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Parental Environmental Effects on Seed Quality and Germination Response to Temperature of *Andropogon gerardii*

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Abstract: Parental environments (PEs) affect seed quality and might alter the re-establishment of big bluestem grass due to impacts on seed germination. An in vitro study was conducted to quantify the temperature response of seed germination and its interaction with the PE in big bluestem. Seeds developed under eight PEs consisting of a combination of four day/night growth temperatures (GTs) (20/12, 25/17, 30/22, and 35/27 °C) and two CO_2 levels (360 and 720 µL L^{-1}) were germinated at eight temperatures (germination temperatures (GRTs)) ranging from 10 to 42.5 °C. Quadratic and modified bilinear regressions best described the cardinal temperatures for the estimated maximum seed germination (MSG) and seed germination rate (SGR), respectively. The average MSG and SGR showed differential responses to the PEs and significantly declined above a 35 °C GRT across the PEs. For the SGR, the minimum and optimum temperatures showed significant differences from other treatments but the opposite response to elevated CO_2, while maximum temperatures significantly declined at high (35/27 °C) and low GTs (20/12 °C). Seed quality parameters, individual seed weight, and C and N contents showed a high correlation (R^2 > 60) with the average percentage of seed germination and the SGR. Thus, high temperatures for both the PEs (>30/22 °C) and GRTs (>30 °C) could significantly reduce germination, affecting the re-establishment of big bluestem.

Keywords: changing climate; C_4 grass; growth temperatures; germination temperatures; parental environments; thermal

1. Introduction

The maternal growing environment affects not only plant productivity but also seed quality traits of C_3 and C_4 crops [1,2]. Quality seed production is crucial for the initial establishment of plants in the natural environment, such as prairies, where big bluestem establishes naturally from seeds and spreads through vegetative growth [3]. Results from a previous study showed that non-optimal temperature decreased big bluestem seed production regardless of carbon dioxide (CO_2) enrichment [4]. Seed quality is determined by parental environments during seed development and maturation [1]. The

growing temperature strongly affects the rate and duration of the seed filling period, thus affecting seed mass and composition, such as nitrogen and carbon contents [5,6]. Also, elevated CO_2 has been found to decrease seed constituents, such as mineral elements and organic compounds [7]. The current levels of global mean surface air temperature and atmospheric CO_2 are likely to exceed 1.1 to 5.4 °C, depending on representative concentration pathway scenarios, by the end of the 21st century [8]. Huxman et al. [9] found an increased C/N ratio in elevated-CO_2-developed *Bromus rubens* L. grass seeds, which resulted in reduced seed quality and seedling performance. Moreover, Hampton et al. [10] suggested that elevated CO_2 during seed development might produce greater ethylene, which induces early seed germination in many species. The adverse impacts of high temperatures on seeds during development might be attributed to the limited supply of photosynthetic assimilates [3,11] and physiological damage leading to a loss of seeds' ability to germinate [1]. Thus, the combined impacts of temperature and elevated CO_2 are likely to alter seed traits essential for initial plant establishment.

Seed mass, composition, viability, germination, and seedling vigor are some useful seed quality indicators for the establishment purposes of plants [12,13]. Seed germination characteristics are easy to determine under a laboratory set-up and have been widely exploited for the selection of cultivars with environmental stress tolerance in several crops, including rice [14], cotton [15], soybeans [16], peppers [17], and switchgrass [18]. Seed germination studies have evaluated the percentage of germination, the germination rate, and three cardinal temperatures (the minimum, maximum, and optimum) that govern the temperature range across which germination can occur. These germination matrices are also useful in evaluating the impacts of maternal environmental conditions on seed quality traits [1,6,10,19]. Since temperature and CO_2 fluctuations are anticipated influencing crop productivity under natural settings, evaluation of seed germination characteristics at a range of temperature regimes would provide insight into the adaptability of big bluestem and a possible link to maternal environments.

Big bluestem (*Andropogon gerardii* Vitman) is a dominant perennial warm-season C_4 grass species that contribute ≈80% of the biomass in the natural and managed grasslands of the United States [20]. However, big bluestem requires cold temperatures to break down seed dormancy for quick re-establishment in grassland regions [21,22]. Rincker [23] reported favorable germinability of C_4 grass seeds when stored in cold temperatures. Decreased seed germination has been found in native warm-season grasses when seeds were stored at 20–30 °C [24]. Although a favorable impact of elevated CO_2 on the productivity of C_4 grasses is limited [25], it might promote the seedling establishment, especially in water-limited environments [26,27]. Studies evaluating the impacts of maternal environments, such as temperature and CO_2, on seed quality, including germination, have been extremely limited and are not available for big bluestem [28].

Moreover, the seed germination adaptability range, as defined by cardinal temperatures for big bluestem, is still unclear. The present study was built upon our previous work, where big bluestem was grown to maturity under five temperature regimes (mean T between 17 °C and 36 °C) across ambient and elevated CO_2 [4]. This provided a unique opportunity to evaluate the impacts of paternal environments as well as to conduct germination trials across temperatures to determine the cardinal points, which defined the adaptability range for seed germination. We hypothesized that seeds developed (maternal environment, irrespective of CO_2 enrichment) and germinated (regardless of the maternal environment) under high temperatures would have poor germination characteristics. The objectives of this study were to evaluate the impacts of maternal environments on big bluestem seed germination characteristics and to quantify seed germination in response to temperature. The relationship between seed germination characteristics and seed mass, N, and carbon contents were also evaluated.

2. Materials and Methods

2.1. Parental Experimental Details

Seed material for this study was obtained from plants grown in controlled environmental conditions using a sunlit, soil-plant-atmosphere research (SPAR) facility [29] at the Rodney Foil Plant Science Research Center (38°28′ N, 88°47′ W), Mississippi State University, Mississippi, USA. Ten different treatment combinations of five levels of day/night growth temperature treatments (20/12, 25/17, 30/22, 35/27, and 40/32 °C) and two levels of carbon dioxide (CO_2) (360 and 720 μL L^{-1}) were randomly imposed in 10 SPAR units at the time of the first true leaf stage. At the final harvest, 125 days after emergence (DAE), total dry weight per plant, and seed weight per plant were recorded. Because the SPAR units were not statistically different for the sorghum growth traits observed in the previous study, which was conducted to test uniformity among SPAR units [4], individual parent plants grown in a given SPAR unit were used as replicates for statistical analysis. No seeds were produced on the plants grown at 40/32 °C across CO_2 concentrations. The details of parental environmental conditions have been described by Kakani and Reddy [4].

