Evidence for microbiome-dependent chilling tolerance in Sorghum

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Abstract

Early season chilling stress is a major constraint on sorghum production in temperate climates. Chilling-tolerant sorghum is an active area of development, but the potential for early season microbial-enhanced chilling tolerance in sorghum has not yet been explored. In this study, we characterized traits of field-grown sorghum accessions in response to chilling and non-chilling temperatures, then we characterized the effects of chilling temperatures and microbial inoculation on sorghum accession traits in a growth chamber experiment. By comparing sorghum trait responses under chilling stress with and without soil microbial inoculation, we were able to detect a potential microbe-dependent sorghum response to chilling stress. Four sorghum genotypes show a negative response to chilling stress with vs. without microbial inoculation, while five sorghum accessions show increased shoot biomass or/ and leaf area under chilling stress when inoculated with a soil microbiome.

Keywords: Grain Sorghum, Chilling Stress, Microbiome, Seedling vigor

Introduction

Sorghum [(*Sorghum bicolor* L.) Moench] is one of the five major crop species in the world and can be grown for grain or biomass. Diverse uses of sorghum for food, energy, paper, fertilizer, and feed makes it an economically important crop (Tari et al. 2013). However, as a plant that evolved in the African tropics, sorghum is sensitive to chilling temperatures, below 15°C (Yu and Tuinstra 2001). Temperature sensitivity has limited the geographic range of sorghum in colder geographic regions, like the upper Midwest in the US, where it could otherwise be an attractive alternative crop for grain and biomass. In these colder regions, sorghum requires later planting dates due to seedling chilling sensitivity, which ultimately decreases yield (Maulana et al. 2013, Tarhi et al. 2013). A recent crop modelling exercise using sorghum planting dates from 1986-2015 in Kansas, recorded a 7 to 11% increase in yield with early planting, compared to normal and late planting (Raymundo et al. 2021). The study concluded that incorporating early-stage chilling tolerance in sorghum would allow for early planting, and hence a means to increase productivity by reducing the risk of terminal droughts and extended growing season.

While sorghum germplasm resources are diverse and under development for early season chilling tolerance (Chopra et al. 2015, Marla et al. 2019, Moghimi et al. 2019, Ostmeyer et al. 2020), the potential for early season microbial-enhanced chilling tolerance in sorghum has not yet been explored. Simultaneously evaluating diverse sorghum genotypes for genetic chilling tolerance and microbe-enhanced chilling tolerance would leverage a novel synergistic approach to early season chilling tolerance. Plant genotype-environment-microbe interactions in field conditions are highly complex (Bakker et al. 2020, Hohmann et al. 2020, Oyserman et al. 2019)). Developing broadly effective microbial enhancements for plant abiotic stress will require incorporating plant genotype – microbe – environment interactions in controlled experiments.

Plant-associated microbes can confer or improve abiotic stress tolerance in plants and exploiting these interactions promises new ways to improve crop production and agricultural practices (Backer et al. 2018, Lata et al. 2018). Various studies have addressed microbial effects on chilling tolerance on plants; focusing on potential mechanisms from a single microbe in conferring resilience against adverse conditions (Barka et al. 2006, Ding et al. 2011, Kang et al. 2015, Wang et al. 2015, Subramanian et al. 2016, Zhou et al. 2021). However, few studies have assessed changes in plant response to chilling or frost associated with bacterial community-level

interactions (Beirinckx et al. 2020, Cloutier et al. 2020). No studies, to our knowledge, have attempted to isolate the overall effects of the presence of a microbial community on the ability of seedlings to tolerate chilling stress. A basic understanding of how the presence of a diverse microbiome affects sorghum seedling vigor under chilling stress, and how the sorghum microbiome changes in response to chilling temperatures, is important context for further work on individual microbial isolates and mechanisms of chilling tolerance.

Prior studies have identified candidate genes and assessed agronomic outcomes for early season chilling tolerance in grain sorghum diversity panel (Moghimi et al. 2019). We utilized a subset of these sorghum accessions based on their field phenotypes (Bheemanahalli et al., 2019) to attempt to isolate the contribution of microbes to the plant chilling stress response and compare the microbial contribution across accessions. We hypothesized that grain sorghum accessions will exhibit diverse phenotypic responses to chilling stress, and that these diverse responses to chilling stress can be broken down into accession-specific component (microbe-independent chilling tolerance) and an accession-microbe interaction component (microbe-dependent chilling tolerance).

In this study, we characterized field-grown sorghum accessions in response to chilling and non-chilling temperatures and further, the effects of chilling temperatures and microbial inoculation interaction on sorghum performance were explored in a growth chamber experiment. By comparing sorghum trait responses under chilling stress with and without microbial inoculation, we were able to detect a microbe-dependent sorghum response to chilling stress. Further, these results were compared to multi-environment field studies to provide a realistic context for interpreting the microbe-dependent chilling response.

Methods

Field experiment design

Twenty sorghum accessions (15 tolerant and 5 sensitive selected from the sorghum association panel of 350 accessions based on field trials in 2018) were grown at Kansas State University, Manhattan, Kansas and South Dakota State University West River Research Farm, Sturgis, South Dakota. Planting was conducted in two intervals as early and regular planting in 2019. Based on

the sorghum production practices at each site, regular planting dates were June 11 in Kansas and June 5 in South Dakota. The early planting date was April 24 in Kansas and May 16 in South Dakota. The early planting date was considered as chilling stress and regular planting as the optimal sorghum growing period. Early planting dates were selected based on historic average air temperatures at the respective field sites. The target air temperature for early planting (chilling stress) was 13ºC and that for regular planting (control) was 20ºC. The experiment was laid out in a split-plot randomized complete block design with three replications. Each genotype was planted in four (Kansas) or two (South Dakota) rows per plot, and replicated thrice for each planting type (temperature treatment). Each row was 3.6 m (12 ft) long and accommodated 48 seeds (Kansas) or 7.3m (24 ft) long and accommodated 101 plants (South Dakota) with an inter-row spacing of 0.75 m. At the Kansas site, the maximum/minimum soil temperature at ~10 cm depth for different planting dates was recorded at 15 min intervals using soil temperature data loggers (Onset Computer Corporation, Bourne, MA, USA). Air and bare soil (4-inch depth) temperatures for the South Dakota West River Research Farm were obtained from the Mesonet weather station at the site (South Dakota Mesonet, https://mesonet.sdstate.edu/archive). Minimum and maximum air temperatures for each day were averaged (Fig. 1 and 2).

