Genetic variation in stomatal density and nighttime stomatal conductance in switchgrass (*Panicum virgatum*): climate and trait associations

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Introduction

Stomata regulate exchange of carbon and water between leaves and the atmosphere. The size and density of stomata could be important predictors of leaf gas exchange. The purpose of this study was to determine genetic implications affecting nighttime stomatal conductance (g_{sn}), and to explore whether switchgrass from different habitats vary in stomatal density and size, and whether this variation is associated with home climate and or other aspects of morphology that vary among genotypes. This study sought to answer three main questions:

(1) To what extent do day and nighttime gas-exchange parameters and stomatal traits covary across genotypes?

(2) Is genotypic variation an important source of variation in g_{sn} and other gas-exchange and stomatal traits?

(3) Is genotypic variation in g_{sn} and other traits associated with climate conditions at the genotype's geographic origin?

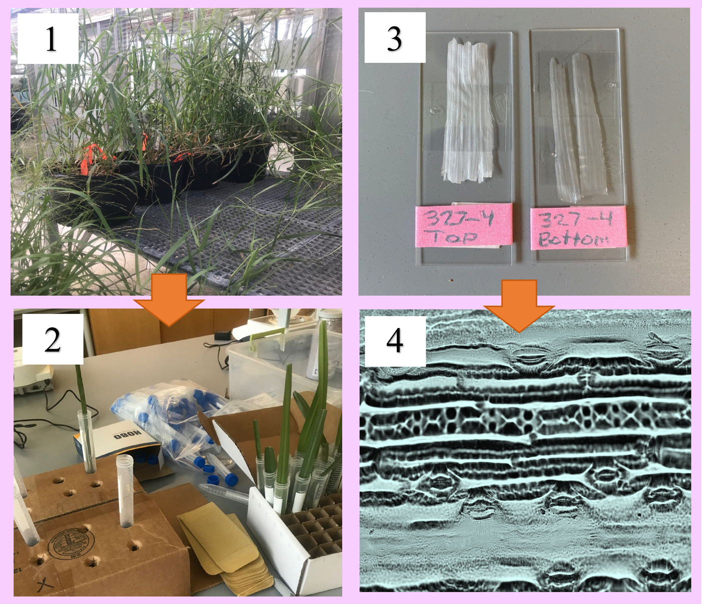
Climates of Origin

Eleven switchgrass genotypes (3-6 replicates per genotype) representing different home-climates from different North American locations were collected and cultivated in the biology greenhouse located at the University of North Florida.

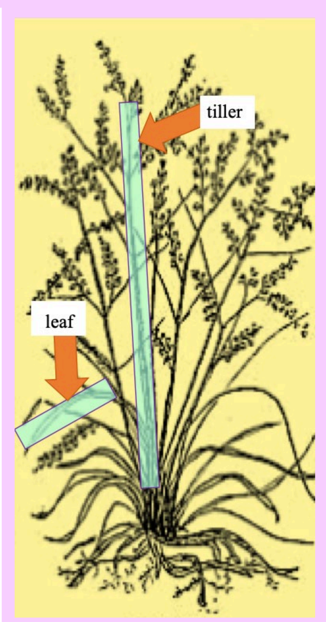
Genotype	State	County	Elevation (m)	Mean Annual Precipitation (mm)	Average Minimum Daily Temperature (°C)	Average Daily Temperature (°C)	Average Maximum Daily Temperature (°C)
001	Florida	Hernando	26	1341.1	14.5	21.2	27.8
020	Florida	Martin	3	1511.8	17.6	22.9	28.3
171	Florida	Duval	3	1138.7	16.9	21.3	25.7
181	Florida	Volusia	1	1263.9	16.9	21.8	26.7
183	Florida	Levy	19	1290.3	15	21.8	28.7
239	Texas	San Patricio	5	838.2	16.2	21.9	27.5
242	Texas	Kenedy	301	947.9	12.7	19.3	25.9
268	Coahuila, Mexico		440	477.8	14.7	21.2	27.7
293	Texas	Bexar	293	819.7	14.8	20.8	26.8
303	Texas	Kenedy	5	721.4	15.8	22.5	29.2
327	Texas	Victoria	29	1043.4	14.9	20.9	26.9

