crops

MDPI

Article

Evaluation of Diverse Sorghum for Leaf Dhurrin Content and Post-Anthesis (Stay-Green) Drought Tolerance

Chad Hayes [1,*], Yves Emendack [1], Jacobo Sanchez [1], John Burke [1], N. Ace Pugh [1], Zhanguo Xin [1] and William Rooney [2]

[1] Plant Stress & Germplasm Development Research Unit, USDA-ARS, Lubbock, TX 79415, USA; yves.emendack@usda.gov (Y.E.); jacobo.sanchez@usda.gov (J.S.); nicholas.pugh@usda.gov (N.A.P.); zhanguo.xin@usda.gov (Z.X.)
[2] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77845, USA; wlr@tamu.edu
* Correspondence: chad.hayes@usda.gov

Abstract: Post-flowering drought tolerance (stay-green) in grain sorghum (*Sorghum bicolor* (L.) Moench) is an important agronomic trait in many arid and semiarid environments throughout the world. Stay-green has been associated with increased grain yields, as well as resistance to lodging and charcoal rot disease. Nonetheless, the relative effects of genotype, environment, and genotype × environment interactions are not well understood for this trait; similarly, the relationship between various leaf sugars and stay-green has not been sufficiently evaluated in diverse germplasm. Thus, the goals of this study were to determine the genotype, environment, and genotype by environment (GxE) effects for leaf dhurrin, sugars, and stay-green in ten diverse grain sorghum breeding lines, to evaluate the Pearson's correlation coefficients (r) between these traits, and to determine entry-mean repeatability (R) for each of these traits. Of the compositional traits studied, we determined that leaf dhurrin had the highest correlation with the stay-green phenotypes ($r = -0.62$). We found that stay-green sorghum lines contained approximately 2–3 times as much dhurrin as non-stay-green lines, with B1778 containing the highest concentration of dhurrin (84.8 $\mu g/cm^2$) and Tx7000 containing the least (20.9 $\mu g/cm^2$). The differences between the environments for several of the traits were high, and all the traits examined had high repeatability ($R = 0.89$–0.92). These data demonstrate a relationship between leaf dhurrin and the stay-green phenotypes in sorghum, and further study will allow researchers to determine the causal effect that dhurrin has on post-flowering drought tolerance in sorghum.

Keywords: sorghum; dhurrin; drought; stay-green

Citation: Hayes, C.; Emendack, Y.; Sanchez, J.; Burke, J.; Pugh, N.A.; Xin, Z.; Rooney, W. Evaluation of Diverse Sorghum for Leaf Dhurrin Content and Post-Anthesis (Stay-Green) Drought Tolerance. *Crops* **2023**, *3*, 241–250. https://doi.org/10.3390/crops3030022

Academic Editor: Christian Cilas

Received: 26 July 2023
Revised: 28 August 2023
Accepted: 6 September 2023
Published: 21 September 2023

1. Introduction

A drought is defined as a prolonged or chronic shortage of rainfall, and droughts can occur in areas with high and low average levels of precipitation. Drought conditions are relative to long-term average rainfall patterns and evapotranspiration demands [1]. Drought stress is the limitation of water that is available for plant growth, and it is the primary constraint to productivity in cereal crops globally. Drought stress can negatively impact crop growth, but its magnitude and effect can differ depending on the plant species and development stage. As the world population continues to increase, it is important to identify crop species with multiple end uses that possess an ability to produce sufficient yields under variable climatic conditions. One example of a plant species that has been proven as a multi-use crop with inherent drought tolerance is sorghum (*Sorghum bicolor* (L.) Moench) [2].

Sorghum is a C_4 cereal grass species that has many uses, including food, feed, forage, and feedstock [3]. It ranks fifth in importance for cereal crop species in the world after rice, wheat, maize, and barley [4]. Sorghum is traditionally cultivated on marginally arable lands and is routinely affected by drought stress. Fortunately, sorghum is commonly known to be drought-tolerant, especially compared to other cereal grains, like rice and maize [5,6].