Field measurements

Emergence percentage, leaf area, and shoot biomass were measured under both early planting (EP) and regular planting (RP) conditions. Seedling emergence percentage was calculated as the ratio of the total number of emerged seedlings to the total number of planted seeds, multiplied by 100. Destructive sampling was done 45 days after planting (DAP) for both planting conditions. Leaf area was estimated by a leaf area meter (LI-3100 area meter, LI-COR. Inc., Lincoln, NE, USA) before oven drying. The shoot was separately oven-dried at 60°C for 72 h, and shoot biomass (g plant^{-1}) was recorded after reaching constant weight. Shoot biomass per row was calculated as a product of average seedling shoot biomass x emergence count per row.

Statistical analysis

The experimental layout was a split-plot with the randomized complete block design, with three replications. An ANOVA was performed for seedling emergence percentage, leaf area, and shoot biomass to estimate the significant difference between early and regular planting using the library

(agricolae) in RStudio 3.6.1 (https://rstudio.com/). Means were separated using the least significant difference (LSD) when treatments and interactions were significant at $P \leq 0.05$. The same set of analyses were conducted in JMP (SAS Institute, Inc.) or R (with the same methods as Kansas) for South Dakota field data.

Growth chamber study

Plant material and growth conditions

In this study, chilling (20/10°C, day/night) and control (30/20°C, day/night) temperature treatments were used to create temperature conditions similar to early and regular planting conditions in the field experiment. Three seeds of similar size for each of the nine-selected sorghum accessions (five high biomass and three low biomass, one sensitive accession, RTx430) were sown at a depth of 2-3 cm in a pot (10 cm×10 cm×30 cm, square pot) filled with 150 g of 3:1 proportion of double-autoclaved vermiculite: perlite mix. The pots were placed in four growth chambers, with three pots maintained per accession in each chamber, totaling 24 replicate pots across treatments (2 inoculum x 2 temperature treatments). The chambers were programmed to reach the daytime (08:00 h to 17:00 h) target temperature, following a gradual increase from 20°C to 30°C (control) and from 10°C to 20°C (chilling stress) over 3 h (05:00 h to 08:00 h). A similar gradual transition was followed from the maximum daytime temperature [30°C (control) or 20°C (chilling)] to night-time temperature (20°C or 10°C, respectively) over a 3 h (17:00 h and 20:00 h) period. Both control and chilling stress chambers were maintained at a 12 h photoperiod, with 800 $\mu mol\ m^{-2}\ s^{-1}$ light intensity at 5 cm above the canopy and 60% relative humidity (RH). Air temperature and RH were recorded every 15 min using HOBO UX 100-003 temperature/RH data loggers (Onset Computer Corp., Bourne, MA, USA) in all growth chambers. The pots were watered throughout the experiment as required using distilled water.

Soil microbe extraction and treatments

Two potting mixes that were identical except for their microbial communities were used in the study. Microbial cells were extracted following the soil wash method as described by Wagner et al. (2014). In brief, six soil subsamples representing ~1 acre were collected at a depth of ~10-30 cm from the same Manhattan, KS field used for the field experiment. Collected samples were homogenized by hand after removing rocks and other large debris. The homogenized soil samples

were sieved through 3.5 mm wire mesh before microbial extraction. A soil subsample of 75 g was stirred into 1 L of 2.5 mM MES monohydrate (Sigma Aldrich, St. Louis, MO, USA) in sterile distilled water (diH_2O) (pH adjusted to 5.7 with KOH). The soil suspensions were vacuum filtered to remove particulates after 30 min. Filtrates were centrifuged for 20 min, 4000 rpm at 25°C to pellet down microbial cells. Distilled water and MES solutions were sterilized via autoclave. Supernatants were discarded and the microbe-enriched pellets were re-suspended in sterile 2.5 mM MES solution. Pots were saturated with sterile distilled water (1 L; free from microbial contamination) one day before planting sorghum seeds. One day post-planting, planting, each microbial inoculation treatment pot received 50 mL diluted inoculum solution (100 mL of microbial suspension and 900 mL of sterile diH_2O). An additional 5 mL of undiluted microbial suspension was pipetted into each microbial inoculation pot (top-dressed). For the non-inoculated control, pots were saturated with an inoculum-free solution containing 100 mL sterile 2.5 mM MES and 900 mL of sterile diH_2O. An additional 5 mL sterile 2.5 mM MES was pipetted into each pot (top-dressed). Treatments derived by this process are termed "+ Microbiome" and "- Microbiome" to indicate high microbial diversity and low microbial diversity, respectively.

Growth chamber experiment design

Chlorophyll index (Model 502, Spectrum Technologies, IL, USA) and effective quantum yield (QY) of photosystem II (FluorPen FP 100, Photon System Instruments, Ltd., Brno, Czech Republic) were measured on the topmost fully expanded leaf before harvest. The seedlings of all the accessions were harvested exactly 45 DAP. The total leaf area was measured using a leaf area meter (LI-3100 area meter, LI-COR, Lincoln, NE, USA). The shoot dry weight was recorded after oven drying at 60°C.

