

Improved bioenergy sorghums for the Southeast

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Introduction

Sorghum is an attractive bioenergy crop for the SE US because of its short establishment time (4-6 months), high biomass potential and limited need for irrigation. Biomass sorghums can reach 4-6 m and can be produced as either sweet or non-sweet stem types. Use of sweet sorghums offers the benefit of generating both readily fermentable sugars and cellulosic biomass. Sweet-stem sorghum present advantages over not sweet sorghum lines: higher carbon-capture ability than grain types¹; stem sugar accumulation can benefit grain yield by providing a source of carbohydrates for grain fill should photosynthesis be limited during this period due to environmental stresses²; sucrose accumulation enhances tolerance to drought and salinity by acting as a protectant to maintain leaf water status and sweet sorghums delay the onset of stem rooting diseases, a major cause of lodging and panicle breakage.

Most of the current high-biomass sweet and forage lines were not developed for use in the SE US. As a result, their production is limited by fungal pathogens, in particular *Colletotrichum sublineolum*, which causes anthracnose leaf blight and stem rot in susceptible cultivars. Commercial seed of sweet sorghum is not readily available due to the high cost of harvesting seed from tall pure-line varieties. In addition, the use of pure lines as opposed to hybrid seed does not take advantage of the increase in yield resulting from heterosis. Adoption of hybrid seed is responsible for large yield increases observed in major crops during the early and mid-1900's. Availability of hybrid bioenergy sorghum seed is vital for the successful establishment of a bioenergy agroindustry. This projects focuses in the elucidation of the genetic relationship observed between height controlling genes (*Dw* genes) and stem sugar concentration^{3,4}, the mapping of genes underlying resistant to fungal pathogens as well as the creation of short stature sweet-stemmed parental lines for hybrid seed production. As outputs, this project will provide several essential tools that will lower the threshold for commercial seed companies to invest in the production of seed for biomass sorghums.



Figure 1. Examples of height phenotypes in sorghum. A. Tall cultivar, potential biomass sorghum. B. Short cultivar, grain line.

Objectives

1. to determine the relationship between height and sugar accumulation in the stem.
2. to map genomic regions associated with anthracnose resistance.
3. to select appropriate short-stature female lines to produce high-biomass, sweet-stem hybrid varieties adapted to the SE US.

Methods

Objective 1

Two populations of F3:4 families were created by crossing two short grain parent with contrasting *Dw* genes, and a panel of tall sweet sorghum lines. The two population are segregating for different *Dw* genes, allowing us to dissect the relationship between the *Dw* genes and sugar concentration.