***Table 1**. Respective climates of origin (home-climates) for each genotype included in this study.*

Materials and Methods



Leaf clippings were collected from the third leaf from the top of the tallest tiller (**1**). Leaf area was measured with a Li-3000 Leaf Area Meter (Li-Cor, Inc., Lincoln, Nebraska, USA) (**2**). Leaf stomatal prints were established through the use of nail varnish peels. These stomatal peels were placed on a glass slide, labeled, and viewed through a compound light microscope (**3**). SPOT Basic Image Capture Software (SPOT Imaging Inc., Sterling Heights, Michigan, USA) was used to capture stomatal images in three different locations of the peel. Stomatal images were analyzed through Image J (NIH) in order to record data on stomatal density, stomatal widths, and stomatal heights (**4**). Nighttime and daytime measurements of leaf gas-exchange were measured on all replicates of all genotypes at three timepoints during summer 2019 using three portable photosynthesis systems; one Li-6400XT and two LI-6800 systems (LI-6800 or LI-6400XT, Li-Cor, Inc, Lincoln NE, USA).



(1) To what extent do day and nighttime gas-exchange parameters and stomatal traits covary across genotypes?

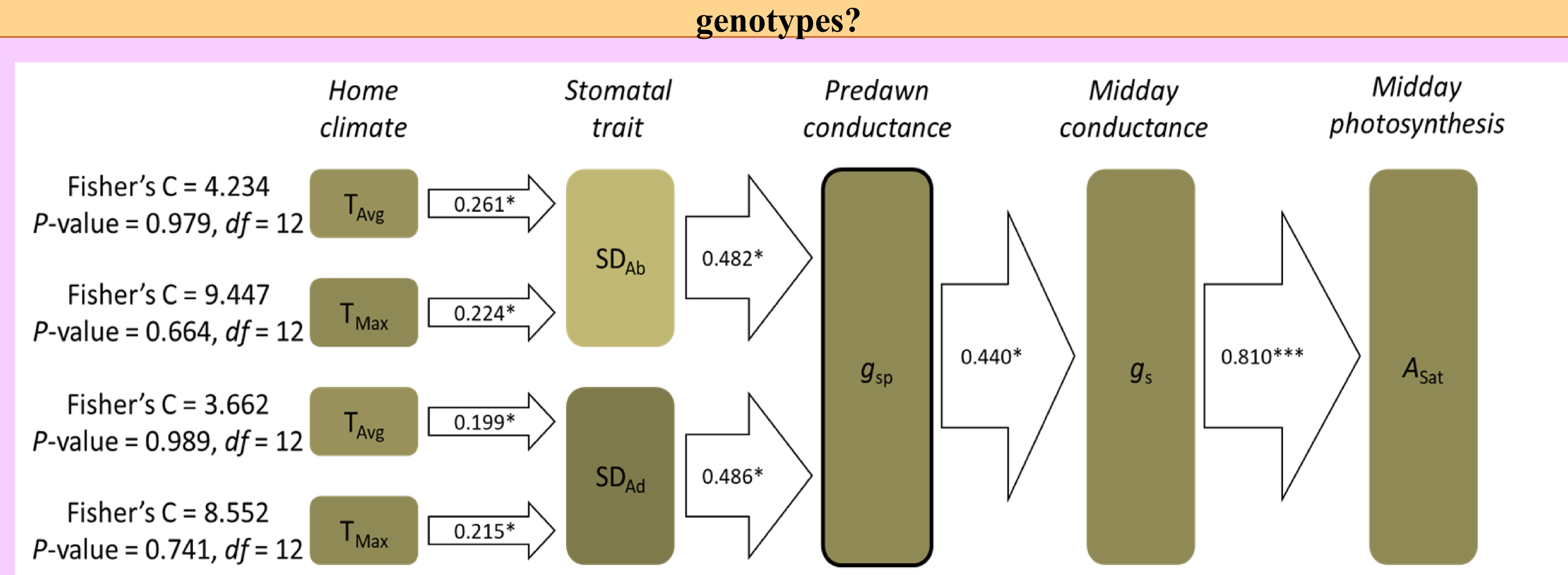


Figure 1.** Overall visualization of significance which indicates how home-climate and stomatal traits affect predawn stomatal conductance (g_{sp}) in Panicum virgatum which integrate with daytime gas-exchange parameters. Significance of pathways (arrows) are denoted as '+' (0.05 < P-value < 0.1), '*' (0.01 < P-value < 0.05), '**' (0.001 < P-value < 0.01), '' (P-value < 0.001). Pathways are all positive, indicating positive relationships.*

Results

(2) Is genotypic variation an important source of variation in g_{sn} and other gas-exchange and stomatal traits?