2.2. Seed Quality Parameters

The seeds developed in the eight parental environments (PEs) were collected to estimate seed quality parameters, including individual seed weight and the concentration (%) and content (mg $seed^{-1}$) of seed carbon (C) and nitrogen (N). The weight of 100 individual seeds was recorded for each PE before the seed germination experiment. One gram of seeds was ground, homogenized, and sieved, and the material was analyzed for C and N concentrations using an automated carbon and nitrogen (C/N) combustion analyzer (Perkin Elmer 2400; Perkin Elmer Corp., Norwalk, CT, USA; software: Eager 300 ver. 1.01) at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Laboratory, Mississippi State, Mississippi State, USA. Endogenous seed reserves (seed N and C contents) were then calculated by multiplying the individual seed weight by the seed C and N percentage. Three individual parent plants were harvested from a given SPAR unit and were used as replicates in statistical analysis for the respective parental environment treatments.

2.3. Seed Germination Experimental Details

2.3.1. Seed Material

Seeds from the eight parental environments, a combination of four different levels of growth temperatures (GTs) (20/12, 25/27, 30/22, and 35/27 °C) and two carbon dioxide (CO_2) levels (360 and 720 μL L^{-1}), were numbered from PE 1 to PE 8 (described in Table 1) and were used to determine seed germination characteristics. Initially, seeds were treated with fungicide captan{cis-N-[(trichloromethyl)thio-4-cyclohexene-1,2-dicarboximide]} at a rate of 2.5 g kg^{-1} seed to avoid any fungicidal infection and were stored in cold conditions (14 days at 5 °C) to maintain quality and to break down dormancy (if any) before subsequent testing.

2.3.2. Seed Germination Time Course Concerning Temperature Treatments

The germination test was conducted under in vitro conditions and consisted of two-factor treatments (8 PEs × 8 levels of germination temperatures (GRTs)) and four replications arranged in a completely randomized design. Four individual parent plants were harvested from a given SPAR unit and were used as replicates in statistical analysis for respective parental environment treatments. The eight levels of GRTs ranged from 10 to 42.5 °C, with an increment of 5 °C until 40 °C, with the eighth level being 42.5 °C. They were maintained using a germination chamber (Fisher Scientific, Suwanee, GA, USA). One-hundred seeds of each of the eight parental environments (PEs) were placed on a double-layered paper towel moistened with distilled water and were then placed on a plastic tray. The plastic trays were covered to prevent moisture loss and then vertically stacked in the germination

chamber, which was set to a constant germination temperature (GRT) treatment. At six-hour intervals from the time of incubation, seeds were examined for germination, filter papers were examined for moisture, and temperature treatment was monitored using data loggers (WatchDog Model 100, Spectrum Technologies, Inc., Aurora, IL, USA) placed in the germination chamber. Seeds with radicle length more than half of the seed length were considered germinated.

Table 1. Description of seed quality parameters, such as individual seed weight, nitrogen (N) and carbon percentages, and N and C content, obtained from plants harvested for each parental environment in the parental experiment.

PE	GT	CO_2	Weight	N	C	N Content	C Content
	(day/night °C)	(μL L^{-1})	(mg $seed^{-1}$)	——-(% $seed^{-1}$)——		——-(mg $seed^{-1}$)——	
1	35/27	720	1.443 ± 0.02	5.095 ± 0.15	50.155 ± 0.28	0.074 ± 0.01	0.724 ± 0.01
2	30/22	720	2.360 ± 0.03	5.422 ± 0.39	50.328 ± 0.13	0.128 ± 0.01	1.188 ± 0.01
3	25/17	720	2.510 ± 0.02	5.954 ± 0.45	50.183 ± 0.24	0.149 ± 0.01	1.259 ± 0.01
4	20/12	720	1.767 ± 0.02	6.431 ± 0.32	50.152 ± 0.40	0.114 ± 0.01	0.886 ± 0.02
5	35/27	360	1.537 ± 0.01	4.803 ± 0.51	49.693 ± 0.37	0.074 ± 0.01	0.764 ± 0.01
6	30/22	360	2.067 ± 0.07	5.780 ± 0.30	50.058 ± 0.69	0.119 ± 0.01	1.033 ± 0.02
7	25/17	360	2.327 ± 0.03	5.360 ± 0.30	49.717 ± 0.40	0.125 ± 0.01	1.157 ± 0.03
8	20/12	360	1.603 ± 0.05	5.792 ± 0.11	50.168 ± 0.35	0.093 ± 0.01	0.805 ± 0.03
Mean			1.952 ± 0.04	5.580 ± 0.34	50.057 ± 0.35	0.109 ± 0.01	0.977 ± 0.02
ANOVA †							
GT			***	*	NS	***	***
CO_2			***	NS	NS	*	***
GT × CO_2			***	NS	NS	NS	***

† Results of the analysis of variance (ANOVA), indicated as *, ***, and NS, representing significance levels at $p \leq 0.05$, $p \leq 0.001$, and $p \geq 0.05$ (NS-Nonsignificant), respectively. PE, parental environment; GT, growth temperature; CO_2, carbon dioxide; N, nitrogen; C, carbon. Data are means ± standard error (SE) of three plants ($n = 3$) harvested from a given soil-plant-atmosphere-research (SPAR) unit and used as replicates in statistical analysis for a respective parental environment.