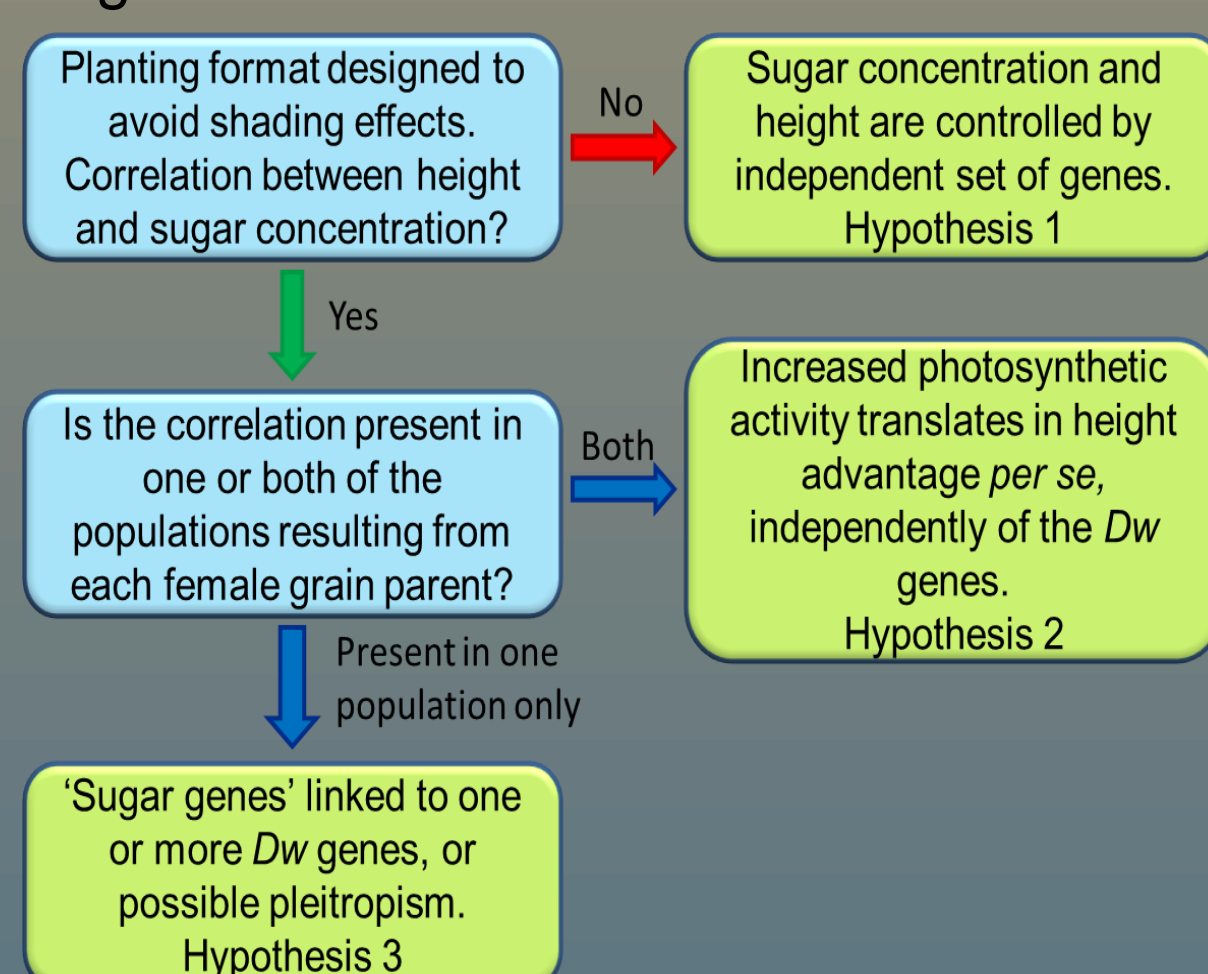


Figure 2. Experimental strategy to determine the relationship between height and sugar concentration.