Estimation of indices to explore microbiome dependent and independent effect

Different indices were calculated to determine the microbiome influence on various parameters measured under control and chilling using the following five different equations. The Microbiome Response Index (MRI, Control or Chilling) was calculated using equations 1 and 2. Trait values in the microbial inoculation treatment are expressed as a percentage of that in control treatment. A positive value indicates a benefit from the microbial inoculation and *vice versa*. Equations 1 and 2 identify accessions that can benefit from microbial inoculation under control and chilling,

respectively. Chilling Tolerance Index without microbe inoculation (CTIm; Equation 3) was used to evaluate the microbiome-independent effect under chilling tolerance of each accession for a given trait. Chilling Tolerance Index with microbe inoculation (CTIM; Equation 4) represents the combined effect of microbiome-dependent and microbiome-independent chilling tolerance of each accession. Therefore, equation 5 (CTIM minus CTIm) values indicate the level of microbiome-dependent chilling tolerance for each trait. Accessions with positive values for a given trait are those that indicate an enhanced microbiome-dependent chilling tolerance.

$$\text{MRI Control (\%)} = \left(\frac{\text{Trait Control+Microbiome}}{\text{Trait Control}-\text{Microbiome}} \text{ x } 100\right)\text{-}100 \quad \rightarrow \text{ Equation 1}$$

$$\text{MRI Chilling (\%)} = \left(\frac{\text{Trait Chilling+Microbiome}}{\text{Trait Chilling}-\text{Microbiome}} \text{ x } 100\right)\text{-}100 \quad \rightarrow \text{ Equation 2}$$

$$\text{CTI No microbe inoc. (CTIm \%)} = \frac{\text{Trait Chilling}-\text{Microbiome}}{\text{Trait Control}-\text{Microbiome}} \text{ x } 100 \quad \rightarrow \text{ Equation 3}$$

$$\text{CTI Microbe inoc. (CTIM \%)} = \frac{\text{Trait Chilling+Microbiome}}{\text{Trait Control}-\text{Microbiome}} \text{ x } 100 \quad \rightarrow \text{ Equation 4}$$

$$\text{CTIM minus CTIm} = (\text{CTIM} - \text{CTIm}) \quad \rightarrow \text{ Equation 5}$$

Statistical analysis

An ANOVA was performed for all traits to estimate the impact of the microbiome (+/-) of control and chilling using the library (agricolae) in RStudio 3.6.1 (https://rstudio.com/). A split-plot with the randomized complete block design was followed to analyze the data, considering the microbiome as the main plot, accessions as the subplot under each temperature treatment. Means were separated using the least significant difference (LSD) when treatments and interactions were significant at $P \leq 0.05$.

Results

Field evaluation of sorghum accessions identified those with consistent chilling tolerance phenotypes in both KS and SD locations

The goal here was to evaluate a set of twenty sorghum accessions with varying levels of chilling tolerance at KS and SD field sites to identify those that show consistent chilling response phenotypes at both locations. Early planting soil and air temperatures were lower by 8°C and 7°C, respectively, compared to regular planting conditions in Kansas (Fig. S1). In South Dakota, early planting soil and air temperatures were lower by 9°C and 8°C compared to regular planting (Fig. S2). Early planting had a significant ($P<0.01$) negative impact on leaf area and shoot biomass (Table 1). In contrast, the lack of a significant effect of chilling stress on the emergence percentage was observed (Table 1). In South Dakota, early planting had a significant ($P <0.01$) negative impact on emergence percentage, leaf area, and shoot biomass (Table 2). Emergence in the early planting treatment ranged from 6 % to 62% and 4% to 34% in the regular planting conditions in Kansas. A similar trend was found in South Dakota, with early planting emergence ranged between 0 - 66% and 0 – 60% emergence in regular planting (Table 2). Under early planting conditions, the emergence percentage of PI576376 was significantly greater than PI533831, where all other accessions were statistically the same at the Kansas field site (Table 1). In South Dakota, accession PI656014 had emergence percentage significantly higher than any other accession (Table 2). However, accessions showed a significant difference among them for the leaf area across sites. Leaf area due to chilling stress conditions was reduced by >90% in all the accessions in Kansas and between 70-91% in South Dakota (Table 1, 2). Similar to emergence percentage, the leaf area of PI576376 was significantly greater than PI533831 under early planting conditions in Kansas. The leaf area of PI656014, PI576376, and PI642998 were significantly higher than other accessions in South Dakota (Table 2). Shoot biomass was significantly ($P<0.001$) affected by chilling stress. However, there was no significant difference in seedling shoot biomass among sorghum accessions . On average, seedling shoot biomass across accessions was reduced by 96-98% with early planting over regular planting in Kansas and 78-92% in South Dakota (Table 1, 2). However, shoot biomass per row of PI576376 and PI642998 was significantly greater than nine of the other accessions under early planting conditions, in Kansas (PI566819, PI533755, PI533831, PI597971, PI642992, PI655991, PI656023, PI656035, PI656121, Table 1). In early planting in South Dakota, PI656014 had significantly greater shoot biomass per row than PI655991 and PI597971 (Table 2). All field physiological measurements showed a significant treatment x accession interaction (Table 1, Table 2).

When accessions were ranked based on shoot biomass per row, five accessions (PI534117, PI642998, PI656014, PI565019 and PI576376) consistently ranked among the top six at both Kansas and South Dakota. Similarly, PI656121, PI597971, and PI566819 performed consistently poorly at both Kansas and South Dakota, showing lower shoot biomass per row. These eight accessions and the chilling sensitive check RTx430 were evaluated for chilling tolerance in the presence/absence of microbiomes in in a controlled environment growth chamber experiment (Table 1 and 2, accessions are underlined).

Sorghum accessions tested had varying levels of microbiome-dependent chilling tolerance

The goal here was to evaluate if microbiomes contributed at least in part to chilling tolerance phenotypes exhibited by these accessions. Chilling stress significantly reduced all plant growth traits evaluated irrespective of the presence or absence of microbiomes and the extent of reduction varied significantly between accessions. Chlorophyll index was not significantly affected by the microbiome treatment in both control and chilling conditions (Supplementary Table 1). However, accessions differed significantly within temperature treatments, with significant interaction between accession and microbiome treatment was observed only under control conditions ($P<0.001$) (Supplementary Table 1). Quantum Yield was significantly affected by the microbiome ($P<0.01$-0.05), accession ($P<0.001$), and their interaction ($P<0.001$) both under control and chilling conditions (Supplementary Table 1).

