A Simple *in vitro* Seed Germination Assay to Classify Switchgrass Genotypes for Thermotolerance

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Introduction

- Switchgrass (*Panicum virgatum* L.) is a potential lignocellulosic biofuel feedstock selected for its desirable agronomic, production, adaptability and energy attributes.
- The immense interest in switchgrass as a feedstock fostered breeding and selection of genotypes for specific environments, resulting in the need to screen large numbers of genotypes for abiotic stress tolerance.
- Field screening can be tedious, inconsistent and seasonally limited. A simple, rapid and reliable technique is required to identify sources of tolerance and evaluation of breeding materials.
- Coefficient-derived indices from *in vitro* seed germination assay have been successfully used to screen for various abiotic stresses including cold- and heat-tolerance in other species (Emerson and Minor,1979; Tiryaki and David, 2001)
- Functional algorithms quantifying thermal responses and thermotolerance classification using seed-based parameters of diverse switchgrass genotypes are currently lacking.

Objectives

The objectives of this study were to:

a) quantify the effects of temperature on switchgrass seed germination capacity and rate,
b) determine the cardinal temperatures for seed germination capacity and rate, and
c) classify genotypes for thermotolerance using cumulative temperature response index concept.

Materials and Methods

Seed material

- Majority of the seeds were collected from the plants that were grown during 2006-2007 growing season at Mississippi State University. Seeds of few genotypes were obtained from the Ernst Seed Company, Meadville, PA.

Germination testing

- Stratified seeds (5 °C for 14 days) were germinated at constant temperature (10, 15, 20, 25, 30, 25, 40, 45 °C) and constant light at photon flux density of 35 2.6 µmol $m^{-2} s^{-1}$ in a germination chamber.
- Genotypes were completely randomized within the germination chamber for each temperature and replicated four times with 100 seeds per replication.
- Germinated seeds were counted, recorded and discarded every 6 hours.
- A seed was considered germinated when the coleoptile or coleorhizae was at least 2 mm long.

Data analysis and curve fitting procedures

- A three parameter sigmoidal function was fitted to cumulative percent seed germination and time, and maximum seed germination percentage (MSG), the shape and steepness of the curve and the time to reach 50% of maximum germination were estimated. Germination rate (GR) was derived by the reciprocal of time to 50% of MSG.
- Maximum seed germination was modeled by a quadratic function (mean r^2 = 0.93 and RMSE = 5.2) while germination rate was modeled by a modified bilinear function (mean r^2 = 0.95 and RMSE = 1.00).
- Quadratic and modified bilinear equations constants were estimated by PROC NLIN in SAS by a modified Newton Gauss iterative method.
- For the quadratic model, the three cardinal temperatures (T_{min}, T_{opt} and T_{max}), were estimated following the protocol used by Salem et al. (2007).
- For the modified bilinear model , T_{opt} was generated in SAS while T_{min} and T_{max} were estimated following the protocol used by Kakani et al. (2002).
- Heat and cold cumulative stress response indices (CTRI) were estimated from the summation of individual stress index values derived from MSG and GR cardinal temperatures, and genotypes were classified as cold-tolerant, moderately cold-tolerant, moderately cold-sensitive and cold-sensitive using standard deviation as the classification criteria. Three groups were obtained based on the range of CTRI for heat tolerance classification and genotypes were classified as heat-tolerant, intermediate and heat-sensitive.
- Percentage seed germination data were arcsine transformed prior to analysis. Replicated values of cardinal temperatures, TAR and MSG were analyzed using one-way ANOVA procedure (PROC GLM) in SAS to determine the effect of temperature treatment on MSG and GR and their respective cardinal temperatures.

Results

Germination time course

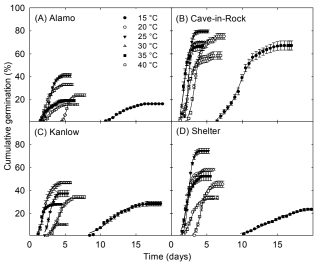

Figure 1. Temperature effects on cumulative seed germination responses over time of four selected switchgrass genotypes.

- The 3-parameter sigmoid curve fitted the cumulative germination time course (r^2 = 0.96 - 0.99) of genotype response to temperature efficiently.
- Temperature affected the germination time course, though the magnitude of the response was genotype-specific (Figure 1).
- There was no germination at 10 or at 45°C.

Maximum seed germination (MSG) and germination rate (GR) responses to temperature

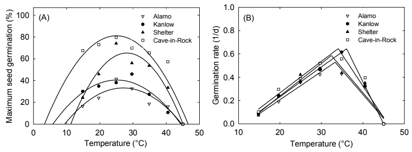

Figure 2. Temperature effects on (A) maximum seed germination and (B) germination rate along with their fitted quadratic and modified bilinear curves, respectively, of four switchgrass genotypes.

- The quadratic function best described the response of MSG to temperature (r^2 = 0.93) while a modified bilinear equation best described the relationship between GR and temperature (r^2 = 0.95).
- Maximum seed germination and germination rate were temperature dependent, though the response to temperature was different. Optimum for maximum seed germination occurred over a range of temperatures while germination rate optimum was sharply defined (Figures 2A and 2B).

Cardinal temperature estimation

Table 1. Maximum seed germination (MSG), cardinal temperatures and temperature adaptability range (TAR) for maximum seed germination and germination rate (GR) determined from *in vitro* seed germination assay of 14 switchgrass genotypes in response to temperature.