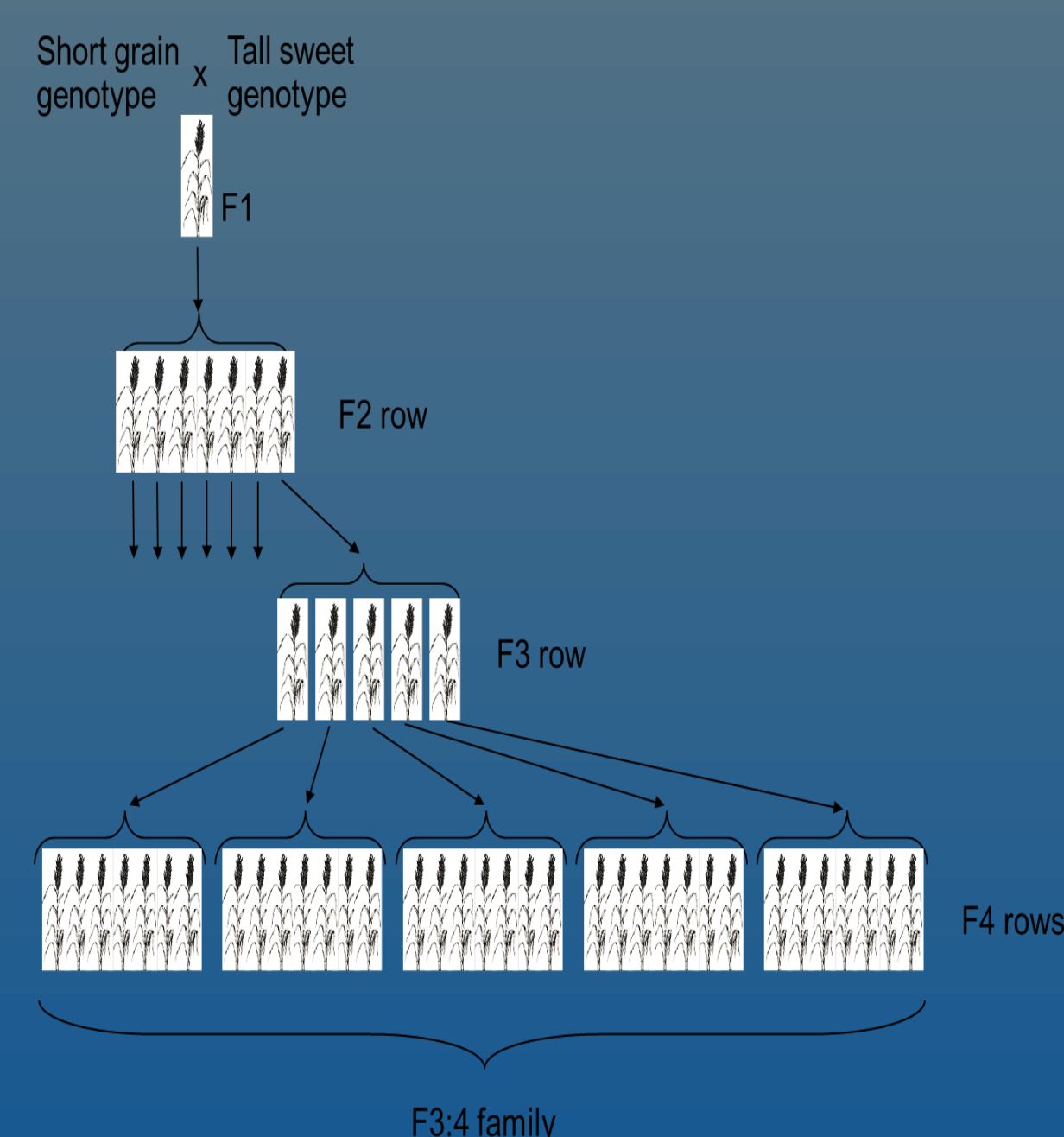


Figure 3. Schematic representation of the creation of one F3:4 family

Objective 2

We identified sources of resistance in grain lines developed in Florida. A recombinant inbred lines (RIL) population from a susceptible x resistant cross (Fig. 4. C and D) and a genome-wide SNP genotyping assay is being used to dissect the genetic control of the resistance via QTL study.