The ability of a plant to maintain green leaf area at maturity, also known colloquially as "stay-green", has been used in sorghum breeding programs for many years as a field-based indicator for post-flowering drought tolerance [7]. Stay-green in sorghum is an economically important drought tolerance trait in production regions where post-anthesis drought events are common [8]. Stay-green sorghum lines maintain green leaves longer under post-anthesis drought, remain photosynthetically active longer, and produce a higher grain yield under drought conditions when compared with sorghum lines that are not stay-green [7,8]. Multiple physiological differences between stay-green and non-stay-green genotypes have been postulated as the causal mechanisms for the stay-green trait. Examples of potential stay-green mechanisms include increased leaf nitrogen at anthesis, higher chlorophyll content in leaf tissue, increased photosynthetic activity and leaf greenness, and a reduction in total leaf area [6,7,9].

Stay-green is considered an important trait, as the ability to maintain green leaf area during periods of post-flowering drought stress is associated with increased grain yields at harvest [8]. It has been hypothesized by some plant breeders that while stay-green hybrids yield more under post-flowering drought conditions, they are less responsive to fully irrigated and optimal conditions. If this is correct, one explanation for lower yields in stay-green hybrids under optimum conditions could be that the sources of stay-green used in breeding programs are relatively few. Since the genetic sources of stay-green are limited, fewer elite combinations of seed parents and pollinators can ultimately be used. The inclusion of new stay-green breeding lines with variable genetic backgrounds would increase genetic variance and potentially lead to more favorable hybrid combinations and a higher yield potential.

The current methodology for identifying and selecting stay-green lines in this field is with visual stay-green ratings [10]. These ratings are assigned after a period of post-flowering drought stress. However, field-based stay-green screening nurseries are often difficult to manage and require multiple test environments, often spanning multiple years, to encounter conditions that can accurately assess the stay-green phenotype. This difficulty is caused by variations in the environment; for example, rainfall after anthesis and natural field variation can eliminate the stress and drastically influence the stay-green ratings. There remains a need for a simple, quantitative assay that accurately identifies these stay-green lines without the need to grow them under post-flowering drought conditions. In order to be considered useful, this assay needs to be reproducible, less expensive than conventional field screening, and provide the ability to screen at early developmental stages.

Dhurrin is a cyanogenic glucoside produced by *Sorghum bicolor* and other sorghum species [11]. Dhurrin is a non-volatile compound in isolation, but physical disruption of plant tissue by animal herbivory or drought stress allows production hydrogen cyanide (HCN), which is produced by the interaction between dhurrin and catabolic dhurrinase enzymes [12,13]. Dhurrin has also been proposed to be an available source of N with osmoprotective properties [14]. Burke et al. [15] identified multiple stay-green breeding lines that contained elevated leaf dhurrin levels. Stay-green ratings from previous studies and environments were used to associate these elevated leaf dhurrin levels with the visual stay-green ratings. Commonly used stay-green germplasm, such as BTx642, B4R, and SC1154-14E, contained 3–4× higher dhurrin levels compared to known senescent sorghum varieties [15]. Recent research has also identified a major stay-green QTL (Stg5) on SBI01 that co-localizes with known dhurrin biosynthetic genes [16].

Although an association between stay-green and leaf dhurrin was observed by Burke et al. [14] and Hayes et al. [17], additional research is needed to evaluate a diverse set of breeding lines for leaf dhurrin content and stay-green within the same growing environments. Therefore, the objectives of this study were as follows: (i) to analyze the genotype, environment, and GxE effects for leaf dhurrin, leaf sugars, and stay-green for ten diverse grain sorghum breeding lines, and (ii) to evaluate correlations between these traits.

2. Materials and Methods

2.1. Plant Material and Experimental Design

A set of ten diverse breeding lines that varied for the described stay-green phenotypes were used in this study (Table 1). These breeding lines varied in terms of their adaptation, maturity, and stay-green. These stay-green lines also varied for their genetic source of stay-green based on their known pedigree history.

Table 1. List of ten diverse breeding lines evaluated for dhurrin, leaf sugars, and stay-green in 2014.

Line	Pedigree	Class	Reference
BTx642	BTx406/IS12555	Stay-green	Xu et al., 2000 [10,18]
R9188	SC599-6sel	Stay-green	Rosenow et al., 1983 [6]
1790E	SC56/SC33	Stay-green	Rosenow et al., 1983 [6]
B4R	BTx406/Rio	Stay-green	Burke et al., 2013 [15]
B1778	SC56/SC33	Stay-green	Rosenow et al., 1983 [6]
Tx7000	Caprock, Kafir-Milo	Senescent	Xu et al., 2000 [10,18]
RTx437	SC120sel/RTx430	Senescent	Burke et al., 2013 [15]
BTx623	BTx3197/SC170-6-4	Senescent	Rosenow et al., 1983 [6]
BTx3042	Redbine, Kafir-Milo	Senescent	Burke et al., 2013 [15]
BTx378	Redlan, Kafir	Senescent	Tenkouano et al., 1993 [19]