2.3.3. Curve Fitting Procedure for Germination Time Course

The seed germination time course was analyzed using a three-parameter sigmoidal function (Equation (1)) in SigmaPlot 13 (Systat Software, Inc., San Jose, CA, USA) to estimate maximum seed germination (*MSG*), time to reach 50% germination (t_{50}), and steepness of the curve (G_{rate}) at each GRT and PE. The reciprocal of t_{50} was taken as the seed germination rate (SGR):

$$Y = MSG/\{1 + \exp[-(t - t_{50})/G_{rate}]\} \quad (1)$$

2.3.4. Determination of Cardinal Temperatures

The maximum seed germination (MSG) and seed germination rate (SGR) were fitted to the best regression models against germination temperatures (GRTs) to determine the cardinal temperatures for all the parental environments. Based on the highest coefficient of determination (R^2) and lowest root mean square error (RMSE), quadratic models (Equation (2)) best described MSG responses to the GRT (mean $R^2 = 0.72$ and mean RMSE = 14.02), while a modified bilinear model (Equation (3)) best described SGR responses to the GRT (mean $R^2 = 0.87$ and mean RMSE = 1.05), which was similar to previous seed germination studies conducted on various crops such as rice, switchgrass, corn, and ornamental pepper [14,17,18,30]. The regression constants for each replicate within each parental type were estimated using PROC NILN with a modified Newton–Gauss iterative procedure in SAS (SAS Institute Inc., Cary, NC, USA). Using a modified bilinear equation (Equation (3)), the SGR and the regression constants were further analyzed to estimate the optimum temperature (T_{opt}) in SAS using PROC NILN, while the minimum temperature (T_{min}) and maximum temperature (T_{max}) were estimated using Equations (4) and (5), respectively:

$$MSG = a + bT + cT2 \quad (2)$$

$$SGR = a + b_1 (T - T_{opt}) + b_2 \times ABS (T_{opt} - T) \quad (3)$$

$$T_{min} = [a + (b_2 - b_1) \times T_{opt}]/b_1 - b_2 \quad (4)$$

$$T_{max} = [a - (b_2 + b_1) \times T_{opt}]/b_1 + b_2 \quad (5)$$

where T is the germination temperature; and a, b_1, and b_2 are regression constants for each replicate within each parental type (obtained using the PROC NILN procedure in SAS).

2.4. Data Analysis

Replicated values of all of the observed and estimated parameters were analyzed using an ANOVA procedure in JMP Pro 12.0 (SAS Institute, Inc., Cary, NC, USA). Post-ANOVA means separations were determined using Fisher's least significant difference ($\alpha = 0.05$). Linear relationships were generated to determine the correlation between seed quality parameters and seed germination parameters using SigmaPlot 13.

3. Results

3.1. Seed Quality Parameters

There were significant effects of growth temperature (GT), carbon dioxide (CO_2), and their interaction on seed weight (Table 1). Both high and low GTs resulted in lower individual seed weights. However, elevated CO_2 increased individual seed weight by 8–14% across GTs, except at the highest GT (35/27 °C) (Table 1). The GT significantly affected seed N%, exhibiting greater values at lower GTs across CO_2 levels (Table 1). However, no substantial effects of parental environments (GT and CO_2) on seed C% were observed. The endogenous seed reserves (seed nitrogen and carbon content per seed) were significantly affected by both GT and CO_2 levels and followed a pattern similar to individual seed weight (Table 1).

3.2. Seed Germination Time Series

Figure 1 illustrates seed germination time series data for the optimum temperature (30/22 °C) across carbon dioxide (CO_2) levels. Seeds obtained from all parental environments (PEs) showed similar patterns. However, the initiation of seed germination and the time to reach the maximum germination were highly varied among germination temperatures (GRTs). For instance, seed germination started approximately within 3–4 days after incubation at and above a 25 °C GRT, whereas it took about eight days at 15 °C and ≈20 days at 10 °C GRT when averaged across the PEs. A three-parameter sigmoidal curve best described (averaged across GRTs and PEs, $R^2 = 0.98$) the seed germination time series data across GRTs and PEs. When plotted against the time course, the seed germination averaged across GRTs clearly showed a sigmoidal response across PEs (Figure 2). The germination time response patterns substantially varied among the PEs, such as for the shape of the curves and percentage germination (Figure 2).

3.3. Maximum Seed Germination

There was a significant effect of parental environment (PE), germination temperature (GRT), and their interaction on maximum seed germination (MSG) (Table 2). MSG percentages were not significantly different ($p > 0.5$) between 10 and 30 °C (>62% MSG), followed by a rapid and significant decline ($p < 0.001$), with an increase in each level of GRT from 35 to 42.5 °C (58% to 6% MSG, respectively) when averaged across the PEs (Figure 3). On average across GRTs, PEs 2, 3, 6, and 7 showed significantly higher MSGs (>60%) than PEs 1, 4, 5, and 8 (<46% MSG). During seed development, elevated carbon dioxide (CO_2) at a 30/22 °C growth temperature significantly ($p < 0.05$) increased MSG by 15.7% but decreased it at 20/12 °C by 30% when compared to ambient CO_2 levels. MSG was the highest for PE

2 at a 20 °C GRT and was lowest for PE 4 at a 42.5 °C GRT. Moreover, elevated CO_2 at 30/22 °C and 20/12 °C caused an increase and decrease in MSG, respectively, across all GRTs except 42.5 °C.

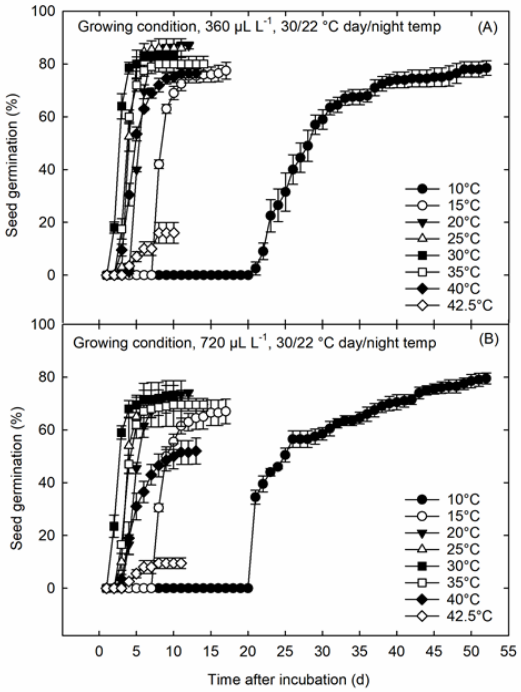


Figure 1. Big bluestem seed germination time course obtained from plants grown under two parental environments representing 30/22 °C day/night temperatures under (**A**) 360-µL L^{-1} and (**B**) 720-µL L^{-1} CO_2 concentrations. Data are means ± standard error (SE) of four plants (n = 4) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

Table 2. Description of eight different parental environments (PEs), CO_2 (µL L^{-1}), day/night growth temperature (*GT*), measured mean *GT* in the chamber, maximum seed germination percentage (*MSG*), temperature adaptability range for maximum seed germination (TAR_{MSG}), quadratic equation constants (a, b, and c), coefficients of determination (R^2), and cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for the MSG of big bluestem seeds grown from eight parental environments.