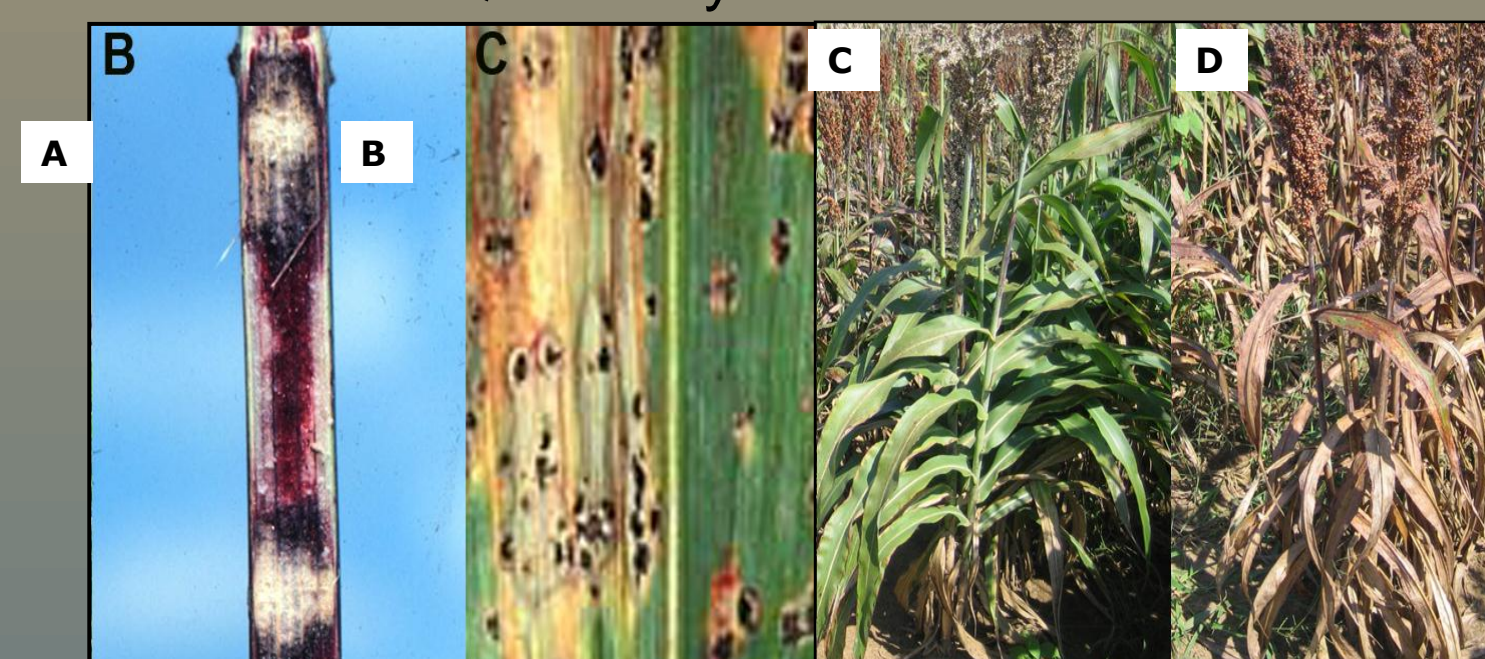


Figure 4. A. Split sorghum stem showing anthracnose infection. B. Foliar lesions. C. Resistant cultivar Bk7. D. Susceptible cultivar 'Early Hegari' at the same maturity and location.

Objective 3

Diverse genotypes are tested for their capacity to restore fertility (R or B reaction, Fig. 5, A). Once the B- or R-reaction of has been defined, it is crossed with a short parent with a defined combination of *Dw* alleles: a B-line containing *dw1*, *Dw2*, *dw3*, *dw4* (B pool), and an R-line containing *Dw1*, *dw2*, *Dw3*, *dw4* (R pool). Short lines from such crosses inherit the *Dw* genes from the corresponding short parent. F1 hybrids between the newly selected B and R lines will be heterozygous for most of their *Dw* genes, and therefore tall (Fig. 5, B).

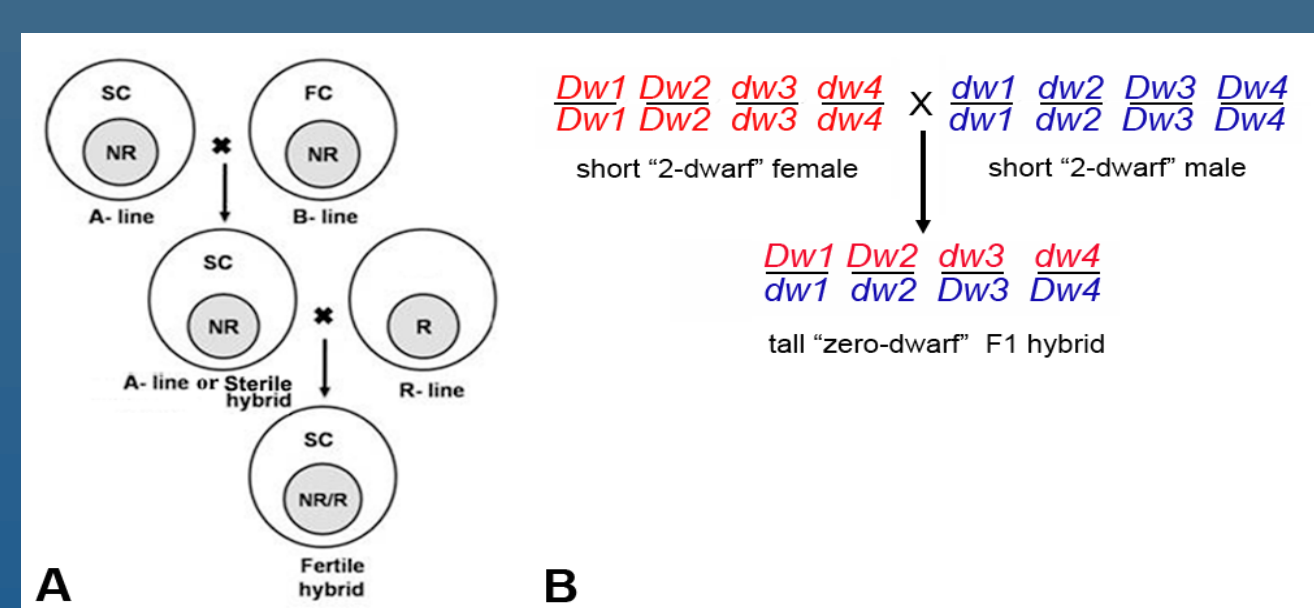


Figure 5. A. Cytoplasmic male fertility in sorghum. SC = 'sterile' cytoplasm; FC = 'fertile' cytoplasm. R (in the nucleus; grey inner circle) indicates a fertility-restoring *R*-gene, whereas NR indicates lack of an *R*-gene. B. Crossing two short inbred parents with contrasting *dwarf* alleles (female in red, male in blue) results in tall F1 progeny.