The lines were grown in replicated field trials in four locations in 2014 (Figure 1). These environments were designated as 14WE, 14CA, 14CW, and 14LB. Weslaco (WE) is in the Rio Grande Valley in the southern tip of Texas and is a humid, arid sub-tropical environment. Corpus Christi Annex and Corpus Christi West (CA and CW, respectively) are also known as humid, semi-arid environments located along the Texas gulf coast. Lubbock (LB) is situated in the Texas High Plains region and has a dry and temperate climate. All locations are established sorghum production regions of Texas and commonly experience post-flowering drought stress. All locations had weather conditions during the period of the experiments that was expected for their region. In all locations, a randomized complete block design (RCBD) with three replications was used. Sorghum seeds were planted at a rate of 70 seeds per plot at a depth of 4 cm. A plot in each environment was defined as a single row that was 5.2 m long. The row spacing in each environment was 1.0 m wide. The Weslaco and Lubbock environments were furrow irrigated as needed to minimize visual drought stress until flowering. The two Corpus Christi environments were only rain fed and did not receive supplemental irrigation. All seeds were treated with a seed treatment mixture of Captan®, Concept®, and Gaucho® to allow for the application of dual herbicides for pre-germination weed control. Standard agricultural practices for sorghum were used in this study.

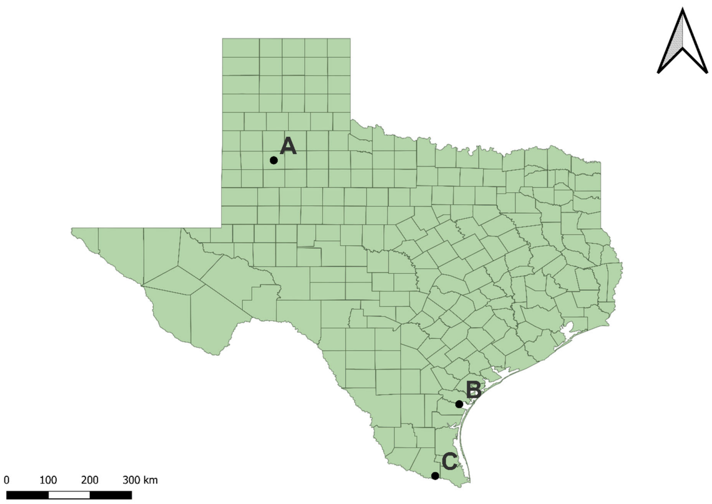

Figure 1. Map of the field trial locations in Texas. This map shows the approximate locations of the four trials in Texas that were conducted in 2014. The four trials were in Lubbock (A), Corpus Christi (B), and Weslaco (C). Two of the four trials were located near Corpus Christi, TX.

2.2. Stay-Green Phenotyping

Stay-green estimates in the form of visual observation ratings were scored in each environment on a plot basis at a scale from 1 to 5. These ratings were based on the degree of visual plant greenness or senescence at physiological maturity, as per Xu et al. [18]. A rating of 1 indicated a completely green plant with green leaves and no senescence (Figure 2). A rating of 5 corresponded to no visual greenness in the leaves, accompanied with complete plant death (Figure 2).

Figure 2. Visual comparison of stay-green and non-stay-green material. A visual comparison of stay-green (**left**) and non-stay-green (**right**) genotypes.

2.3. Leaf Dhurrin and Sugar Analysis

Leaf dhurrin, glucose, sucrose, and fructose were extracted from the lines approximately at mid-anthesis in each location. Five leaf punches that measured 1.0 cm in diameter were collected from five random, representative plants within each plot. Leaf punches were collected on the youngest, fully exposed leaf (excluding the flag leaf). The leaf punches were taken at the midpoint between the blade tip and the base of the leaf and did not include the midrib. The leaf punches were immediately placed on ice in the field and promptly returned to the laboratory. All HPLC analyses were performed at the Cropping Systems Research Laboratory in Lubbock, TX. Dhurrin and leaf sugars from the five leaf punches was extracted in 1 mL of 80% ethanol at 60 °C for one hour followed by five minutes at room temperature. The extract was centrifuged for 10 min at 10,000 RPM and the resulting supernatant was transferred to an Eppendorf tube and dried with a vacuum centrifugation system (Savant Instruments Inc., Hyderabad, India) set on the "low" setting. The extract was suspended in 200 µL of deionized water and separated on a YMC polyamine II column with a mobile phase of 75% acetonitrile (C_2H_3N) at a flow rate of 1.5 mL/min. A standard curve was used to quantify the concentration of dhurrin, and leaf sugars were expressed as µg/cm^2.