PE	*GT*	CO_2	Mean *GT*	*MSG*	TAR_{MSG}	Equation Constants			R^2	Cardinal Temperatures		
						a	*b*	*c*		T_{min}	T_{opt}	T_{max}
	(day/night, °C)	µL L^{-1}	(°C)	(%)						——————(°C)——————		
1	35/27	720	30.8	36.18	45.15	1.29	4.73	−0.11	0.83	−1.41	21.17	43.74
2	30/22	720	26.3	72.58	52.71	21.81	6.04	−0.13	0.62	−3.44	22.91	49.26
3	25/17	720	22.1	66.48	51.51	27.23	5.62	−0.13	0.70	−4.53	21.23	46.98
4	20/12	720	17.4	34.73	52.32	25.50	2.79	−0.08	0.86	−8.72	17.44	43.59
5	35/27	360	30.8	38.00	48.21	11.29	3.83	−0.09	0.66	−2.88	21.23	45.33
6	30/22	360	26.3	61.15	56.52	36.35	4.21	−0.10	0.66	−8.34	19.92	48.18
7	25/17	360	21.5	69.34	62.67	50.91	3.66	−0.09	0.61	−12.24	19.10	50.43
8	20/12	360	17.0	46.26	54.65	36.18	3.42	−0.10	0.82	−10.55	16.78	44.11
Mean				53.09	52.97	-	-	-	0.72	−6.51	19.97	46.45
ANOVA †			PE	***	*	-	-	-	-	NS	*	***
			GT	***	*	-	-	-	-	NS	**	***
			CO_2	NS	*	-	-	-	-	NS	NS	NS
			GRT	***	-	-	-	-	-	-	-	-
			PE × GRT	***	-	-	-	-	-	-	-	-

† Results of the analysis of variance (ANOVA), indicated as *, **, ***, and NS, representing significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and $p \geq 0.05$ (NS—nonsignificant), respectively. The dash ("-") sign indicates that the ANOVA or the given term was not assessed. PE, parental environment; GRT, germination temperature; MSG, maximum seed germination; TAR_{MSG}, temperature adaptability range; T_{min}, minimum temperature; T_{opt}, optimum temperature; T_{max}, maximum temperature; R^2, coefficient of determination.

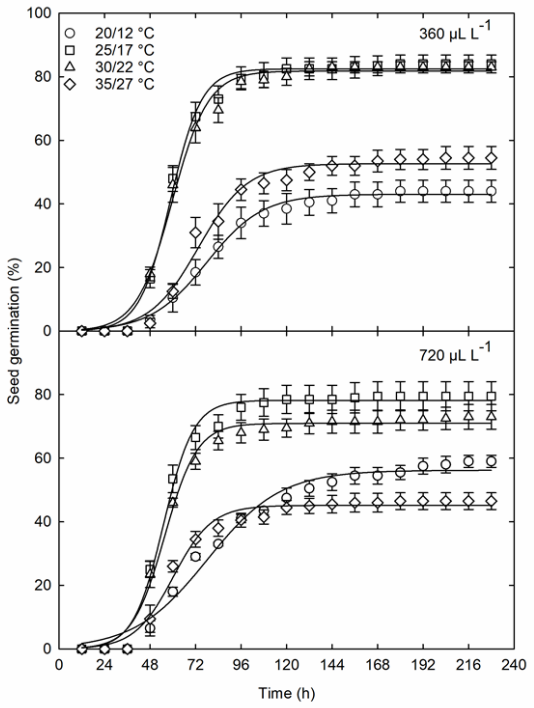


Figure 2. Germination time courses of eight parental environments when averaged across a range of germination temperatures (10–42.5 °C). Lines fitted through a three-parameter sigmoidal curve represent germination time course, and symbols represent germination data. Data are means ± standard error (SE) of four plants (n = 4) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

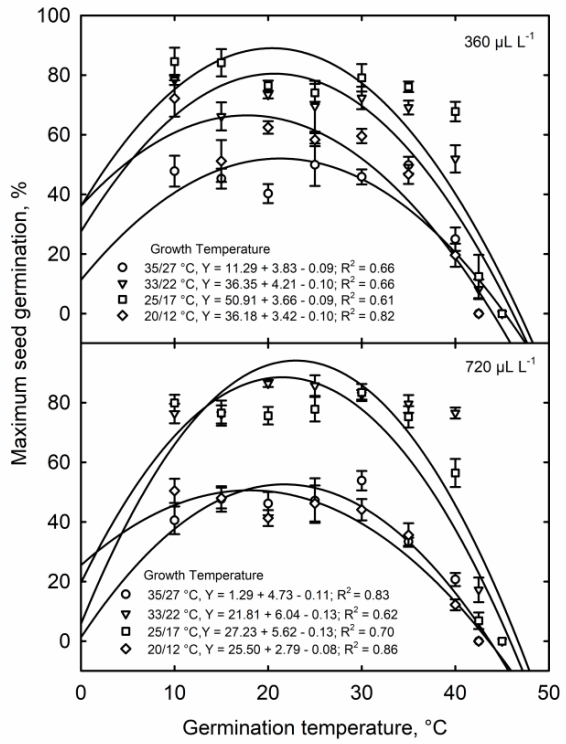


Figure 3. Effect of germination temperatures on the maximum seed germination of eight parental environments described by quadratic functions. Data are means ± standard error (SE) of four plants (n = 4) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

3.4. Seed Germination Rate

The seed germination rate (SGR) showed a positive linear increase with germination temperature (GRT) from 10 to 30 °C, followed by a rapid linear decline as GTR increased further to 42.5 °C across PEs (Figure 4). Averaged across parental environments (PEs), the highest (0.33 day^{-1}) and the lowest (0.03 day^{-1}) SGR were estimated under a GRT of 30 °C and 10 °C, respectively (Figure 4). The effects of the PE, GRT, and their interaction were also significant ($p < 0.01$) for the SGR (Table 3). Similarly to maximum seed germination (MSG), the SGR was also significantly higher for PEs 2, 3, 6, and 7 compared to PEs 1, 4, 5, and 8. The interaction of the parental environment with germination temperature was significant for SGR responses to temperatures in a manner where parental environments 6 and 7 under a 30 °C germination temperature had significantly the highest SGRs, and parental environments 4 and 8 under a 42.5 °C GRT had the lowest SGRs (Table 3).