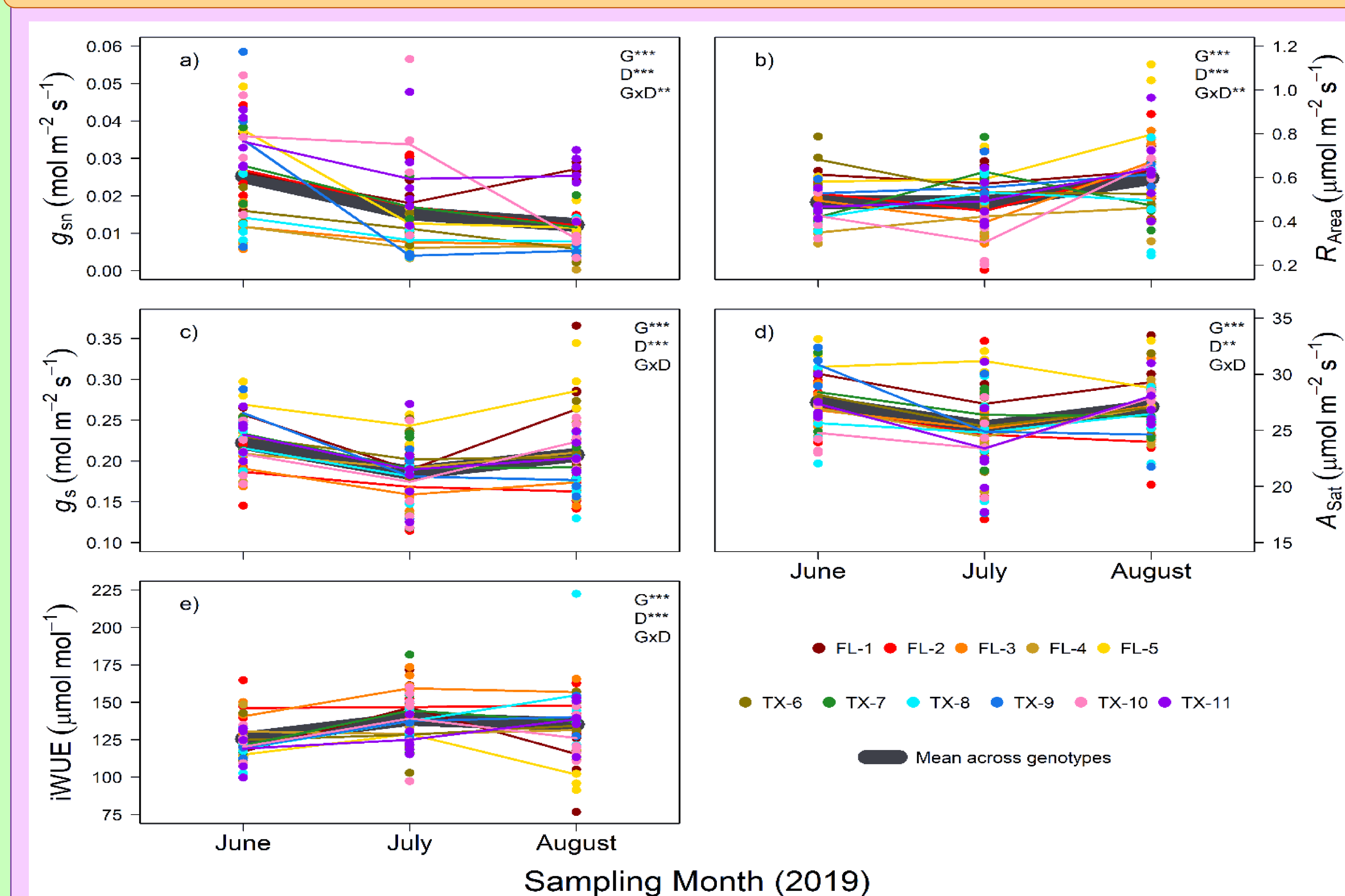


Figure 2.** Genotypic variation in nighttime (measured predawn) stomatal conductance (panel a, g_{sn}), area-based respiration (panel b, R_{area}), daytime stomatal conductance (panel c, g_s), saturated photosynthesis (panel d, A_{sat}), and intrinsic water-use efficiency (panel e, iWUE) for 11 genotypes of Panicum virgatum (switchgrass) and the overall mean value across genotypes. The effects of genotype ('G'), sampling date ('D'), and their interaction ('GxD') on gas-exchange parameters are denoted within each panel. Significance of terms are denoted as '*' (0.01 < P-value < 0.05), '**' (0.001 < P-value < 0.01), '' (P-value < 0.001).*