2.4. Statistical Analysis

Individual data from each location were analyzed to determine whether the variances were homogeneous. Since heterogeneity of error variances was not detected using a Bartlett's test for equal variances, a combined analysis was performed. The statistical model used for the combined analysis was:

$$\text{Dependent Variable} = \mu + \text{Genotype} + \text{Environment} + \text{Genotype} \times \text{Environment} + \text{Rep[Env]} + \text{Error}. \quad (1)$$

JMP® Version 10.0.0 was used for all statistical analyses. Genotype was considered a fixed effect and all other sources of variation were considered as random effects. Raw HPLC data was log Box–Cox transformed to ensure that the ANOVA normality assumption was satisfied. Multivariate analysis was performed using the Pearson's correlation coefficient method. Coefficient of variation (CV) values were calculated. Repeatability of traits was calculated using the following equation:

$$\text{Repeatability} = \sigma^2_{G}/[\sigma^2_{G} + (\sigma^2_{GxE/E}) + (\sigma^2_{error/ER})] \quad (2)$$

where σ^2_G is the genotype effect, σ^2_{GxE} is the genotype × environment effect, σ^2_{error} is the error, E is the number of environments, and R is the number of replications.

3. Results and Discussion

In the combined analysis, genotype effects were found to be significant for all traits measured (Table 2). The environment effect was also found to be significant for all traits except for stay-green. The interaction between the genotype and the environment (GxE) was found to be significant for dhurrin and fructose; although, the magnitude of this interaction was deemed to be minimal (Table 2). Repeatability for all traits was high, ranging from 0.87 (fructose) to 0.95 (glucose). As mentioned, reliable and consistent drought screening nurseries are notoriously difficult to generate due to rainfall and other agronomic factors. In 2014, specifically, three locations were not included in the analysis as the stay-green phenotype was not sufficiently expressed in those specific environments due to rainfall occurring late in the season. In contrast, 2014 was an excellent year for the evaluation of stay-green in some environments, as evidenced by the clear separation within the breeding lines and a relatively low CV. The inclusion of environments with moderate or no differences for stay-green would decrease repeatability and made effects due to the environment more profound.

Table 2. Mean squares for leaf composition traits and stay-green from combined data from the stay-green vs. non-stay-green test in four environments in 2014.

Source of Variation	df	Dhurrin †	Glucose †	Fructose †	Sucrose †	Stay-Green ‡
Environment (E)	3	2.12 ***	4.03 ***	8.75 ***	15.47 ***	0.25
Rep[E]	8	0.08 **	0.32 ***	2.76 ***	0.89 ***	0.49 *
Genotype (G)	9	3.23 ***	0.48 ***	2.82 ***	0.51 ***	9.0 ***
GxE	27	0.13 ***	0.07	1.7 **	0.13	0.32
Error	72	0.04	0.05	0.04	0.08	0.21
R^2		0.93	0.85	0.86	0.92	0.86
CV (%)		5.0	9.6	5.0	7.5	15.3
Repeatability		0.89	0.95	0.87	0.92	0.92

*, **, *** significant at 0.10, 0.05, and 0.01, respectively. †: μg/cm² of leaf tissue. ‡: stay-green rating where 1 = green and 5 = fully senesced.

Specific environmental differences for leaf dhurrin content did not receive further investigation as nitrogen fertilizer, which is known to greatly affect dhurrin accumulation, was not applied equally in each environment due to varying agronomic practices for each production region.

Dhurrin content was strongly correlated (r = −0.62) with stay-green ratings in this study (Table 3). Strong positive associations were numerically negative in this study due to the stay-green rating system used, where a rating of one is considered highly stay-green, and a rating of five is considered fully senesced. Associations between leaf dhurrin and stay-green have been previously observed; thus, the strong correlation observed in this study between dhurrin and visual stay-green corroborates previous results [15,20]. Stay-green ratings were only modestly correlated (r = −0.14) with leaf sucrose concentrations (Table 3). Previous research has indicated that BTx642 and 1790E, which are known stay-green lines, contained high leaf sucrose concentrations at anthesis compared to Tx7000 and BTx623, which are known senescent lines [21]. Burke et al. [21] also identified that quantum efficiency (Fv/Fm), which serves as a predictive bioassay that is used in identifying stay-green breeding lines, is significantly correlated (r = 0.67) with leaf sucrose levels.