Results

Objective 1

The positive correlation between plant height and sugar concentration measured as brix is present in both populations (Table 1), regardless of the *Dw* genes present in the female parent. However, within families, the strength and direction of the relationship vary, with a predominance of positive correlations. These results suggest the absence of linkage of an individual *Dw* gene and a "brix gene". The increased concentration of photosynthates available for growth could have a direct positive effect on the final height of the plant (hypothesis 2).

Table 1. Observed relationship between plant height and sugar concentration measured as brix in two population of F3:4 families resulting from the cross of two short non-sweet sorghum females with a panel of sweet sorghums.

Early Hegari population r=0.52 R ² =.27 Average height=242 cm						Btx631 population r=0.43 R ² =0.18 Average height=223 cm					
Male	F3:4 Family	Number of F4 lines	Average height (cm)	Average brix	R	Male	F3:4 Family	Number of F4 lines	Average height (cm)	Average brix	R
Bethlana	1	3	0.05	243	14.1	Brandes	1	2	negative	168	11.0
	2	4	0.57	198.1	12.5		2	5	0.74	253	13.8
	3	3	-0.61	326	15.0		3	2	negative	146	13.5
	across fam				0.88		across fam				0.53
Brandes	1	4	0.03	276	13.1	MERB-4	1	6	0.83	198	10.0
	2	3	0.99	266	15.7		2	5	-0.67	177	13.5
	3	4	-0.05	215	15.2		3	4	0.89	292	12.1
	across fam				0.45		4	2	negative	168	11.4
MERB-4	1	5	-0.92	153	9.1		5	4	0.99	127	10.3
	2	5	-0.50	158	9.6		6	2	positive	248	9.8
	3	5	0.95	323	14.0		7	5	0.97	297	12.5
	across fam				0.99		8	7	0.71	218	10.9
Muremba	1	2	positive	138	5.4		9	11	0.10	235	13.6
	2	2	n/a	124	7.2		10	2	negative	320	15.5
	3	2	n/a	200	14.7		11	2	positive	114	11.0
	4	2	negative	107	10.8		across fam				0.61
	5	5	0.73	328	13.9	Muremba	1	3	0.96	236	17.2
	6	3	0.86	223	12.1		2	2	negative	154	14.8
	7	2	positive	127	12.6		across fam				positive
	across fam				0.73		1	5	0.47	184	14.0
Rox Orange	1	4	0	225	14.2		2	7	0.78	304	13.6
	2	2	positive	221	10.8		3	5	0.04	167	12.0
	3	4	0.66	160	10.2		across fam				0.44
	across fam				0.46	Rio	1	4	0.95	274	11.0
Smith	1	3	0.64	312	17.4		2	3	0.69	190	10.8
	2	3	0.83	326	14.1		3	4	na	267	13.0
	3	4	0.7	150	12.7		4	3	0.05	214	10.5
	4	2	positive	343	13.9		5	2	positive	253	13.1
	5	3	-0.97	347	14.6		6	6	0.98	235	12.3
	across fam				0.53		7	3	0.82	137	16.5
							across fam				-0.51
Sugar Drip	1	5	0.76	283	11.9						
	2	6	0.51	229	11.1						
	3	2	negative	244	9.7						
	4	5	0.45	337	13.5						
	5	2	positive	259	13.4						
	6	6	0.41	254	10.0						
	7	2	positive	274	12.8						
	8	3	0.05	299	13.7						
	9	2	positive	248	15.6						
	across fam				0.45						
Upendo red/corn	1	3	0.78	224	8.5						
	2	5	0.14	270	9.2						
	across fam				positive						