Leaf area was significantly affected by microbiome ($P<0.05$), accession ($P<0.01$) and their interactions ($P<0.001$) under both control and chilling conditions (Supplementary Table 1). In the microbiome inoculation treatment, PI566819, PI642998, PI656019, and PI656121 had significantly ($P<0.05$) higher leaf area under the control condition (Fig. 1A). In contrast, the microbiome inoculation reduced ($P<0.05$) the leaf area under chilling stress in PI597971, PI642998, and PI656019 (Fig. 1B). Assessment based on microbiome response index suggested that microbiome inoculation had a positive impact on leaf area both under control and chilling stress conditions for accessions PI534117, PI656014, PI656121 (Fig. 3C). The microbiome inoculation promoted leaf area under control conditions but reduced leaf area under chilling stress conditions in PI566819, PI597971, PI642998, PI656019 and RTx430 (Fig. 3C). In one accession, PI576376, the microbiome inoculation reduced the leaf area under both control and chilling stress (Fig. 1C).

Similarly, seedling shoot biomass was significantly affected by inoculation treatment ($P<0.05$), accession $P<0.01$) and their interactions ($P<0.001$) under control condtions. In the chilling condition, seedling biomass was only affected by accession (Supplementary Table 1). On average, shoot biomass across accessions with microbiome inoculation was increased by 53% in control and 17.5% in chilling stress conditions (Fig. 1D, E). The microbiome inoculation promoted shoot biomass under control conditions, but reduced biomass under chilling stress conditions in PI534117, PI566819, PI597971, PI642998 and PI656019 (Fig. 1F). Whereas the microbiome inoculation had a positive impact on biomass both under control and chilling stress conditions in PI656014, PI656121 and RTx430 (Fig. 1F). On the other hand, microbial inoculation had a positive effect on leaf area and shoot biomass under both control and chilling stress conditions in PI656014 and PI656121 (Fig. 3C, F). The microbiome had a largely neutral or negatve effect on cholorophlly content and Quantum Yield (Fig. S3).

CTIM minus CTIm values indicate the level of microbiome-dependent chilling response for each of the measured traits (Fig. 2). A positive microbiome-dependent chilling tolerance was observed for chlorophyll index in three accessions PI576376, PI642998, PI656014. Three accessions, PI534117, PI656014 and PI656121, displayed enhanced microbiome-dependent chilling tolerance for leaf area. For shoot biomass, PI576376, PI656014 PI656121, and RTx430 showed microbiome-dependent chilling tolerance (Fig. 2). No positive responses were observed for QY, although PI576376, PI656014, and PI642998 had the least negative response to microbial inoculation (Fig. 2). Apart from QY, one accession (PI656014) showed positive microbiome-dependent chilling tolerance for all other traits. All other accessions that showed microbiome-dependent chilling tolerance rank as follows: PI656121 positive index for leaf area, shoot biomass; PI576376 positive index for shoot biomass, chlorophyll; RTx430 positive index for shoot biomass; PI642998 positive index for chlorophyll; PI534117 positive index for leaf area.

Discussion

Early season chilling stress is a yield-limiting factor for sorghum in temperate climates. Tapping into sorghum genetic resources and microbes that enhance chilling tolerance could expand solutions for such yield limitations. This study identified sorghum accessions with microbiome-dependent responses to chilling stress. Overall, sorghum accession response to temperature treatments was accession-specific in both field and growth chamber experiments. In the field, early

planting imposed significant chilling stress on sorghum plants, resulting in an overall 70-90% decrease in shoot biomass and leaf area compared to regular planting. A similar trend was found for sorghum in the growth chamber temperature treatments in the current and previous studies (Chiluwal et al. 2018, Moghimi et al. 2019, Ostmeyer et al. 2020).

The performance of sorghum accessions was broadly similar across the field sites. Generally, accessions with higher shoot biomass, leaf area, and percent emergence in early planting in Kansas also tended to have higher trait values in South Dakota. Sorghum accessions that had the highest leaf area and shoot biomass in early planting conditions also tended to perform better in growth chamber chilling stress in the absence of microbial inoculation (PI577356, PI642998). This suggests that microbial inoculations can have a negative growth impact on some accessions. Interestingly, these genotypes performed much worse in growth chamber chilling stress with microbial inoculation.

PI656014 and PI656121 showed a positive index for microbiome-dependent chilling tolerance in the growth chamber (Fig. 4), however, these accessions were neither the best nor worst-performing accessions in the field. For example, PI576376 had the highest shoot biomass in early planting in Kansas and the fourth highest in South Dakota, and showed a positive shoot biomass response to microbial inoculation in chilling stress. The accessions with greater shoot row biomass in early planting field conditions (PI656014, PI576376) also had positive microbiome-dependent chilling trait responses in the growth chamber experiment (PI576376: biomass, chlorophyll, PI656014: biomass, chlorophyll, leaf area). This suggests that some accessions will be amenable to chilling tolerance enhancements via microbial inoculants.

Plants are thought to primarily obtain their microbiomes from the soil (Beirinckx et al. 2020). The microbial vs. control inoculation treatments in the growth chamber experiment likely created a large difference in soil microbial diversity available to the plant in the growth chamber experiment. The sterilized potting media should harbor very few bacteria and fungi, while soil-extracted inoculum added diverse soil microbial taxa to the potting media. The temperature has been shown to shift bacterial and fungal community composition in soils and plants (Beirinckx et al. 2020, Cloutier et al. 2020), and we would assume such differences occurred in the field and growth chamber plant microbiomes. In this study, we focus on the plant physiological outcome of these microbial treatments to assess the potential for microbial-enhanced chilling tolerance in sorghum.

To our knowledge, this is the first study to compare microbial inoculation treatments across plant genotypes under chilling stress. We observed diverse responses among sorghum accessions to microbial inoculation that were temperature condition dependent. For example, most accessions had increased in leaf area and shoot biomass with microbial inoculation under control (30ºC/20ºC, day/night) temperatures, with 3 and 4 accessions exhibiting a statistically significant response in leaf area and shoot biomass, respectively (Fig. 1A, D). Under chilling stress (20ºC/15ºC, day/night), a positive effect of microbial inoculation on at least one trait is observed qualitatively in six accessions out of nine. PI656014 performed best under chilling stress: three out of four traits had higher values with microbial inoculation. Other accessions showed different combinations of traits that respond positively to microbe inoculation under chilling stress. Overall, the diverse trait response of accessions to microbial inoculation in the growth chamber under different temperature conditions suggests that microbes may enhance plant chilling tolerance via genotype-specific mechanisms.

