2014-03-492

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 hence improves breeding efficiency.

Start Date: 2014
Project Duration: 3 years (with the fourth year extended without extra fund)
Total Funding: $30,000

In 2017, a number of experiments were performed to further evaluate the hypothesis regarding the possible linkage of bermudagrass (*Cynodon dactylon* (L.) Pers.) for its responses to aryloxyphenoxypropionate (AOPP) herbicide and cold temperature stresses. Built upon the segregating population resulting from a cross between “A12935” and “A12936” bermudagrass collections, we selected eight progenies with four representing AOPP-tolerant and another four representing AOPP-susceptible genotypes. Assessment of AOPP responses was previously conducted. The entry “A12396” is a breeding line selected from the Oklahoma State University (OSU) bermudagrass germplasm known for its cold hardiness. The entry “A12395” is a collection from Puerto Rico which is susceptible to low temperature stress. Our previous research has determined the LT_{50} (Lethal temperature to 50% of the plants in the population) to be -11.1 ºC and -8.2 ºC for cold-hard “A12396” and cold-sensitive “A12935”, respectively. The four AOPP-tolerant progenies, T27, T36, T41, and T44, collectively showed greater cold-tolerance than the four AOPP-susceptible progenies, S1, S66, S99, and S103, evidenced by the segregation of the LT_{50} (Fig 1). This result further confirmed the correlation between bermudagrasses’ responses to the two stresses, and indicating its likelihood of inheritability.

To decipher the possible mechanism for such a correlation, we have also performed lipid profiling of the two previously-studied bermudagrass cultivars, “Riviera” and “Celebration”, by subjecting them to chilling stress at 4 ºC for a total of 3 weeks. Experimental design was a complete randomized design with 4 replications. Alteration in membrane phospholipids content and increasing in unsaturated membrane lipids has been found in various plant species, including bermudagrass, contributing to their supreme cold tolerance. These changes in lipids increase membrane stabilization and maintain membrane fluidity under cold temperatures. Our results found an increase of phosphatidylcholines (PC) for both cultivars, although “Riviera” showed 10% greater PC at 3 weeks after treatment (WAT) (Table 1). For phosphatidylglycerols (PG) and phosphatidylethanolamines (PE), however, only “Riviera” showed a consistent increase as cold temperature stress was extended. Under cold temperatures, both cultivars showed an increase for double bond index (DBI) of most phospholipids tested, “Riviera” appeared to accumulate a greater amount of double bonds with PC, PG, and PE (Table 2). At 3 WAT, “Riviera” produced 10% or 37% greater double bonds in PC and PG, respectively, compared to “Celebration”. Compared to the DBI prior to treatment application, “Riviera” accumulated 1.9 times greater double bonds of PE at 3 WAT, while “Celebration” only increased 1.4 times. Collectively, these results indicate that alteration of certain membrane lipids or increasing unsaturated lipids likely

explains the supreme cold-tolerance of “Riviera” compared to “Celebration”. Based on this knowledge, we have recently shifted our focus to decipher the molecular basis of such a mechanism.

Various attempts were made to identify, clone, and sequence putative genes; part of the difficulty in conducting this portion of the research is the absence of genomic information for bermudagrass. Our recent findings, however, indicate that we might have identified a putative gene that encodes fatty acid desaturase (FAD) from both of the bermudagrass cultivars (Fig 2). The putative genes cloned from “Riviera” and “Celebration” were identical, and shared 75-90% homology with the genes encoding stearoyl-acyl carrier protein desaturase from different crops including wheat and maize. Beyond the funding period, we are planning to confirm the putative *FAD* gene we cloned from bermudagrass. We also plan to evaluate the expression of *ACCase* which we have previously cloned. Our previous results found no mutation which was the known resistance mechanism for other AOPP-resistant crops.

Summary:

- We have generated abundant evidence that indicates the likelihood of the linkage between bermudagrasses’ tolerance to AOPP herbicide and cold temperature;
- Utilizing a segregating population, we produced in-depth evidence that AOPP herbicide can be used as a practical method to select bermudagrass breeding lines based on their cold-tolerance;
- Beyond the funding period, we will continue working on the possible mechanism at the molecular level, and we expect an in-depth discovery sometime in the future.

Table 1. Effects of chilling temperature on phospholipids of 'Riviera' and 'Celebration' bermudagrasses at 0, 1, 2 and 3 weeks after treatment (WAT). PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid.