Table 3. Pearson's correlation coefficients (r) for leaf dhurrin, leaf sugars, and stay-green ratings in four environments in 2014.

Trait	Dhurrin †	Fructose †	Glucose †	Sucrose †	Stay-Green ‡
Dhurrin	-				
Fructose	−0.11	-			
Glucose	−0.16 *	0.97 ***	-		
Sucrose	0.05	0.09	0.04	-	
Stay-green ‡	−0.62 **	−0.08	−0.08	−0.14 *	-

*, **, *** significant at 0.10, 0.05 and 0.01, respectively. †: μg/cm^2 of leaf tissue. ‡: stay-green rating, where 1 = green and 5 = fully senesced.

The breeding lines varied for dhurrin in this study (Table 4). Across the four environments, leaf dhurrin ranged from 84.8 μg/cm^2 (B1778) to 20.0 μg/cm^2 (Tx7000) and separated into two distinct classes based on the presence/absence of stay-green in most cases (Table 4). BTx642, a standard for the stay-green phenotype, averaged 60.0 μg/cm^2 and was statistically higher than all senescent lines.

Table 4. Averages for leaf composition traits and stay-green of 10 breeding lines varying for stay-green evaluated across four environments in 2014.

Pedigree	Class	Dhurrin †	Glucose †	Fructose †	Sucrose †	Stay-Green ‡
B1778	Stay-green	84.8	73.4	57.6	64.5	2.5
1790E	Stay-green	66.7	46.5	40.0	42.5	2.3
BTx642	Stay-green	60.0	82.1	70.4	78.4	1.5
B4R	Stay-green	51.7	80.6	60.4	78.9	2.3
R9188	Stay-green	45.9	95.8	63.8	65.8	2.4
RTx437	Senescent	28.3	80.3	64.9	44.9	3.7
BTx378	Senescent	23.5	75.1	59.5	39.2	3.8
BTx623	Senescent	23.1	57.8	47.5	63.9	3.8
BTx3042	Senescent	23.0	71.2	55.3	54.5	3.6
Tx7000	Senescent	20.9	90.2	67.5	67.3	3.8
$HSD_{p\,<\,0.05}$		9.8	27.1	19.6	39.8	0.5
CV (%)		5.0	10.4	9.5	19.9	17.1

†: μg/cm^2 of leaf tissue. ‡: stay-green rating, where 1 = green and 5 = fully senesced.

The relative ranks of breeding lines for dhurrin concentration differed between the environments, but the magnitude was minimal (Table 2). Among the stay-green lines, R9188 had the lowest dhurrin concentrations in three of the four environments in this study (Table 5). In the WE environment, R9188 had the second-highest dhurrin levels, and B1778 was the highest (Table 5). This shift in the concentrations in R9188 may account for the significant GxE effect. Although B1778 was determined to be the highest in three of these environments, it did not have the highest stay-green rating in the CA environment (Table 5).

The relatively low coefficient of variation observed for dhurrin in this study further justifies its use as a bioassay associated with stay-green (Table 4).

Table 5. Least significant differences (LSD) for leaf dhurrin content for 10 different grain sorghum lines varying for the stay-green phenotype in Corpus Christi annex (CA), Corpus Christi West (CW), Lubbock (LB), and Weslaco (WE) in 2014. Genotypes in bold format and marked with an * were classified as stay-green and genotypes in non-bold bold format were classified as non-stay-green, senescent genotypes.