Objective 2

125 F4 recombinant inbred lines were created from the cross susceptible x resistant lines. The population and parental lines were scored for natural infection, as well as their response to artificial inoculation⁵. The parents maintained their previously observed reaction (Early Hegari, 5: highly susceptible, Bk7, 1: highly resistant, Fig. 6). The scores of the symptoms of the artificial inoculation with isolated anthracnose pathogen presented a clear 1:2:1 ration (χ^2 p-value= 0.79). However, the majority of the progeny RILs presented natural infection scores intermediate between the parent. This could be due to the presence of multiple pathogens in the area, which may compound the overall disease score of the plant. As the Bk7 parent presented a high level of resistance to natural infection, it is expected that this line contains several resistance genes to diverse fungal pathogens. Independent segregation of these genes in the progeny could explain the intermediate levels of resistance observed in the RILs.

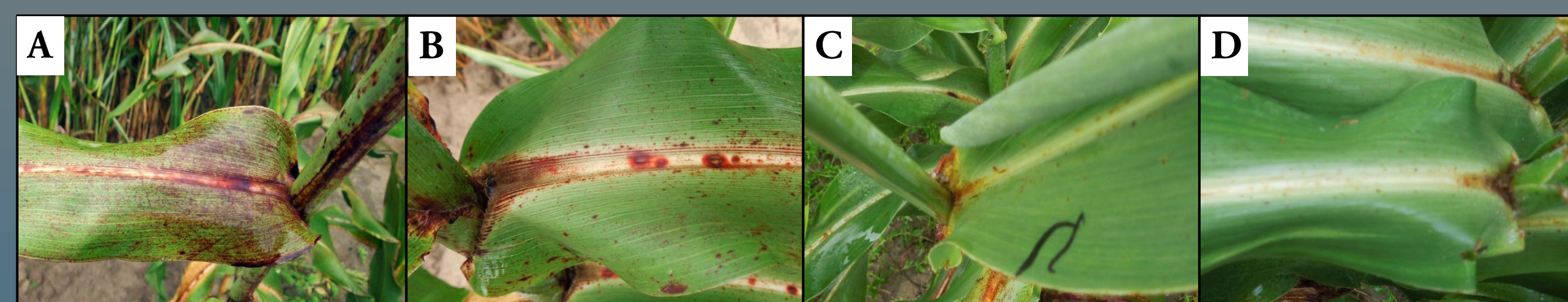


Figure 6. Parental lines response to artificial inoculation (A, C) and natural infection (B, D). A and B. Early Hegari. C and D. Bk7.