	0 WAT[†]	1WAT	2 WAT	3WAT
	----------PC[§]----------			
Celebration	4.63b1	5.30a1	5.50a1	5.47a2
Riviera	4.63c1	4.46c2	5.34b1	6.03a1
	----------PG----------			
Celebration	2.36bc1	2.68a1	2.39bc1	2.25c2
Riviera	2.36c1	2.33c2	2.54bc1	2.87a1
	----------PE----------			
Celebration	1.18c1	1.72ab1	1.92a1	1.68b1
Riviera	0.88c2	1.26b2	1.83a1	1.71a1
	----------PI----------			
Celebration	0.45b1	0.47b1	0.53a1	0.48ab1
Riviera	0.44a1	0.38c2	0.40bc2	0.43ab2
	----------PA----------			
Celebration	0.06c1	0.10ab1	0.10a1	0.10ab1
Riviera	0.08b1	0.07b1	0.07b2	0.12a1
	----------PC/(PE+PA)----------			
Celebration	3.79a2	2.98bc2	2.72c1	3.15b1
Riviera	4.90a1	3.45b1	2.88c1	3.44b1

[†]Means in the same rows labeled by the same letters are not significantly different according to Fisher's Protected LSD ($P = 0.05$); Means in the same columns for each variable labeled by the same numbers are not significantly different according to Fisher's Protected LSD ($P = 0.05$).
[§]The units of phospholipids presenting in the table are mol% except PC/(PE+PA).

Table 2. Effects of chilling temperature on phospholipid double bond indices (DBIs) of 'Riviera' and 'Celebration' bermudagrasses at 0, 1, 2 and 3 weeks after treatment (WAT). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol.

	0 WAT†	1WAT	2 WAT	3WAT
		DBI		
	----------PC----------			
Celebration	0.155c1	0.176b1	0.184a1	0.187a2
Riviera	0.154c1	0.152c2	0.180b1	0.206a1
	----------PG----------			
Celebration	0.052b1	0.059a1	0.051b2	0.049b2
Riviera	0.053c1	0.054bc1	0.058b1	0.067a1
	----------PE----------			
Celebration	0.043c1	0.060b1	0.068a1	0.059b1
Riviera	0.032c2	0.045b2	0.065a1	0.061a1
	----------PI----------			
Celebration	0.0116b1	0.0122b1	0.0135a1	0.0128ab1
Riviera	0.0116a1	0.0103b2	0.0106ab2	0.0116a1

†Means in the same rows labeled by the same letters are not significantly different according to Fisher's Protected LSD ($P = 0.05$); Means in the same columns for each variable labeled by the same numbers are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