Leaf Dhurrin (μg/cm²)			
CA		CW	
Genotype	LS mean	Genotype	LS mean
1790E *	**70.4**	**B1778 ***	**91.6**
B1778 *	**51.0**	**BTx642 ***	**66.9**
BTx642 *	**38.5**	**1790E ***	**60.5**
B4R *	**27.5**	**B4R ***	**52.8**
R9188 *	**24.8**	**R9188 ***	**33.0**
BTx378	17.7	RTx437	29.2
BTx623	16.8	BTx623	24.2
Tx7000	15.3	BTx378	22.4
BTx3042	14.9	BTx3042	20.3
RTx437	14.6	Tx7000	19.8
$LSD_{p\,<\,0.05}$	10.8	$LSD_{p\,<\,0.05}$	19.6
LB		WE	
Genotype	LS mean	Genotype	LS mean
B1788 *	**90.1**	**B1778 ***	**106.4**
BTx642 *	**78.0**	**R9188 ***	**82.8**
1790E *	**73.8**	**B4R ***	**79.6**
B4R *	**46.7**	**1790E ***	**61.6**
R9188 *	**42.8**	**BTx642 ***	**56.4**
RTx437	41.2	RTx437	28.1
BTx3042	30.5	BTx3042	26.2
BTx378	30.0	BTx378	23.7
BTx623	29.5	TX7000	22.9
Tx7000	25.2	BTx623	21.9
$LSD_{p\,<\,0.05}$	17.7	$LSD_{p\,<\,0.05}$	23.0

The breeding lines evaluated in this study also varied greatly for visual stay-green ratings (Tables 4 and 6). BTx642 had the best stay-green rating averaged across all environments (Table 6). Rank differences for stay-green ratings within different environments were observed, but these differences were minimal, and the GxE effect in the combined analysis was non-significant (Table 2).

Leaf sucrose also varied in this study (Tables 4 and 7). B4R contained the highest concentration of sucrose (78.9 μg/cm²), and BTx378 contained the lowest concentration of sucrose (39.2 μg/cm²) (Table 4). B4R is a derivative of Rio, a sweet sorghum line selected for its high sugar (brix) content in its stems. BTx642 and R9188, two known stay-green lines, also consistently contained higher sucrose concentrations in all environments (Table 7). The line 1790E, a known stay-green line, produced relatively low leaf sucrose concentrations in all environments (Table 7). Previous studies have associated high leaf sucrose concentrations with stay-green using the breeding lines BTx642 and R9188. The results from this study indicate that while some stay-green lines contained elevated leaf sugars, other stay-green lines contained relatively low leaf sucrose levels. These findings indicate that leaf sugar content is a contributory factor in stay-green, but it is not the only factor that determines stay-green.

Table 6. Least significant difference (LSD) values for stay-green ratings for 10 different grain sorghum lines varying for the stay-green phenotype in Corpus Christi annex (CA), Corpus Christi West (CW), Lubbock (LB), and Weslaco (WE) in 2014. Genotypes in bold format and marked with an * were classified as stay-green and genotypes in non-bold format were classified as senescent genotypes.

Stay-Green Rating			
CA		CW	
Genotype	LS mean	Genotype	LS mean
BTx623	4.0	BTx378	4.2
BTx378	3.8	BTx623	4.2
Tx7000	3.7	Tx7000	4.0
RTx437	3.7	BTx3042	3.7
BTx3042	3.2	RTx437	3.7
B1778 *	**3.0**	**B1778 ***	**2.7**
B4R *	**2.3**	**R9188 ***	**2.3**
R9188 *	**2.0**	**B4R ***	**2.3**
1790E *	**1.8**	**1790E ***	**2.0**
BTx642 *	**1.3**	**BTx642 ***	**1.7**
$LSD_{p<0.05}$	1.1	$LSD_{p<0.05}$	1.0
LB		WE	
Genotype	LS mean	Genotype	LS mean
BTx3042	4.2	RTx437	4.2
Tx7000	4.0	BTx378	3.8
BTx623	3.8	BTx3042	3.7
RTx437	3.7	Tx7000	3.7
BTx378	3.5	BTx623	3.3
1790E *	**2.7**	**1790E ***	**2.8**
B4R *	**2.7**	**R9188 ***	**2.5**
R9188 *	**2.7**	**B1778 ***	**2.2**
B1778 *	**2.3**	**B4R ***	**2.0**
BTx642 *	**1.3**	**BTx642 ***	**1.8**
$LSD_{p<0.05}$	0.9	$LSD_{p<0.05}$	1.1

Table 7. Least significant difference (LSD) values for leaf sucrose content for 10 different grain sorghum lines varying for the stay-green phenotype in Corpus Christi annex (CA), Corpus Christi West (CW), Lubbock (LB), and Weslaco (WE) in 2014. Genotypes in bold format and marked with an * were classified as stay-green and genotypes in non-bold format were classified as senescent genotypes.