Although few other studies use a diverse microbiome, several studies have tested the effect of individual microbial taxa on plants under chilling stress. These studies show similar plant biomass effects to our study, where the plant growth-promoting bacteria increase the biomass of the inoculated plant relative to non-inoculated plant under chilling stress, while the microbe-inoculated plants in the control temperatures result in an even greater plant biomass increase relative to non-inoculated plants (Barka et al. 2006, Subramanian 2016, Wang et al. 2016). Therefore, further exploration of individual or consortia of microbes and genetic loci responsible for their microbial recruitment and benefit have great promise to enhance chilling tolerance in sorghum.

Conclusion

Pinning down microbial effects on plant phenotype can be difficult due to context-dependent responses and variation in experimental microbial communities. In spite of this variability, we observe accession-specific positive and negative responses to high and low microbial diversity treatments under chilling stress temperatures, which we have termed the microbiome-dependent chilling stress response. Follow-up studies will screen microbial taxa with the diverse panel of sorghum accessions for microbe taxa impact on accession chilling tolerance in the growth chamber

and field experiments. Candidate taxa and sorghum accessions will be assessed for the genetic basis of microbiome-dependent chilling tolerance.

Table 1. Kansas. Emergence percentage, leaf area, and shoot biomass of sorghum accessions grown in the Kansas field site. Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Accessions with different letters are significantly different at P = 0.05 within respective planting conditions. "-": no seedling emergence. Accessions with underlines are those selected for microbiome experiment.

Accession	Emergence (%)			Leaf area (cm^2)			Shoot biomass (g)			Shoot biomass per row (g)		
	Regular	Early	% change	Regular	Early	% change	Regular	Early	% change	Regular	Early	% change
PI533755	14.58cde	20.62efg	-41.43	1296.29d	156.86ab	87.90	29.19abcd	0.81a	97.23	202.31cde	9.74bc	95.19
PI533831	4.17e	6.87g	-64.75	2532.90bc	58.40b	97.69	20.27d	0.29a	98.57	40.73e	1.05c	97.42
PI540816	9.11de	-		2672.02bc	-		40.86a	-		194.37cde	-	
PI655978	-	-		-	-		-	-		-	-	
PI655996	-	-		-	-		-	-		-	-	
PI534117	16.49bcd	54.25abc	-228.99	1914.25cd	156.12ab	91.84	36.81ab	0.85a	97.69	294.89bcd	22.46ab	92.38
PI566819	16.32bcd	31.11cdef	-90.63	3097.40ab	118.14ab	96.19	34.49abc	0.45a	98.70	310.12bcd	8.18bc	97.36
PI576376	18.58bcd	62.57a	-236.76	2474.75bc	167.77a	93.22	26.27abcd	0.83a	96.84	238.12bcde	26.16a	89.01
PI576394	11.46de	28.57defg	-149.30	2413.82bc	139.00ab	94.24	29.08abcd	0.77a	97.35	156.82de	11.36abc	92.76
PI597971	23.96abc	19.17fg	19.99	2591.99bc	95.40ab	96.32	34.02abcd	0.53a	98.44	383.44bc	4.95c	98.71
PI642992	18.06bcd	38.34bcdef	-112.29	3099.80ab	93.43ab	96.99	26.93abcd	0.48a	98.22	232.93bcde	9.23bc	96.04
PI642998	8.85de	54.62abc	-517.18	2572.24bc	151.84ab	94.10	36.68ab	0.84a	97.71	179.52cde	25.51a	85.79
PI651496	19.10bcd	44.49abcde	-132.93	3613.29a	97.58ab	97.30	36.06ab	0.54a	98.50	319.18bcd	11.69abc	96.34
PI655991	17.19bcd	36.89bcdef	-114.60	3065.34ab	103.78ab	96.61	36.30ab	0.51a	98.60	300.15bcd	9.32bc	96.89
PI656014	22.92bc	42.32abcdef	-84.64	2024.61cd	125.46ab	93.80	22.14cd	0.63a	97.15	239.06bcde	14.22abc	94.05
PI656019	23.96abc	56.79ab	-137.02	2593.99bc	114.10ab	95.60	36.10ab	0.63a	98.25	425.29ab	19.29abc	95.46
PI656023	19.44bcd	42.68abcdef	-119.55	3103.51ab	80.44b	97.41	38.80ab	0.48a	98.76	361.51bcd	9.87bc	97.27
PI656035	34.03a	21.34efg	37.29	2973.10ab	91.58ab	96.92	37.26ab	0.45a	98.79	600.80a	4.66c	99.22
PI656038	16.15bcd	49.91abcd	-209.04	2418.47bc	121.33ab	94.98	25.44bcd	0.62a	97.56	192.67ede	15.49abc	91.96
PI656121	26.39ab	22.79efg	13.64	2064.30cd	90.81ab	95.60	28.59abcd	0.51a	98.22	379.74bc	5.57c	98.53
Treatment (T)	NS			< 0.001			< 0.001			< 0.01		
Accession (A)	< 0.001			< 0.01			NS			< 0.01		
T x A	< 0.001			< 0.001			NS			< 0.01		

Table 2. South Dakota. Emergence percentage, leaf area, and shoot biomass of sorghum accessions grown in the South Dakota field site. Accessions with different letters are significantly different at P = 0.05 within respective planting conditions. "-": no seedling emergence. Accessions with underlines are those selected for microbiome experiment.

