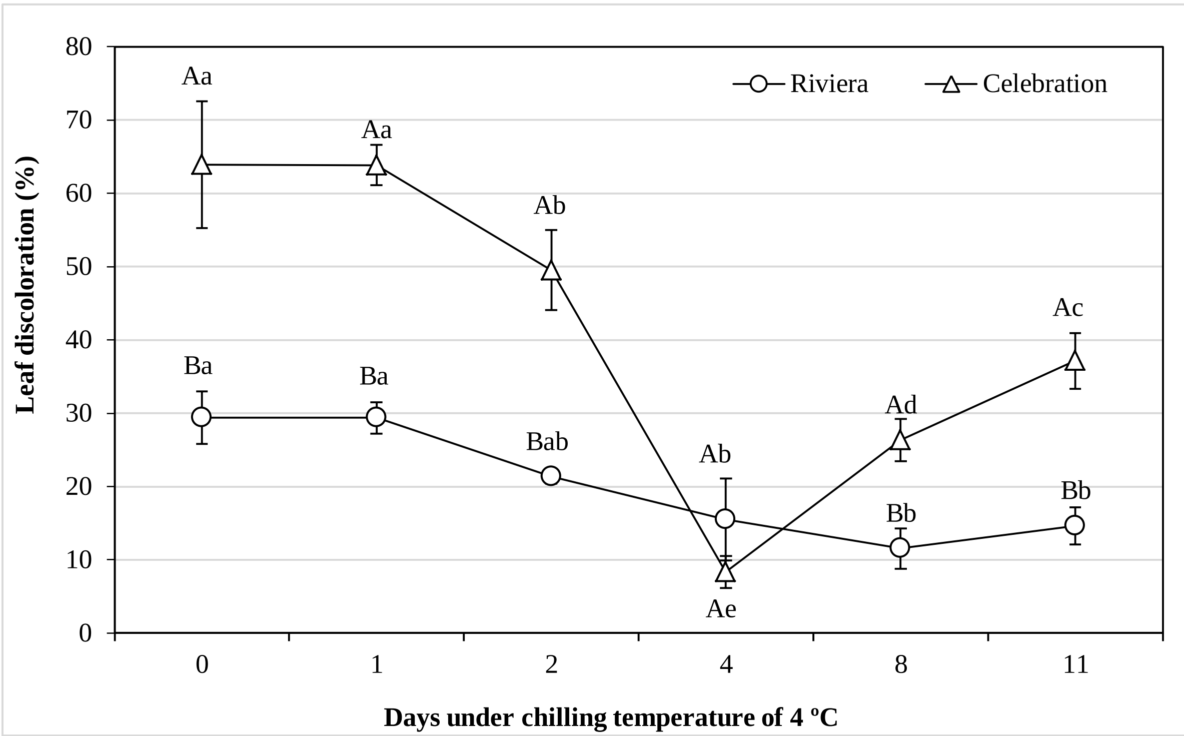

Fig 3. Electrolyte leakage (%) of bermudagrass "Riviera" and "Celebration" stolons at various temperatures ranging from 0 to -28 °C after acclimation at 4 °C for 48 h (n=6).