Leaf Sucrose (μg/cm^2)			
CA		CW	
Genotype	LS mean	Genotype	LS mean
R9188 *	**89.4**	**BTx642 ***	**34.4**
BTx642 *	**83.6**	**B4R ***	**33.0**
B4R *	**69.4**	**R9188 ***	**29.8**
Tx7000	58.4	RTx437	28.6
BTx623	53.4	BTx3042	26.8
BTx3042	51.8	Tx7000	26.8
B1778 *	**42.7**	BTx378	25.5
BTx378	41.9	**B1778 ***	**25.5**
RTx437	37.7	BTx623	25.2
1790E *	**32.8**	**1790E ***	**23.9**
$LSD_{p<0.05}$	51.9	$LSD_{p<0.05}$	7.7
LB		WE	
Genotype	LS mean	Genotype	LS mean
BTx642 *	**48.6**	**B4R ***	**171.0**
B4R *	**42.1**	**B1778 ***	**169.6**
BTx623	40.7	Tx7000	145.9
Tx7000	23.7	BTx623	136.6
BTx3042	23.2	**BTx642 ***	**132.5**
B1778 *	**20.3**	BTx3042	116.4
R9188 *	**20.2**	**R9188 ***	**108.5**
BTx378	18.7	RTx437	100.7
1790E *	**17.5**	**1790E ***	**95.9**
RTx437	12.8	BTx378	66.3
$LSD_{p<0.05}$	31.8	$LSD_{p<0.05}$	45.2

4. Conclusions

This study represents one of the first studies of leaf dhurrin levels and stay-green evaluation within the same environment. As hypothesized, these lines separated into two distinct groups based on stay-green and differentiated in similar groups for leaf dhurrin concentration. In addition, these stay-green lines consistently contained approximately 2–3 times the leaf dhurrin content at anthesis than did the non-stay-green breeding lines.

Burke et al. [15] first described a relationship between elevated leaf dhurrin levels at anthesis and stay-green. This study expands on that previous work by measuring stay-green, leaf dhurrin, and leaf sugars within the same environments. The causal effect of high leaf dhurrin levels at anthesis producing a stay-green response in sorghum is still not fully understood. Results from this study indicate that associations between dhurrin and stay-green are present beyond the BTx642 genetic background, and that further investigations are needed to fully understand the relationship between high dhurrin and stay-green.

Author Contributions: Conceptualization, C.H. and W.R., methodology, J.B., C.H. and W.R., formal analysis, Y.E., C.H. and N.A.P., investigation, C.H., data curation, Y.E., C.H. and J.S., writing-original draft preparation, C.H. and W.R., writing-review and editing, J.B, C.H., N.A.P. and Z.X., funding acquisition J.B. and W.R. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for this project was provided in part by the United Sorghum Checkoff Program.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank the teams at the Plant Stress and Germplasm Development Research unit at USDA-ARS in Lubbock, as well as members of the Bill Rooney laboratory at Texas A & M University for their contributions to this project.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ANOVA—analysis of variance, CA—Corpus Christi Annex, CW—Corpus Christi West, DAP—days after planting, HCN—hydrogen cyanide, HPLC—high-performance liquid chromatography, LB—Lubbock, QTL—quantitative trait loci, RCBD—randomized complete block design, and WE—Weslaco.

References

1. Wilhite, D.A.; Glantz, M.H. Understanding: The drought phenomenon: The role of definitions. *Water Int.* **1985**, *10*, 111–120. [CrossRef]
2. Rooney, W. Sorghum Improvement—Integrating traditional and new technology to produce improved genotypes. *Adv. Agron.* **2004**, *83*, 37–109.
3. Rooney, W.L.; Blumenthal, J.; Bean, B.; Mullet, J.E. Designing sorghum as a dedicated bioenergy feedstock. *Biofuels Bioprod. Biorefining* **2007**, *1*, 147–157. [CrossRef]
4. Doggett, H. *Sorghum*, 2nd ed.; John Wiley and Sons: New York, NY, USA, 1998.
5. Rosenow, D.T.; Clark, L.E. Drought tolerance in sorghum. In Proceedings of the Annual Corn and Sorghum Industry Research Conference—American Seed Trade Association, Corn and Sorghum Division, Corn and Sorghum Research Conference, Washington, DC, USA, 9–11 December 1981; Loden, H.D., Wilkinson, D., Eds.; American Seed Trade Association: Alexandria, VA, USA, 1981; pp. 18–30.
6. Rosenow, D.T.; Quisenberry, J.E.; Wendt, C.W.; Clark, L.E. Drought tolerant sorghum and cotton germplasm. *Agric. Water Manag.* **1983**, *12*, 207–222. [CrossRef]
7. Borrell, A.K.; Hammer, G.L.; Douglas, A.C.L. Does maintaining green leaf area in sorghum improve yield under drought? I. leaf growth and senescence. *Crop. Sci.* **2000**, *40*, 1026–1037. [CrossRef]
8. Jordan, D.R.; Hunt, C.H.; Cruickshank, A.W.; Borrell, A.K.; Henzell, R.G. The relationship between the stay-green trait and grain yield in elite sorghum hybrids grown in a range of environments. *Crop Sci.* **2012**, *52*, 1153–1161. [CrossRef]
9. Borrell, A.K.; Mullet, J.E.; George-Jaeggli, B.; van Oosterom, E.J.; Hammer, G.L.; Klein, P.E.; Jordan, D.R. Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *J. Exp. Bot.* **2014**, *65*, 6251–6263. [CrossRef] [PubMed]