Accession	Emergence (%)			Leaf area (cm^2)			Shoot biomass (g)			Shoot biomass per row (g)		
	Regular	Early	% change	Regular	Early	% change	Regular	Early	% change	Regular	Early	% change
PI533755	21.95ef	14.03fgh	36.09	731.43cdef	120.72bcdef	83.50	7.19bcdef	1cde	86.18	74.22cde	7.02ab	90.55
PI533831	14.2fg	6.61gh	53.49	546.31def	-	-	4.89def	-	-	31.88de	-	-
PI534117	33.83cde	24.92cdef	26.34	857abcd	198.32ab	76.86	9.42abcd	1.73abc	81.70	151.79abcde	22.63ab	85.09
PI540816	0.17g	0.83h	-400.00	-	-	-	-	-	-	-	-	-
PI566819	55.95ab	21.04defg	62.39	909.38abcd	84.31ef	90.73	8.41abcd	0.7e	91.74	233.14abcde	5.81b	97.51
PI576376	61.23a	22.12def	63.88	987.08abcd	214.3a	78.29	11.36ab	1.73abc	84.77	328.95ab	18.45ab	94.39
PI576394	42.08bcd	23.77def	43.53	666.51cdef	107.27def	83.91	7.98abcde	0.75de	90.66	162.75abcde	9.2ab	94.35
PI597971	64.2a	19.64defg	69.41	736.33bcde	66.5f	90.97	5.94cdef	0.55e	90.78	181.82abcde	4.85b	97.33
PI642992	56.44ab	39.61bc	29.82	1055.16abc	112.72cdef	89.32	7.6bcdef	0.74de	90.36	204.03abcde	14.64ab	92.83
PI642998	54.96ab	31.52bcde	42.64	1200.57a	238.96a	80.10	12.48a	1.86ab	85.11	364.83a	28.43ab	92.21
PI651496	53.47ab	41.42b	22.53	838.84abcd	83.62ef	90.03	7.5abcdef	0.61e	91.90	194.28abcde	12.64ab	93.49
PI655978	0.5g	16.09efgh	-3150.00	-	-	-	-	-	-	-	-	-
PI655991	32.51de	15.6efgh	52.03	653.32cdef	103.77def	84.12	5.64cdef	0.85de	84.92	88.81bcde	4.74b	94.66
PI655996	0.67g	-	-	222.46ef	-	-	1.95ef	-	-	1.03e	-	-
PI656014	65.85a	60.4a	8.27	813.2abcde	246.7a	69.66	11.21ab	2.08a	81.49	355.79a	40.9a	88.50
PI656019	51.99ab	33.34bcd	35.87	801.7abcde	184.9abcd	76.94	7.16bcdef	1.59abcd	77.89	178.82abcde	25.06ab	85.98
PI656023	52.65ab	27.07bcdef	48.59	870.47abcd	191.79abc	77.97	9.92abc	1.72abc	82.67	252.85abcd	22.44ab	91.13
PI656035	66.34a	22.12def	66.67	922.25abcd	87.81ef	90.48	8.4abcd	0.73e	91.38	253.95abcd	7.72ab	96.96
PI656038	58.09ab	23.11def	60.23	1131.13ab	166.27abcde	85.30	10.23abc	1.21bcde	88.22	282.36abc	15.27ab	94.59
PI656121	51abc	27.73bcdef	45.63	655.65cdef	63.59f	90.30	6.83bcdef	0.68e	90.03	163.08abcde	9.07ab	94.44
Treatment (T)	0.008			<0.001			<0.001			0.0004		
Accession (A)	0.001			<0.001			<0.001			<0.0001		
T x A	0.001			<0.001			<0.001			<0.0001		

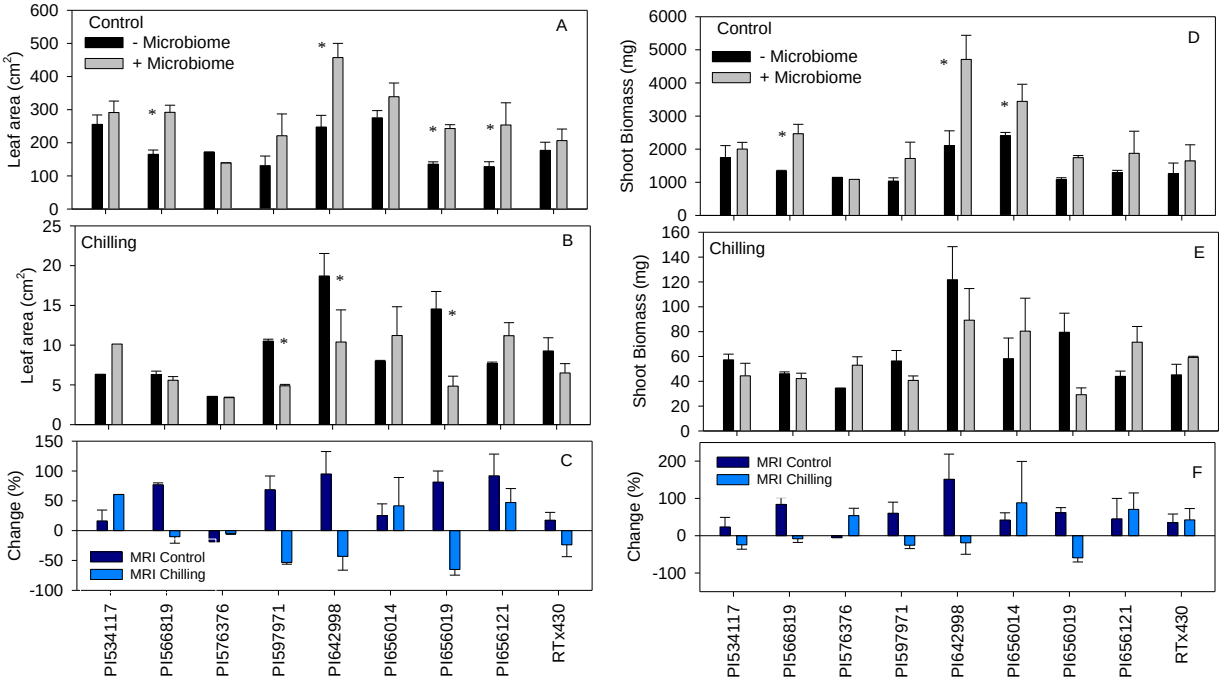


Figure 1. Leaf area (A-C) and shoot biomass (D-F) of sorghum accessions at 45 DAP with and without microbe inoculation under control (A and D; 30ºC/20ºC, day/night) and chilling stress (B and E; 20ºC/10ºC, day/night). C and F Microbiome Response Index (MRI) indicates the percentage change with and without microbe inoculation under control and chilling conditions. * represents a significant difference between treatments (microbiome and accession) at 5%.