Genotypes	MSG	MSG Cardinal Temperatures			TAR	GR Cardinal Temperatures			TAR
		T_{min}	T_{opt}	T_{max}		T_{min}	T_{opt}	T_{max}	
		C							
Alamo	40.97	9.61	27.08	44.55	34.94	11.96	33.02	46.25	34.29
Blackwell	83.23	9.33	27.34	45.34	36.01	12.14	33.91	45.06	32.92
Carthage	55.09	10.20	27.95	45.71	35.51	12.83	30.45	46.89	34.06
Cave-in-Rock	79.48	5.62	25.88	46.14	40.52	10.16	34.43	45.27	35.11
Dacotah	85.68	10.40	27.52	44.64	34.24	9.09	35.34	45.27	36.18
Expresso	93.07	3.69	25.38	47.07	43.38	9.33	35.50	45.09	35.76
Forestburg	80.76	7.68	26.31	44.95	37.27	10.18	34.03	45.35	35.17
Kanlow	53.05	6.40	25.37	44.34	37.94	9.94	35.65	45.00	35.06
Shawnee	50.31	9.90	27.60	45.31	35.41	12.54	30.56	47.55	35.01
Shelter	74.27	11.46	28.19	44.92	33.46	12.92	29.55	48.15	35.23
Summer	67.52	12.83	28.56	44.30	31.47	12.06	30.77	47.08	35.02
Sunburst	86.95	5.49	25.81	46.14	40.65	11.21	30.48	46.55	35.34
Trailblazer	87.46	4.19	25.08	45.97	41.78	9.86	34.21	45.44	35.58
Warrior	89.56	6.27	24.04	41.81	35.54	11.65	35.73	45.16	33.51
Mean	73.38	8.08	26.58	45.09	37.01	11.1	33.1	46.0	34.9
LSD	12.66*	3.09*	1.43*	1.70*	4.09*	2.32*	4.49*	2.17*	2.47*

* $P < 0.05$

- Maximum seed germination varied among genotypes with a mean of 73% and ranged from 41% (Alamo) to 93% (Expresso).
- Cardinal temperatures for MSG and GR differed among the genotypes.
- Cardinal temperatures for MSG were higher relative to GR. Mean minima (T_{min}), optima (T_{opt}) and maxima (T_{max}) were 8.08, 26.58, 45.09 C and 11.13, 33.12 and 46.01 C for MSG and GR, respectively (Table 1).

Results cont'd

Thermotolerance classification using CTRI

Table 2. Classification of switchgrass genotypes into cold tolerance groups based on cumulative temperature response index (CTRI) along with individual score in parenthesis.

Cold-sensitive (CTRI = 4.74 - 5.03)	*Moderately cold-sensitive* (CTRI = 5.04 - 5.32)	*Moderately cold-tolerant* (CTRI = 5.33 -5.62)	*Cold-tolerant* (CTRI = 5.63 - 6.21)
Alamo (4.84)	Cave-in-Rock (5.24)	Trailblazer (5.52)	Expresso (5.64)
Blackwell (4.82)	Forestburg (5.08)		
Carthage (4.78)	Kanlow (5.21)		
Dacotah (5.0)	Sunburst (5.26)		
Shawnee (4.8)	Warrior (5.19)		
Shelter (4.74)			
Summer (4.74)			

Table 3. Classification of switchgrass genotypes into heat tolerance groups based on cumulative temperature response index (CTRI) along with individual score in parenthesis.

Heat-sensitive (CTRI = 4.83 - 5.43)	*Intermediate* (CTRI = 5.44 -5.74)	*Heat-tolerant* (CTRI = 5.75 - 6.05)
Cave-in-Rock (5.01)	Alamo (5.45)	Summer (5.78)
Dacotah (5.36)	Blackwell (5.47)	
Expresso (4.83)	Carthage (5.56)	
Forestburg (5.16)	Shawnee (5.51)	
Kanlow (5.03)	Shelter (5.59)	
Sunburst (5.0)		
Trailblazer (4.85)		
Warrior (5.06)		

- Thermotolerance varied among the genotypes based on cumulative temperature response index classification (Table 2 and 3).
- The genotype, Summer was identified as the most heat-tolerant while Cave-in-Rock, Dacotah, Expresso, Forestburg, Kanlow, Sunburst, Trailblazer and Warrior were identified as heat-sensitive.
- Expresso had the highest cold-CTRI (5.64), and therefore considered as the most cold-tolerant among the 14 genotypes tested, while Summer had the highest heat-CTRI (5.78) and was classified as the most cold-susceptible genotype.
- The inverse relationship between cold- and heat-tolerance cumulative temperature response indices (Figure 3) suggest that these two traits are independent; therefore selection for either trait is a viable objective.

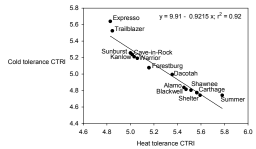

Figure 3.
The relationship between heat- and cold- tolerance CTRI of 14 switchgrass genotypes.

Conclusions

- Thermotolerance classification via *in vitro* seed germination assay is a simple and inexpensive technique for screening large number of genotypes.
- The identified cold- and heat- tolerant genotypes can be used in breeding programs to develop switchgrass germplasm for thermotolerance.
- The functional algorithms can be used to improve switchgrass simulation models to accurately predict seed germination in the field.

References

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