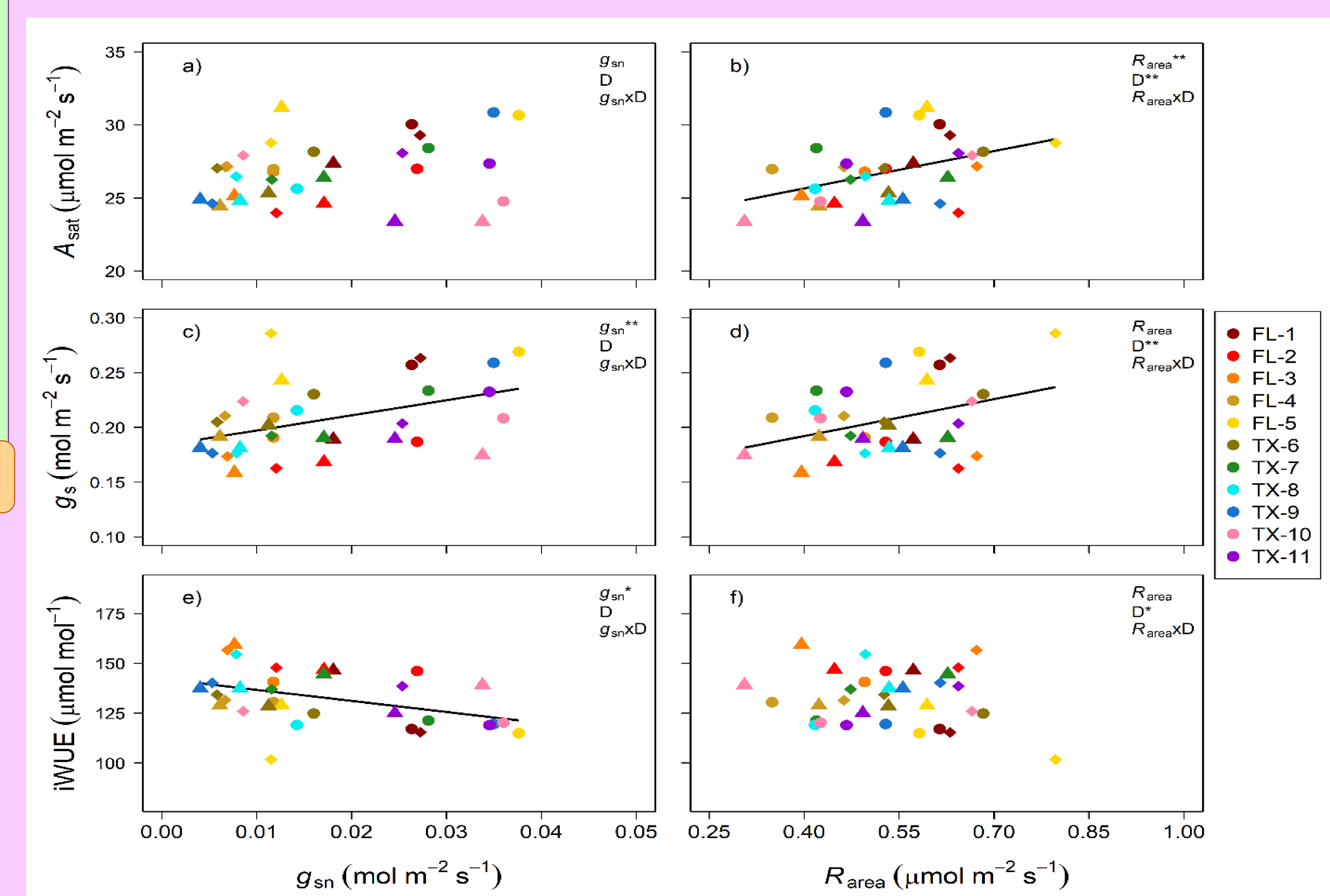


***Figure 3**. Covariation of predawn stomatal conductance (left panels, g_{sp} mol m^{-2} s^{-1}), area-based respiration (right panels, R_{Area} µmol m^{-2} s^{-1}) with saturated photosynthesis (panels a-b, A_{Sat} µmol m^{-2} s^{-1}), daytime stomatal conductance (panels c-d, g_s mol m^{-2} s^{-1}), and intrinsic water-use efficiency (panels e-f, iWUE µmol mol^{-1}) for 11 genotypes of switchgrass (Panicum virgatum) grown in common garden. Data for each genotype (colors) were averaged from data collected in June (●), July (▲), and August (♦).*

(3) Is genotypic variation in g_{sn} and other traits associated with climate conditions at the genotype's geographic origin?