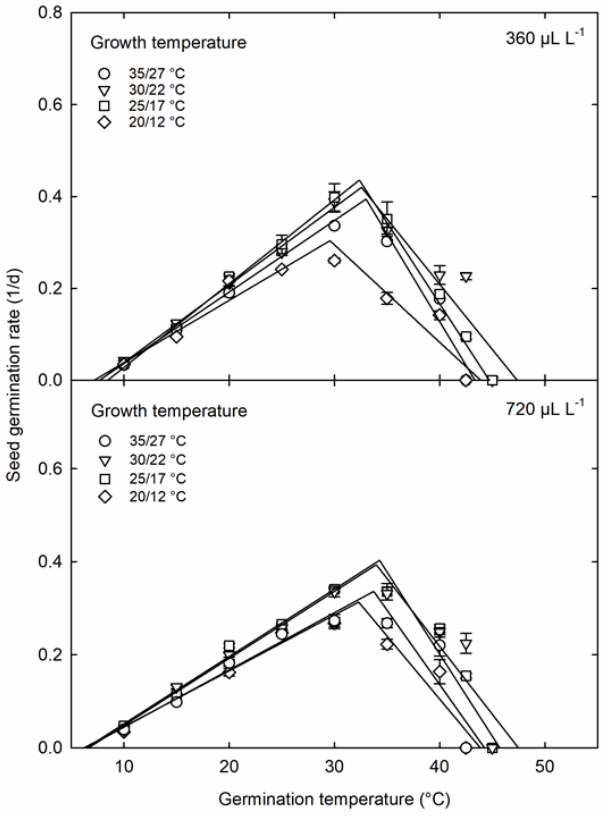


Figure 4. Effect of germination temperatures on the seed germination rate of eight parental environments described by modified bilinear functions. Data are means ± SE of four plants (n = 4) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

3.5. Cardinal Temperatures

Cardinal temperatures predicted for maximum seed germination (MSG) and the seed germination rate (SGR) using parameter estimates of quadratic (mean R^2 = 0.72 and mean RMSE = 14.02) and modified bilinear functions (mean R^2 = 0.87 and mean RMSE = 1.05), respectively, varied significantly between parental environments (Tables 1 and 2). For MSG, the average minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) temperatures estimated were −6.51, 19.97, and 46.45 °C, respectively (Table 2). The optimum temperature significantly differed between parental environments (PEs), ranging from PE 8 (16.7 °C) to PE 2 (22.9 °C) (Table 3), indicating that low growth temperatures (20/12 °C) during seed development could significantly lower the T_{opt} of MSG, with no effect ($p > 0.05$) of CO_2. The T_{max} for maximum seed germination also significantly differed between PEs, exhibiting the highest and lowest values under PE 7 (50.4 °C) and PE 4 (43.5 °C), respectively (Table 1). Further,

the study found that low (20/12 °C) and high (35/27 °C) growth temperatures during seed development could significantly lower the T_{max} of MSG, without a significant effect ($p > 0.05$) of growth CO_2. The temperature adaptability range for maximum seed germination (TAR_{MSG}), calculated as the difference between T_{max} and T_{min}, significantly varied between PEs, ranging from 45.15 °C for PE 1 to 62.67 °C for PE 7 (Table 2). TAR_{MSG} was the lowest at the highest growth temperature under both CO_2 levels. TAR_{MSG} was also lower under elevated CO_2 across growth temperatures (Table 2).

Table 3. Seed germination rate (*SGR*), temperature adaptability range for seed germination rate (TAR_{SGR}), modified bilinear equation constants (a, b, and c) and cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for the SGRs of big bluestem seeds grown from eight parental environments.

PE	*SGR*	TAR_{SGR}	Equation Constants			R^2	Cardinal Temperatures		
			a	b_1	b_2		T_{min}	T_{opt}	T_{max}
	(day^{-1})	(°C)					———(°C)———		
1	0.17	37.96	0.34	−0.01	−0.02	0.82	6.32	33.74	44.27
2	0.22	40.96	0.39	−0.01	−0.02	0.96	6.61	33.99	47.56
3	0.22	40.21	0.40	−0.01	−0.02	0.87	6.40	34.29	46.60
4	0.15	37.81	0.31	−0.01	−0.02	0.87	6.14	32.32	43.95
5	0.18	35.76	0.39	−0.01	−0.03	0.94	7.63	33.01	43.39
6	0.23	40.40	0.42	−0.01	−0.02	0.79	7.50	32.58	47.90
7	0.21	37.36	0.44	−0.01	−0.03	0.87	8.19	32.38	45.55
8	0.15	37.44	0.30	0.00	−0.02	0.86	6.78	29.58	44.22
Mean	0.19	38.49	-	-	-	0.87	6.94	32.74	45.43
ANOVA †									
PE	**	***	-	-	-	-	**	**	***
GT	***	***	-	-	-	-	NS	**	***
CO_2	NS	**	-	-	-	-	***	**	NS
GRT	***	-	-	-	-	-	-	-	-
PE × GRT	***	-	-	-	-	-	-	-	-

† Results of the analysis of variance (ANOVA), indicated as **, ***, and NS, representing significance at $p \leq 0.01$, $p \leq 0.001$, and $p \geq 0.05$ (NS—nonsignificant), respectively. The dash ("-") sign indicates that the ANOVA or the given term was not assessed. PE, parental environment; GRT, germination temperature; SGR, seed germination rate; TAR_{MSG}, temperature adaptability range; T_{min}, minimum temperature; T_{opt}, optimum temperature; T_{max}, maximum temperature; R^2, coefficient of determination.