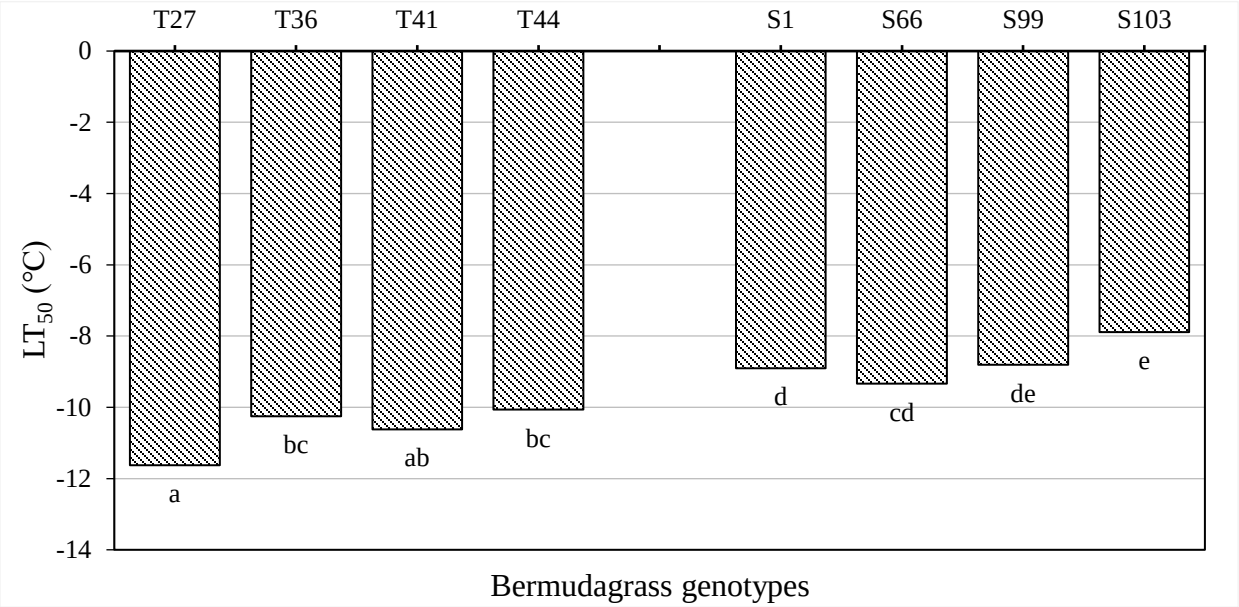

Fig 1. Lethal temperature, based on 50% loss in electrolytes (LT_{50}), of eight bermudagrass genotypes. The eight bermudagrasses represent four genotypes that showed tolerance to aryloxyphenoxypropionate (AOPP) herbicide (T27, T36, T41, and T44), and four genotypes that showed susceptibility to AOPP herbicide (S1, S66, S99, and S103). LT_{50} values marked with the same letters are not significantly different according to Fisher's Protected LSD (P=0.05).

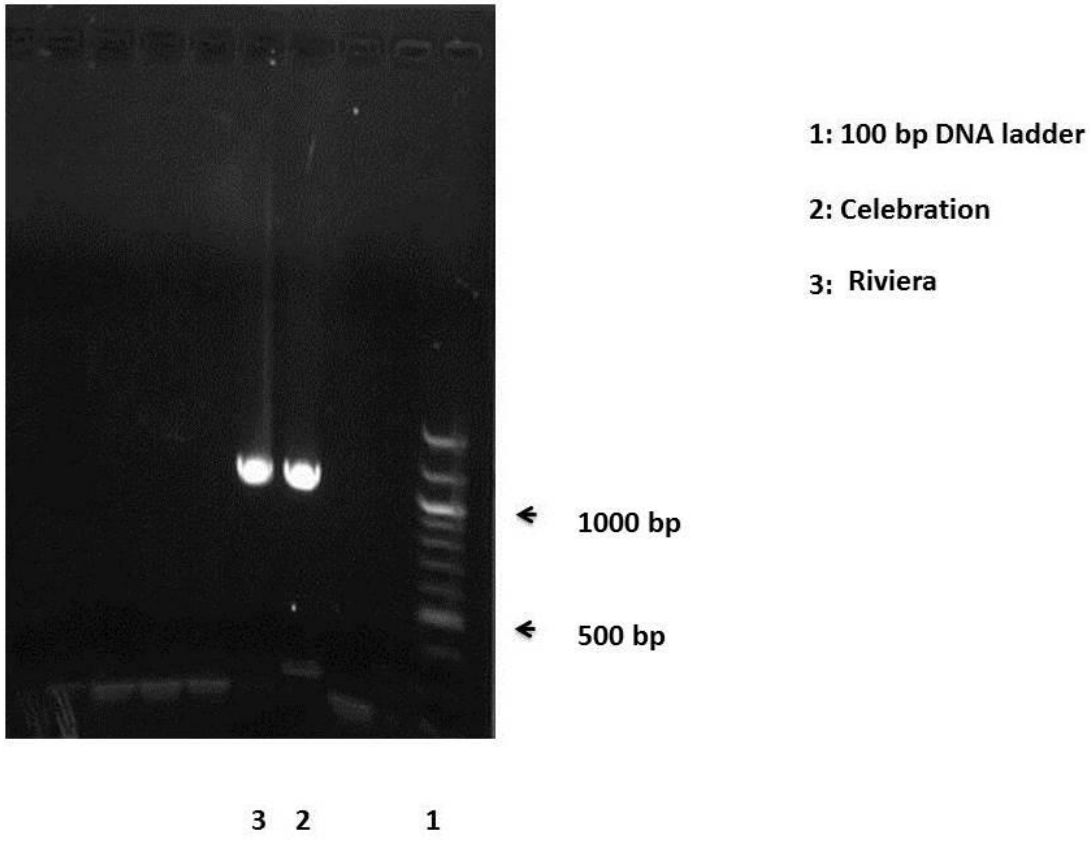

Fig 2. Putative fatty acid desaturase (FADS) genes cloned from “Riviera” and “Celebration” bermudagrasses. The amplified bands were 1,083 base pairs with 361 amino acids.