10. Xu, W.; Rosenow, D.T.; Nguyen, H.T. Stay green trait in grain sorghum: Relationship between visual rating and leaf chlorophyll concentration. *Plant Breed.* **2000**, *119*, 365–367. [CrossRef]
11. Rhykerd, C.L.; Johnson, K.D. Minimizing prussic acid poisoning hazard in forages. In *Publ. AY-96. Forage Information*; Agronomy Extension Purdue University: West Lafayette, IN, USA, 2007.
12. Busk, P.K.; Møller, B.L. Dhurrin synthesis in sorghum leaves is regulated at the transcriptional level and induced by nitrogen fertilization in older plants. *Plant Physiol.* **2002**, *129*, 1222–1231. [CrossRef] [PubMed]
13. Emendack, Y.; Burke, J.; Laza, H.; Sanchez, J.; Hayes, C. Abiotic stress effects on sorghum leaf dhurrin and soluble sugar contents throughout plant development. *Crop. Sci.* **2018**, *58*, 1706–1716. [CrossRef]
14. Burke, J.J.; Payton, P.; Chen, J.; Xin, Z.; Burow, G.; Hayes, C. Metabolic responses of two contrasting sorghums to water-deficit stress. *Crop Sci.* **2015**, *55*, 344–353. [CrossRef]
15. Burke, J.J.; Chen, J.; Burow, G.; Mechref, Y.; Rosenow, D.; Payton, P.; Hayes, C.M. Leaf dhurrin content is a quantitative measure of the level of pre-and postflowering drought tolerance in sorghum. *Crop Sci.* **2013**, *53*, 1056–1065. [CrossRef]
16. Hayes, C.M.; Weers, B.D.; Thakran, M.; Burow, G.; Xin, Z.; Emendack, Y.; Burke, J.J.; Rooney, W.L.; Mullet, J.E. Discovery of a dhurrin QTL in sorghum: Co-localization of dhurrin biosynthesis and a novel stay-green QTL. *Crop. Sci.* **2016**, *56*, 104–112. [CrossRef]
17. Hayes, C.M.; Burow, G.B.; Brown, P.J.; Thurber, C.; Xin, Z.; Burke, J.J. Natural variation in synthesis and catabolism genes influences dhurrin content in sorghum. *Plant Genome* **2015**, *8*, plantgenome2014-09. [CrossRef] [PubMed]
18. Xu, W.; Subudhi, P.K.; Crasta, O.R.; Rosenow, D.T.; Mullet, J.E.; Nguyen, H.T. Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). *Genome* **2000**, *43*, 461–469. [CrossRef] [PubMed]
19. Tenkouano, A.; Miller, F.R.; Frederiksen, R.A.; Rosenow, D.T. Genetics of nonsenescence and charcoal rot resistance in sorghum. *Theor. Appl. Genet.* **1993**, *85*, 644–648. [CrossRef]
20. Mcbee, G.G.; Miller, F.R. Hydrocyanic acid potential in several sorghum breeding lines as affected by nitrogen fertilization and variable harvests. *Crop Sci.* **1980**, *20*, 232–234. [CrossRef]
21. Burke, J.J.; Franks, C.D.; Burow, G.; Xin, Z. Selection system for the stay-green drought tolerance trait in sorghum germplasm. *Agron. J.* **2010**, *102*, 1118–1122. [CrossRef]