For the seed germination rate (SGR), a significant effect of parental environment (PE) on cardinal temperature was observed (Table 3). The mean minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) temperatures across PEs were 6.94, 32.7, and 45.43 °C, respectively (Table 3). The maximum and minimum values of T_{min}, T_{opt}, and T_{max} for the SGR were observed for PEs 7 and 4, 3 and 8, and 6 and 5, respectively. Among the cardinal temperatures, T_{max} was significantly ($p < 0.001$; mean $R^2 = 0.95$) influenced by growth temperature (*GT*), whereas T_{min} and T_{opt} showed significant ($p < 0.001$; mean $R^2 = 0.74$) sensitivity to carbon dioxide (CO_2) (Figure 5). Similarly to the T_{max} of maximum seed germination, low (20/12 °C) and high (35/27 °C) GTs significantly lowered the T_{max} of SGR by 1.4 °C and 1.6 °C compared to the mean T_{max} of the PEs (Table 3 and Figure 5). Relative to ambient CO_2, elevated CO_2 levels significantly lowered T_{min} by 1.16 °C but raised T_{opt} by 1.7 °C when averaged across GTs (Table 3 and Figure 5).

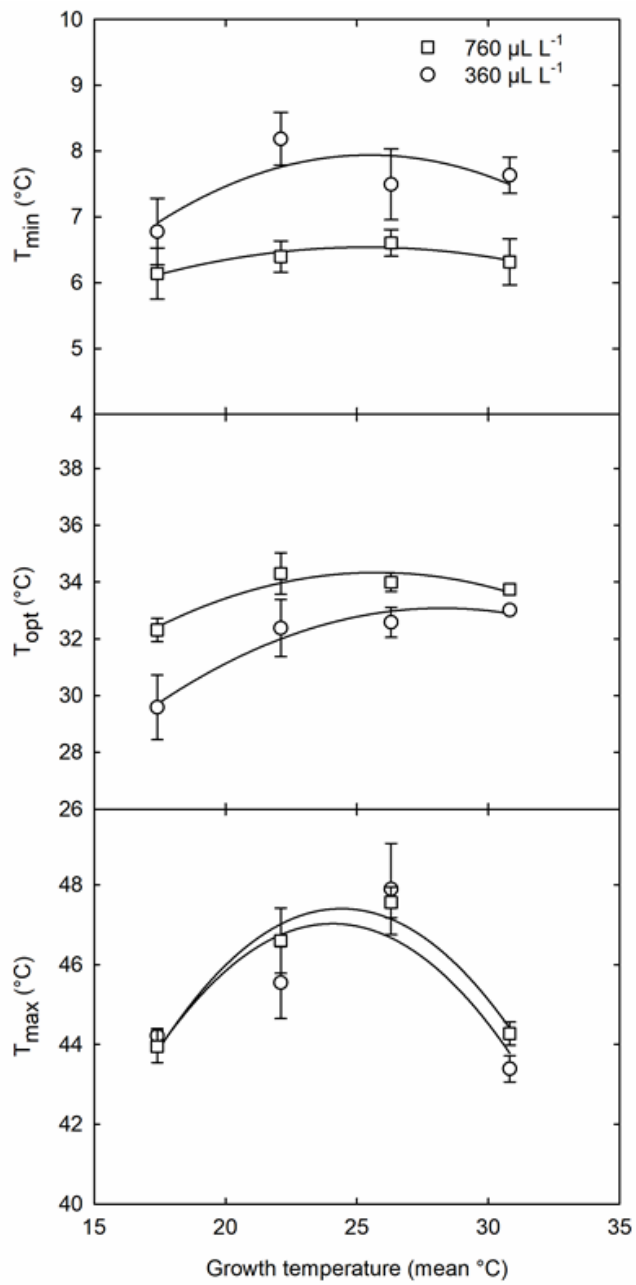


Figure 5. Influence of growth temperature and carbon dioxide during seed development on cardinal temperatures derived from cumulative seed germination percentages and time series across a range of temperatures for seed germination. Data are means ± SE of four plants (n = 4) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

3.6. Correlations between Seed Germination Traits

A significant linear and positive relationship was observed between maximum seed germination (MSG) and the individual seed weight (R^2 = 0.80) and seed carbon (C) (R^2 = 0.79) and nitrogen (N) (R^2 = 0.62) content (Figure 6A–C). Similarly, the relationship between the seed germination rate (SGR) and individual seed weight (R^2 = 0.79) and seed C (R^2 = 0.89) and N (R^2 = 0.79) content was linear and positive (Figure 7A–C). Further, significant ($p < 0.05$) linear relationships between the maximum temperatures of MSG and seed C (R^2 = 0.65) and N content (R^2 = 0.39) were observed, while the relationship between the minimum and optimum temperatures was not significant (regression relationship data not presented). The individual seed weight also had a positive linear relationship

(R^2 = 0.66), with the T_{max} of MSG (R^2 = 0.88) among the cardinal temperatures (regression data not presented).