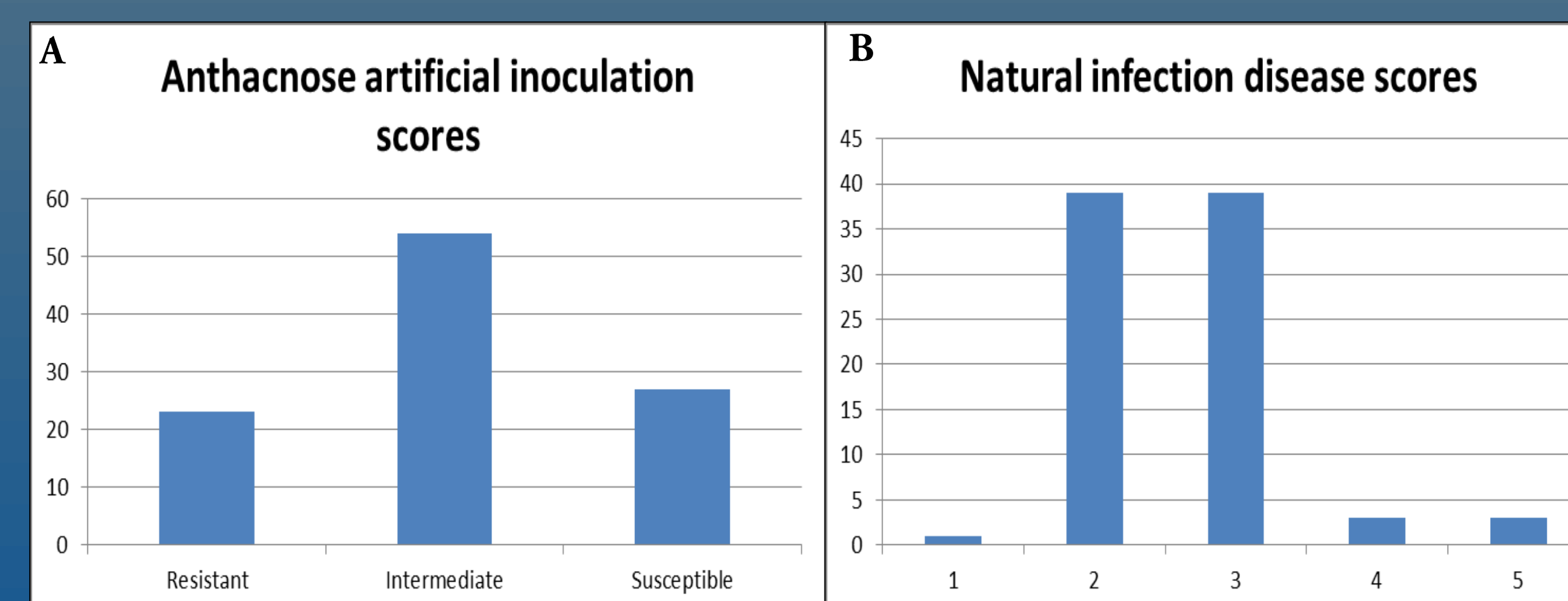


Figure 7. A. RILs scores 4 weeks after inoculation. Resistant: no expanded lesion, no sporulation. Intermediate: Lesions expanding from the inoculation point, no sporulation. Susceptible: Sporulation present in lesions. B. RILs natural infection disease scores at physiological maturity. 1-resistant, 5 extremely susceptible. Y axis: Number of RILs

Objective 3

A diverse panel of progenitor cultivars with advantageous traits in the Southeast has been selected for the production of bioenergy sorghum parental lines. This panel includes traditional and exotic sweet sorghum material, UF-developed disease-resistance grain lines and publicly available grain lines. Each line has been tested for its height system, disease response, fertility reaction and general agronomic traits. Crosses aimed to harness the best traits from the lines for the production of parental lines for hybrids have resulted in the production of over 2000 new lines at different stages of inbreeding. This winter season, 45 short-stature disease resistant, sweet-stemmed B lines at the F4 stage are being evaluated while starting the process of sterilization to produce the A/B pairs for female production. A large number of short-stature disease resistant, sweet-stemmed R lines have been developed and will be selected for their combining ability for sugar and height with a panel of short female lines. As the first step for the commercialization of UF developed bioenergy sorghum, a set of 12 tall sweet sorghum cultivars is available for testing as R (male) progenitors for bioenergy hybrids.



Figure 8. Introgression of disease tolerance into high yielding genotypes. A. Testing of a disease resistant cultivar (left) for its fertility reaction, height system and dominance of the disease resistance by crossing it to a susceptible grain A line (right). B. A UF-developed disease resistant sweet sorghum cultivar, compared to a commercial hybrid not developed in the state (right).

Conclusions

- o The lack of a linkage between a gene or genes controlling sugar concentration and one of the *Dw* genes will allow for the development of short sweet stemmed parental lines for hybrids that would have the advantages of the high sugar content in the stem. Based in empirical observations, having the sweet sorghum trait in both parental lines is necessary to ensure high sugar content in the hybrid. Since the sugar concentration is not linked to an individual *Dw* gene, it is then possible to develop both A and R lines of short stature and high sugar concentration, which would produce a tall bioenergy hybrid.
- o The resistance to anthracnose in Bk7 maybe controlled by one gene or a cluster of tightly linked genes. Mapping the locus associated with the resistance will greatly facilitate our efforts to introgress the high level of resistance from the Bk7 line into bioenergy lines. Additional genes present in Bk7 appear to be responsible for the resistance to other fungal pathogens. The number and genetic relationship of these resistance genes in not know. New single-pathogen inoculation protocols will be needed to allow the phenotyping of the RILs for their reaction to several fungal pathogens. The identification of molecular markers for all the resistance genes is necessary for the efficient pyramiding of the genes into improved lines, and ultimately, for the identification and characterization of the genes responsible for the phenotype.
- o Our strategy to develop short parental lines for tall, high biomass hybrids adapted to the environmental challenges of the southeast has resulted in the production of disease resistant, high brix lines with excellent agronomic performance in Florida. Their combining ability will be tested next season to ensure that hybrids display heterosis for biomass and sugar yield, while maintaining their high levels of disease resistance.

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