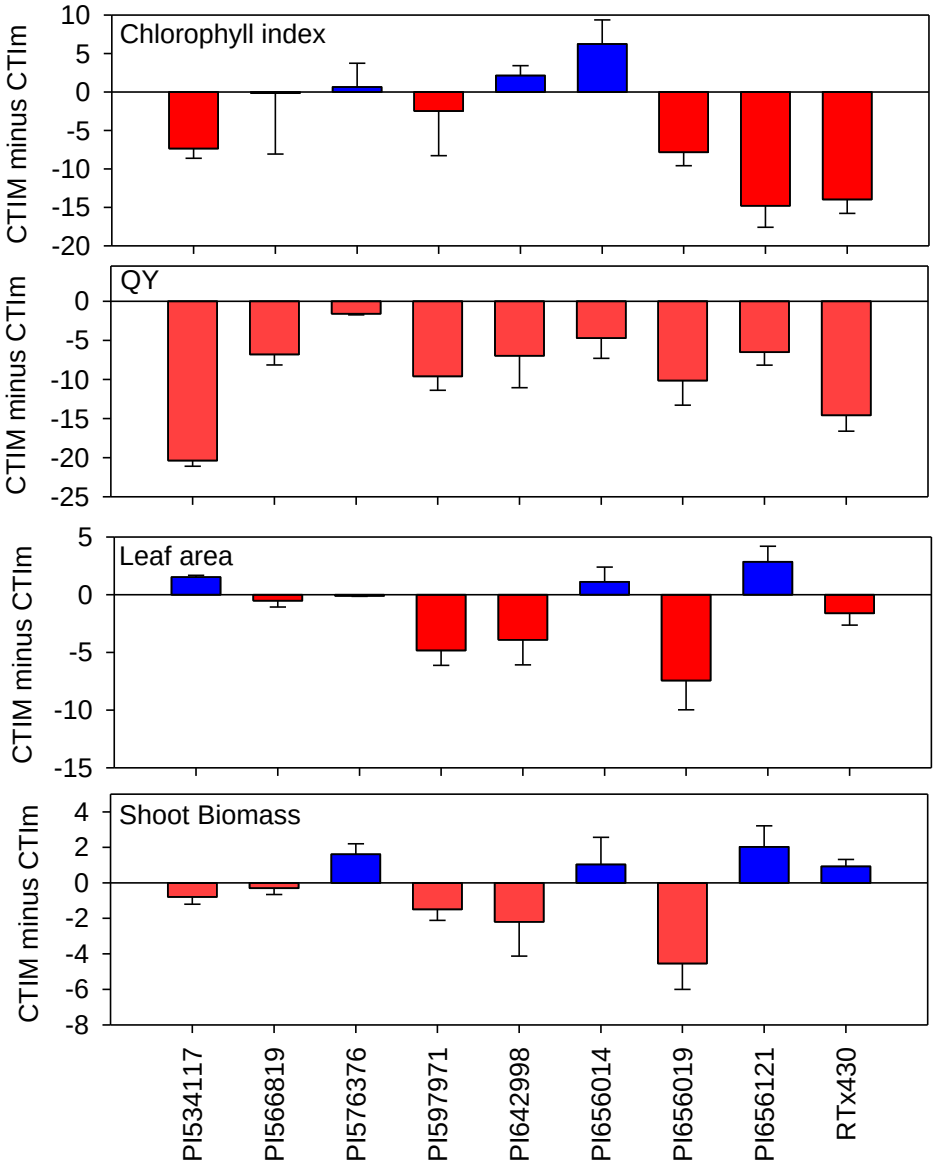
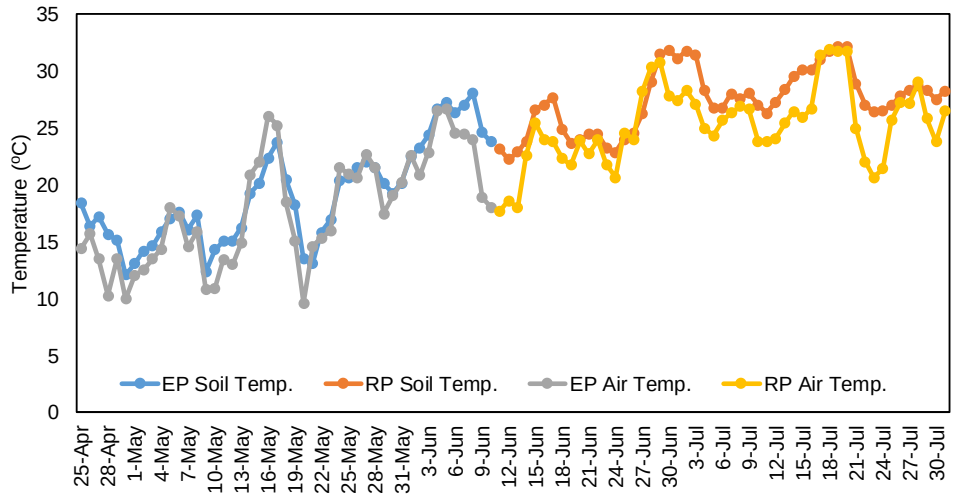
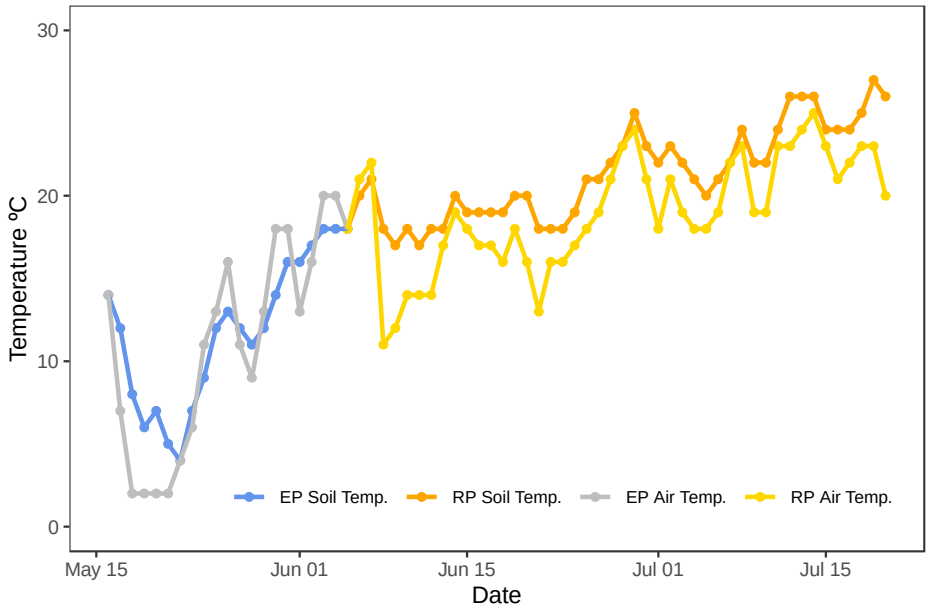