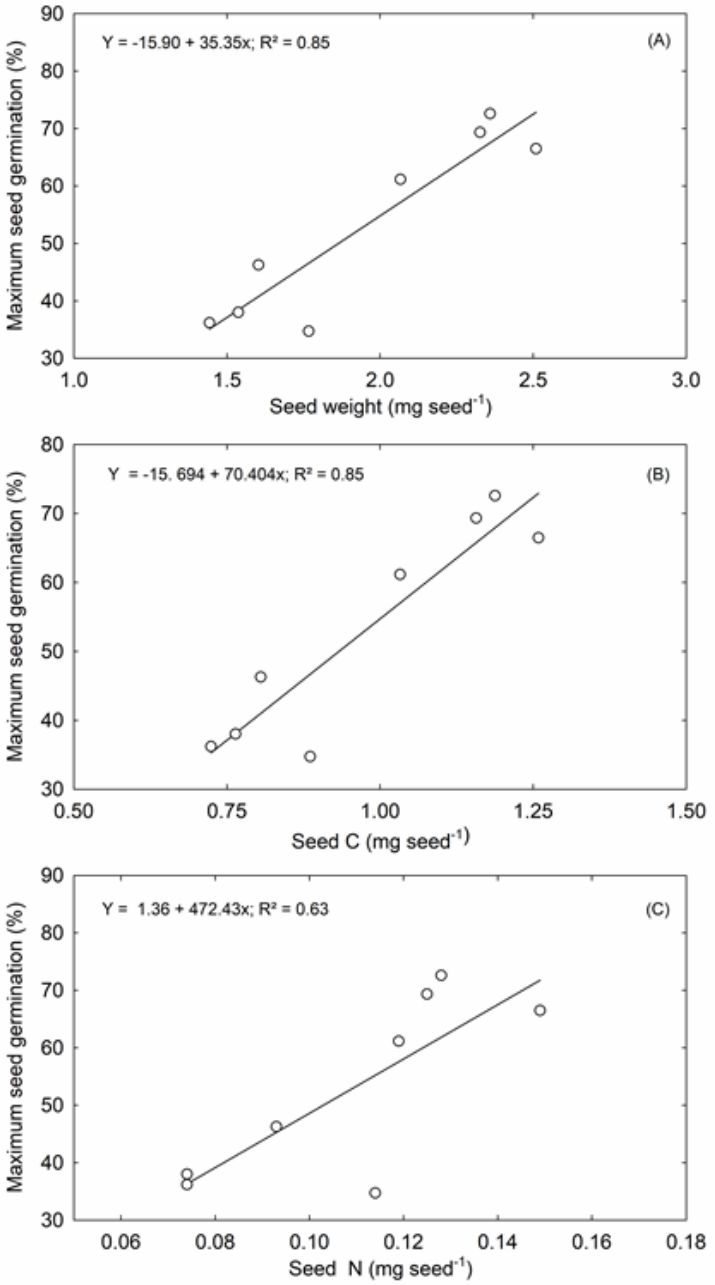


Figure 6. The relationship between maximum seed germination and (**A**) seed weight, (**B**) seed C content, and (**C**) seed N content across eight parental environments. Data are means ± SE of three plants (n = 3) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

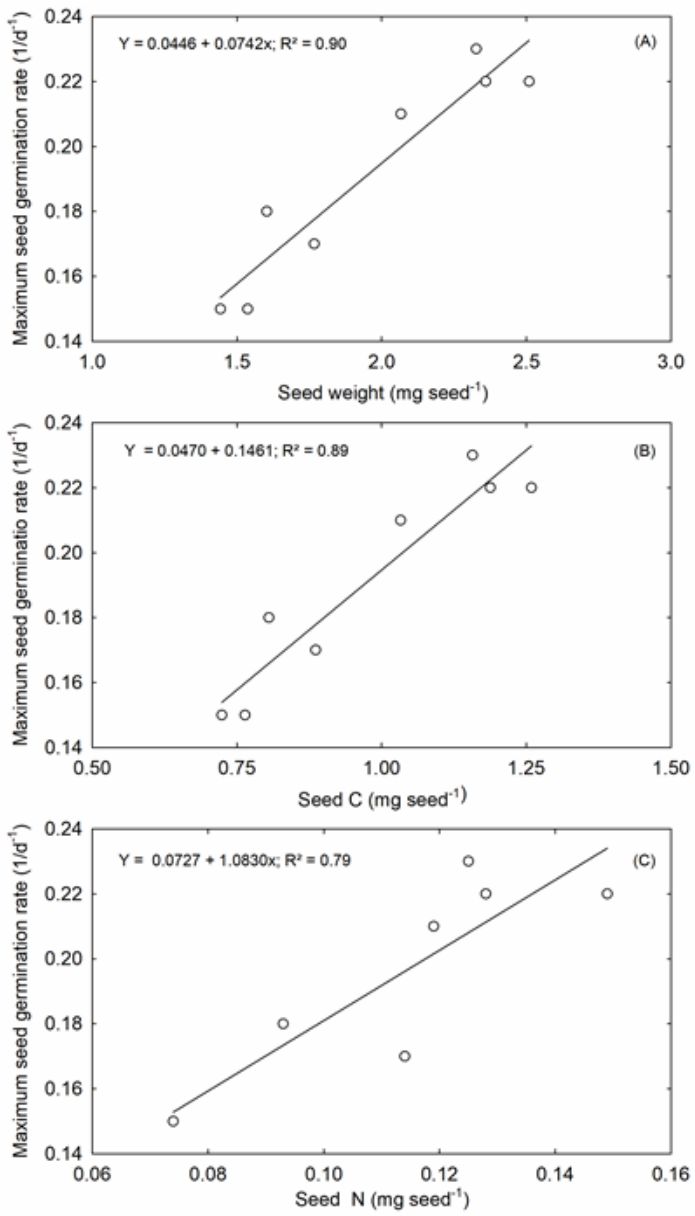


Figure 7. The relationship between seed germination rate and (**A**) seed weight, (**B**) seed C content, and (**C**) seed N content across eight parental environments. Data are means ± SE of three plants (n = 3) harvested from a given SPAR unit and used as replicates in statistical analysis for a respective parental environment.

4. Discussion

Plants have evolved to live in an environment where they are exposed to different environmental factors, either separately or in combination. Being immobile, they are capable of developing certain mechanisms to detect environmental changes and respond to environmental conditions. Plants show morphological and physiological adaptations in response to environmental conditions [4,14,31,32]. For instance, the reproductive development of many crop species is damaged by heat in a way that they produce no viable pollen or no flowers [33–35], or if they produce flowers, they may set no fruit or seed [3,4,6,11]. The major constraints for crop production are global climate changes that give rise to various environmental stress conditions such as global warming, drought, and high UV levels [8,31,36]. Environmental plant physiological approaches can prove to be beneficial and may finally generate models showing the contribution of different signaling pathways that define

"omic" architectural responses to global climatic changes [31]. Studies in the past have recognized an epigenetic contribution related to the adaptation of crops to stressed environments [1,6,9,10,37]. Because seeds are developed in a parental environment, transgenerational epigenetic inheritance provides a mechanism for adaptive parental effects on progeny [38]. Past literature has revealed that transgenerational plasticity in response to parental effects gives rise to offspring phenotypes, such as seed mass and composition, that are adaptive to parental environments [9,38,39].

The present study evaluated the effect of eight parental environments on seed germination characteristics, including seed germination, germination rate, and cardinal temperatures. The parental environments affected the range of traits simultaneously in the present study. However, the effects of parental environments were highly dynamic and context-dependent, which discourages a static view of the parental environment effect [40]. Thus, a transgenerational epigenetic mechanism for a single trait or stress factor may not depict the mechanism for a range of traits or natural environments [40]. Previous studies, including both C_3 and C_4 crops, have mostly focused on the response of seed germination characteristics to a given stress situation, with little or no interaction with the parental environment [14,17,18]. To our knowledge, this is the first study describing the effects of the parental environment on the cardinal temperatures of seed germination in big bluestem.