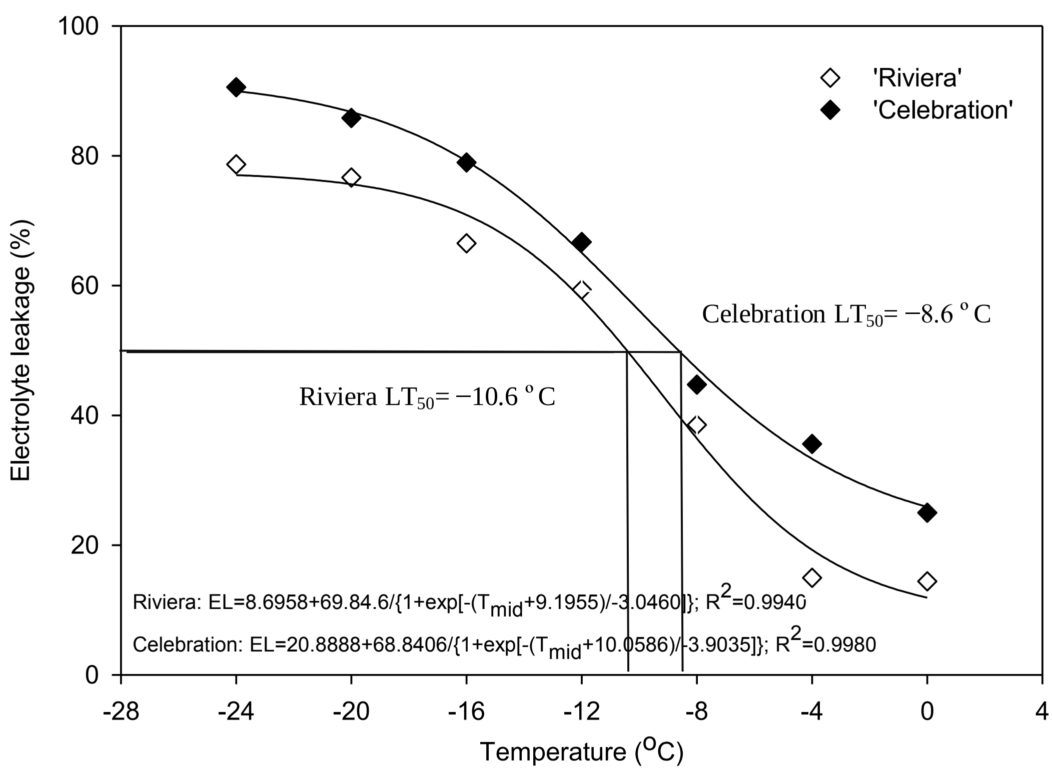

Fig 4. Electrolyte leakage (%) of bermudagrass genotype "A12935" and "A12936" stolons at various temperatures ranging from 0 to -28 °C after acclimation at 4 °C for 48 h (n=6). Genotype "A12935" is a cold-sensitive entry collected from Puerto Rico, and "A12936" is a breeding line selection for cold-tolerance. Both genotypes were provided by Co-PI Wu at the Oklahoma State University.

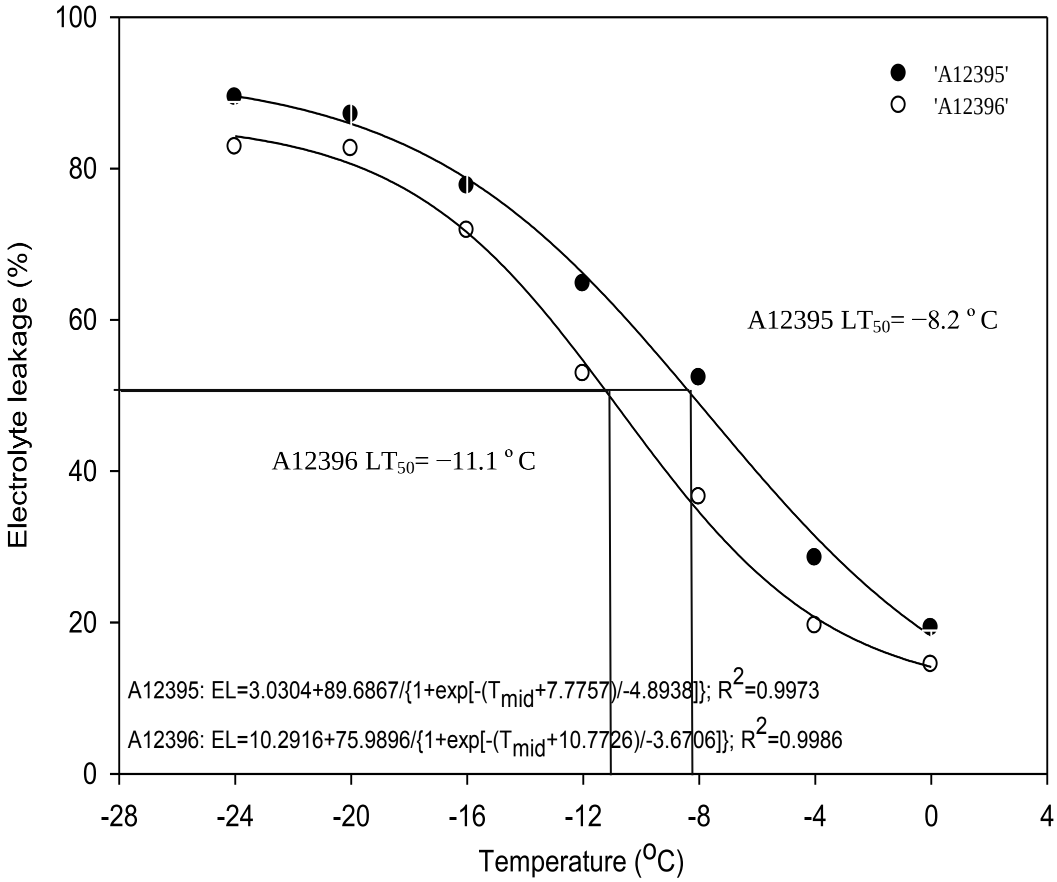

Fig 5. Representative images of bermudagrass progeny plants from the segregating population of "A12935" × "A12936" in response to fenoxaprop-ethyl (Acclaim Extra®) application at 2 or 3 weeks after herbicide application treatment (WAT). Herbicide-tolerant progenies at 2 (A) or 3 (B) WAT; Herbicide-sensitive progenies at 2 (C) or 3 (D) WAT.