Figure 2. CTIM minus CTIm values for chlorophyll index, QY, leaf area and shoot biomass for nine sorghum accessions evaluated under growth chamber conditions. Accessions with blue are the ones showing microbiome-dependent chilling tolerance for traits and red indicates *vice versa*. CTIm -Chilling Tolerance Index with no microbe inoculum; CTIM -Chilling Tolerance Index with the microbe inoculum.

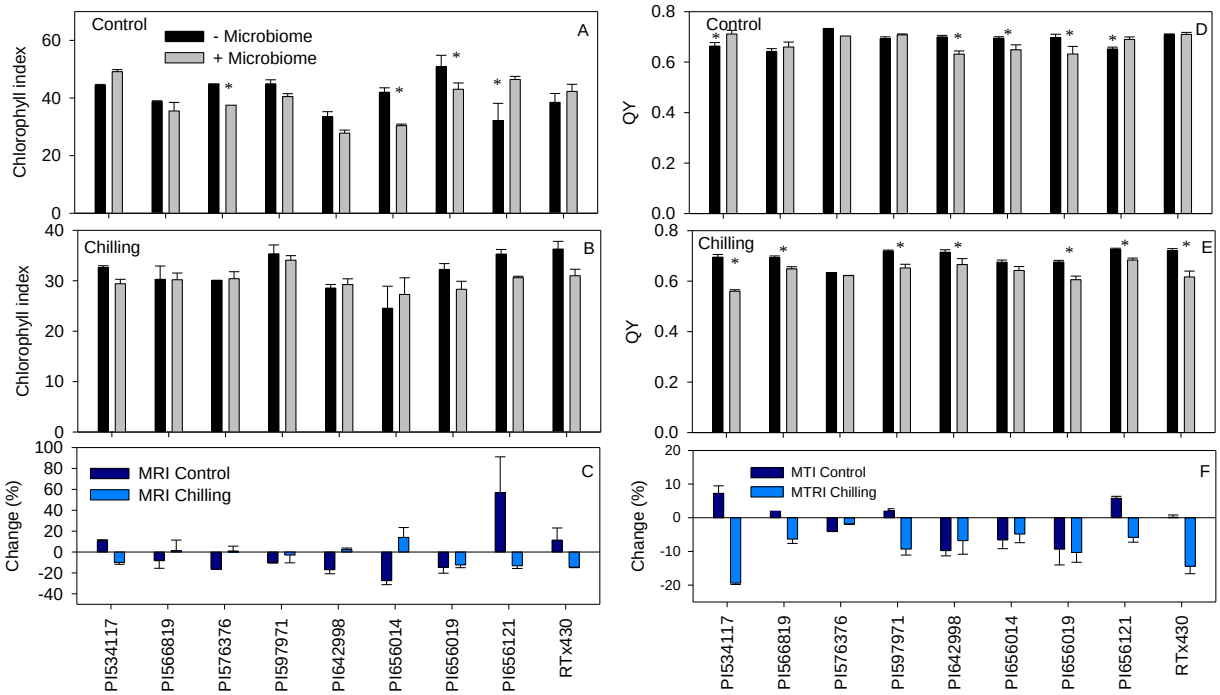
Supplementary information:



Supplementary Figure 1. Average daily air temperature and soil temperature in the field under early and regular planting conditions at the Kansas field site.



Supplementary Figure 2. Average daily air and soil temperature in the field under early and regular planting conditions at the South Dakota field site.



Supplementary Figure 3. Chlorophyll index (A-C) and quantum yield (D-F) of sorghum accessions at 45 DAP with and without microbial inoculation under control (A and D; 30ºC/20ºC, day/night) and chilling stress conditions (B and E; 20ºC/10ºC, day/night). C and F, Microbiome Response Index (MRI) indicates the percentage change with and without microbial inoculation under control and chilling conditions. * represents a significant difference between treatments (microbiome and accession) at 5%.

Supplementary Table 1: Summary of analysis of variance for growth chambers experiment. The split-plot analysis was performed for each temperature treatment separately. Significant at the <0.05 (*), p<0.01 (**) and <0.001 (***) probability level; NS, Non-significant.

	Control	**Chilling**
Chlorophyll index		
Microbiome (M)	NS	NS
Accession (A)	<0.001	<0.001
MxA	<0.001	NS
QY		
Microbiome (M)	<0.05	<0.01
Accession (A)	<0.001	<0.001
MxA	<0.001	<0.001
Leaf area (cm^2)		
Microbiome (M)	<0.05	<0.05
Accession (A)	<0.01	<0.001
MxA	<0.001	<0.001
Shoot biomass (mg)		
Microbiome (M)	<0.01	NS
Accession (A)	<0.001	<0.01
MxA	<0.05	NS

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