Further, this study introduced the correlation of seed weight and mineral composition with the cardinal temperatures of germination parameters. The present study reported an adverse effect of very high growth temperature (40/32 °C) during seed development, resulting in no seed production [4], which was consistent with previous studies that concluded that the limited supply of assimilates into developing seeds under elevated temperatures is one of the main reasons for either no or poor quality seed production [3,11]. Spears et al. [3] observed that seeds of soybean cultivars developed at high temperatures (38/28 °C) were small, wrinkled, and shriveled, which explained reduced soybean quality in the absence of seed-borne disease when compared to normal seeds developed at 33/28 °C. Similarly, exposure of garden peas (*Pisum sativum*) to hourly temperatures of more than 50 °C with a base temperature of 28 °C was strongly correlated to the incidence of hollow heart, a pea seed disorder [11].

The significant reduction of stored seed reserves (seed carbon and nitrogen content) under sub- and supraoptimal conditions observed in this study also agreed with several previous studies. At very high growth temperatures, this study reported no increase in seed production, even with increasing carbon dioxide (CO_2), which may have been because the elevated temperature might have nullified the response to elevated CO_2 [1,36]. Carbon dioxide acts as a source of carbon for biomass accumulation and also regulates water use efficiency [41]. However, past studies on C_4 species (such as big bluestem and *Bouteloua gracilis* (blue grama)) have revealed a lack of significant growth response to increasing CO_2 at elevated temperatures because of the weak response of C_4 photosynthesis under well-watered conditions (such as those maintained in the present study) [4,42,43] compared to water-deficient conditions (Owensby, 1993). Moreover, the present study observed only a slight effect of elevated CO_2 on maximum seed germination (MSG) and the seed germination rate (SGR), which strengthens the argument of Jaggard et al. [25] that seed vigor and yields of C_4 plant species are not expected to change with projected CO_2 enrichment. Hampton et al. [1] also pointed out that changes in the seed quality of C_4 species with elevated CO_2 were negligible when compared to elevated temperatures. Hence, the absence of significant effects of elevated CO_2, particularly at elevated temperatures and well-watered conditions, is common in C_4 species.

The response to the temperature of seed germination observed in this study was similar to responses observed in other C_3 [17] and C_4 crops [18]. Maximum seed germination in switchgrass, Indiangrass (*Sorghastrum nutans* (L.) Nash), and ornamental pepper (*Capsicum annuum* L.) [17,18,44] and pollen germination in *Capsicum* species, soybean (*Glycine max* L.), groundnut (*Arachis hypogaea* L.), and cotton (*Gossypium hirsutum* L.) cultivars [33–35,45] exhibited a quadratic response to temperature, as in big bluestem. Also, the seed germination rate in big bluestem exhibited a modified bilinear response to a temperature similar to the above germination studies in different crops. The variation observed in maximum seed germination, and the seed germination rate between parental environments

(PEs) suggests that sub- and supra-optimal growth temperatures during seed development could limit the rate of re-establishment of big bluestem under field conditions [21]. Rashid et al. [19] and Hampton et al. [1] explained that reductions in seed germination responses and seed weight are mainly due to decreased growth rates and a shortening of the seed filling period under elevated growth temperatures. The strong and positive correlation between maximum seed germination and seed weight and seed reserves indicated variations in seed quality parameters [46] that have usually been ascribed to changes in the parent environment, which was reflected in maximum seed germination (MSG). Due to the effects of the parental environment, maximum seed germination, and seed weight have not been useful for the thermotolerance classification of species or genotypes in previous seed germination studies [17,18].

The greater individual seed weight observed under elevated CO_2 agreed with the previous study and might be attributed to the increased availability of seed reserves during seed development [1]. For instance, Huxman et al. [9] observed that seeds of *Bromus rubens* L. developed under elevated parental CO_2 environments had increased seed C/N ratios. Garbutt and Bazzaz [47] found that the seed mass of *Datura stramonium* and *Abutilon theophrasti* increased with increasing CO_2. Mean cardinal temperatures for maximum seed germination and the seed germination rate describe the wide range of adaptation of big bluestem across the globe [18]. Further, variations in cardinal temperatures among parental environments characterize region-specific effects on the germination process of big bluestem. Seepaul et al. [18] used the variability in cardinal temperatures derived for both MSG and the SGR to classify switchgrass genotypes for temperature tolerance.

Similarly, cardinal temperatures derived for seed or pollen germination in ornamental pepper, cotton, groundnuts, *Capsicum* spp., and soybeans were used to classify genotypes for thermotolerance [17,33–35,45]. A lack of parental environmental effects on the minimum temperature (T_{min}) of maximum seed germination in the present study indicated the inherent tolerance of big bluestem to germination in cooler regions. Therefore, cooler regions could be preferred for the sowing of big bluestem even with climate change in the future.

Additionally, cardinal temperatures estimated for parental environments with elevated growth temperatures and carbon dioxide from this study could potentially be introduced in regions best suited for sowing big bluestem in the future. However, this study reported a reduction in temperature adaptability ranges (TARs) for parental environments with elevated growth temperatures and carbon dioxide. This demands further studies that focus on evaluating management practices and farm operations conducive to optimum germination and the subsequent development of big bluestem under projected changing climates.

5. Conclusions

Changes in growth temperature and carbon dioxide concentrations during seed development can be reflected in the seed quality and germination process of big bluestem. However, different parameters of seed quality and germination traits responded to parental environments. For instance, maximum seed germination and the seed germination rate showed a stronger response to elevated growth temperatures than to elevated carbon dioxide concentrations, while seed weight and seed carbon and nitrogen content were sensitive to both elevated growth temperatures and carbon dioxide. Increasing the germination temperature can adversely reduce the germination process of big bluestem. However, germination temperature interaction with the parental environment can modify the rate of re-establishment of big bluestem under natural conditions. Thus, the seed quality and germination equations developed in this study could be useful in evaluating current management practices to optimize region-specific germination and the subsequent development of big bluestem.

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Abbreviations

CO_2, carbon dioxide; GT, growth temperature; GRT, germination temperature; MSG, maximum seed germination; PE, parental environment; SGR, seed germination rate; TAR_{MSG}, temperature adaptability range for maximum seed germination; TAR_{SGR}, temperature adaptability range for the seed germination rate; T_{min}, minimum temperature; T_{opt}, optimum temperature; T_{max}, maximum temperatures; R^2, coefficient of determination.

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