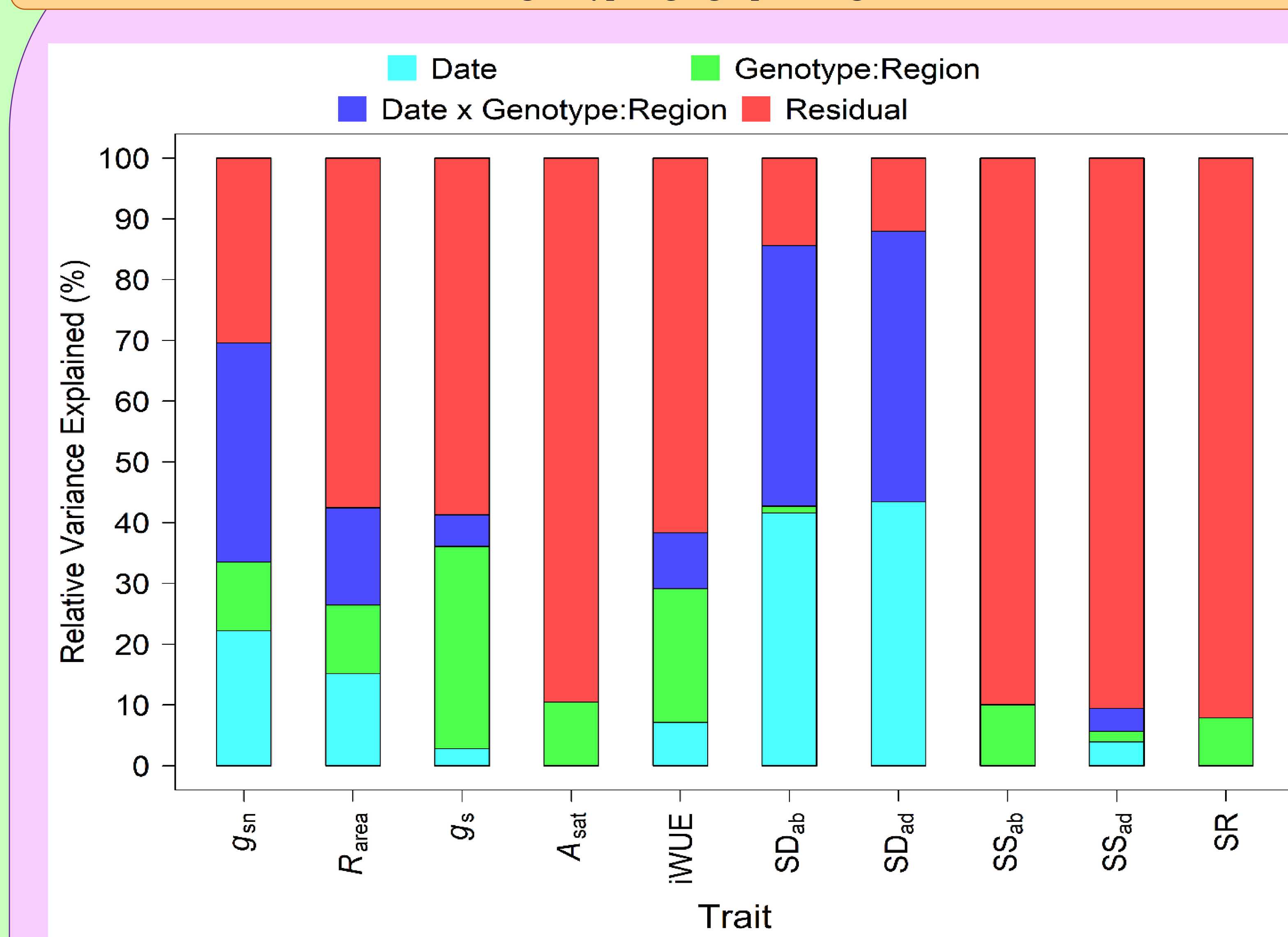


***Figure 4**. Proportion of trait variance explained by sampling month ('Date' light blue), genotype nested within region ('Genotype:Region' green), and their interaction ('Date x Genotype:Region' dark blue) in 11 genotypes of Panicum virgatum (switchgrass) grown in common garden. Unexplained/residual variance ('Residual') is indicated by red. Traits are: g_{sn} = nighttime stomatal conductance (g_{sn} mol m^{-2} s^{-1}), area-based respiration (R_{area} µmol m^{-2} s^{-1}), daytime stomatal conductance (g_s mol m^{-2} s^{-1}), saturated photosynthesis (A_{sat} µmol m^{-2} s^{-1}), intrinsic water-use efficiency (iWUE µmol mol^{-1}), abaxial stomatal density (SD_{Ab} µm^{-2}), adaxial stomatal density (SD_{Ad} µm^{-2}), average abaxial stomatal size (SS_{ab} µm^2), average adaxial stomatal size (SS_{ad} µm^2), and the ratio stomata on the adaxial to abaxial leaf surface (SR).*

Trait	MAP		Date		MAP x Date		T_{avg}		Date		T_{avg} x Date		T_{max}		Date		T_{max} x Date	
	df	*F-value*	*df*	*F-value*	*df*	*F-value*	*df*	*F-value*	*df*	*F-value*	*df*	*F-value*	*df*	*F-value*	*df*	*F-value*	*df*	*F-value*
g_{sn}	1	1.03	2	6.76**	2	0.29	1	0.28	2	7.02**	2	1.22	1	0.43	2	6.60**	2	0.26
R_{area}	1	1.18	2	3.76*	2	0.22	1	0.13	2	3.63*	2	0.24	1	0.11	2	3.81*	2	0.92
g_s	1	1.76	2	4.46*	2	0.57	1	0.63	2	4.27*	2	0.51	1	7.75**	2	5.29*	2	0.37
A_{sat}	1	4.37*	2	4.38*	2	0.63	1	1.29	2	4.02*	2	0.84	1	2.88	2	4.17	2	0.58
iWUE	1	0.02	2	3.57*	2	2.06	1	0.00	2	3.18	2	0.33	1	6.61*	2	3.88*	2	0.09
SD_{ab}	1‡	0.85	1	81.07***	1	0.00	1	8.70**	1	97.76***	1	3.69	1	6.31*	1	95.94***	1	5.51*
SD_{ad}	1‡	1.06	1	147.02***	1	0.43	1	6.83*	1	144.73***	1	3.32	1	8.26*	1	150.13***	1	2.93
SS_{ab}	1	4.40	1	0.07	1	0.01	1	0.00	1	0.06	1	0.18	1	0.58	1	0.06	1	0.05
SS_{ad}	1	0.11	1	0.70	1	0.01	1	0.32	1	0.73	1	0.60	1	0.01	1	0.78	1	2.31
SR	1	0.09	1	0.02	1	0.71	1	0.71	1	0.02	1	0.01	1	0.05	1	0.03	1	1.86