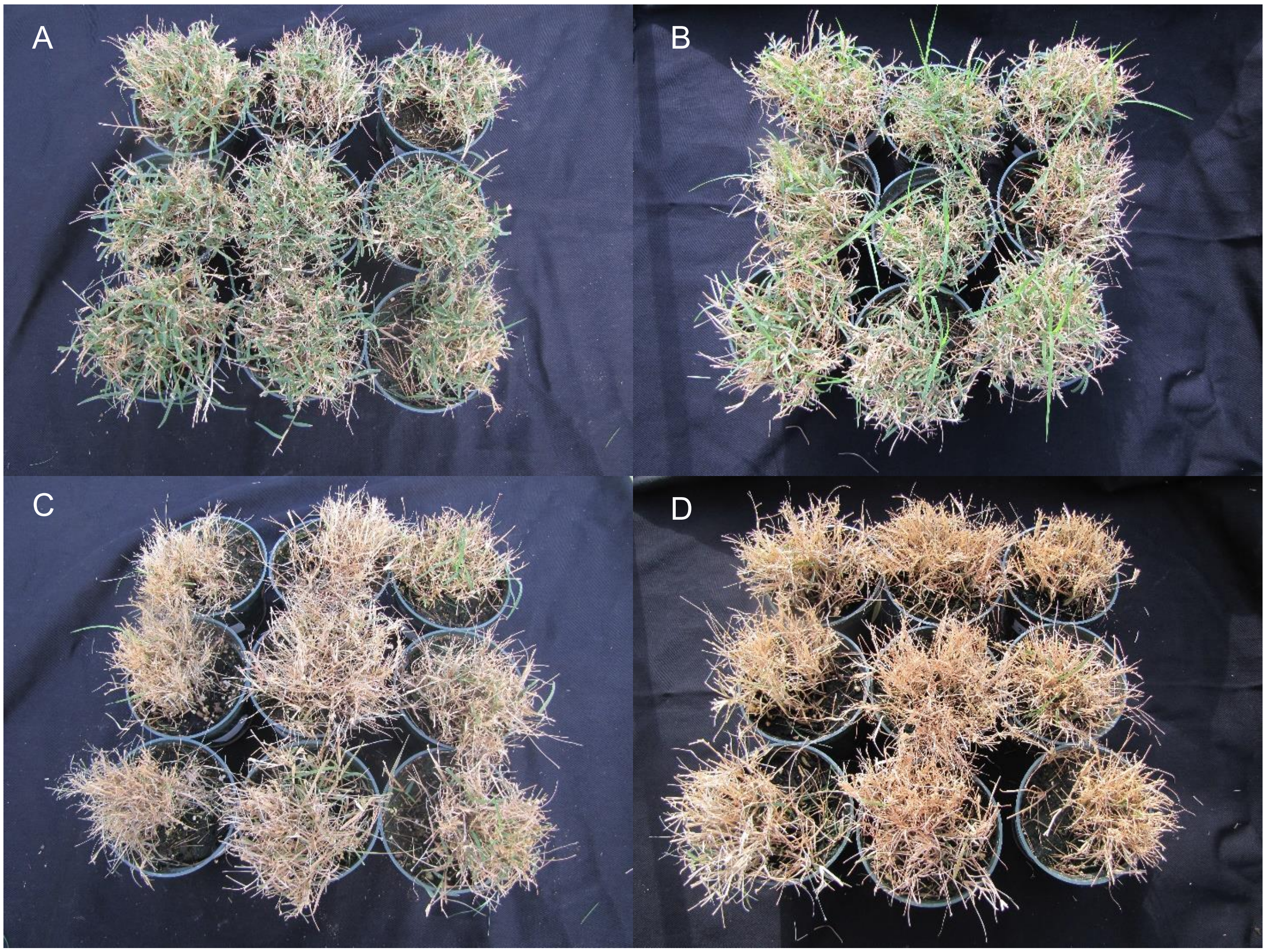

Fig 6. Segregation of 116 bermudagrass progenies (n=4) from population of "A12935" × "A12936" following fenoxaprop-ethyl application at 2 weeks after treatment. Segregation was based on visual injury assessment from 1-9 with 9 being no injury and 1 being plant death.