***Table 2.** Relationship between gas-exchange and stomatal traits with home-climate. Gas-exchange measurements were conducted in June, July and August while stomatal traits were measured in June and August, only. Residual degrees of freedom (df) were 27 for gas-exchange measurements and 68 for stomatal traits.*

While nearly all measures of night- and daytime gas-exchange varied between months we found no relationships between home-climate and nighttime gas-exchange and few correlations between home-climate and daytime gas exchange (**Table 2**). Similarly, we found few significant relationships between genotype home-climate and stomatal traits. Where significant relationships were found, temperature was the primary driver. Stomatal density (SD) on the abaxial and adaxial leaf surface tended to be greater in genotypes from sites with greater T_{Avg}.

Discussion

Switchgrass is such an important topic of study because of its implications and possible future use as a major biofuel. Its appeal as a biofuel comes from its resilience, ability to grow in a majority of locations, lack of requirement for controlled agricultural growing land, and long life span due to its perennial and C4 characteristics. By better understanding the innerworkings and trait derivations of switchgrass, we can better comprehend which genotypes of switchgrass could better be used for mass biofuel production, or if switchgrass is only optimal in its climate of origin. We found genotypic differences in g_{sn} were time-dependent, and the interaction of sampling date and genotype was a major source of variation in g_{sn}. We found no relationship between home-climate and nighttime gas-exchange; however, stomatal density tended to be greater in genotypes from warmer climates which in turn drove higher g_{sn} values resulting in greater daytime stomatal conductance (g_s) and net photosynthesis (A_{sat}). In future studies, we hope to examine the stomata found in the stems of switchgrass and their relation to leaf stomata as well as the strength and importance of their gas-exchange.

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