

Biomass, Cell Wall Components and Gene Expressions of Switchgrass in Response to Water Deficit Conditions

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Objective:

To determine effects of water deficit on biomass and biofuel components of switchgrass (*Panicum virgatum* L.)

Rationale:

Switchgrass is a warm-season perennial grass indigenous to the tall grass regions of the Great Plains and the Midwest. It is a highly productive grass that requires low maintenance. Researchers at the US Department of Energy (DOE) have identified switchgrass as a promising energy crop for use in producing cellulosic ethanol. Extensive production of switchgrass for biofuels will likely occur on marginal soils that are not well-suited for production of conventional row crops. However, these marginal soils are often shallow, coarse textured, and relatively infertile. Switchgrass grown in these soils will frequently be subjected to adverse environmental conditions such as water deficit, which reduces plant growth and biomass yield and alters the amount of cell wall components. Optimizing plant biomass for more efficient bioethanol processing requires a better understanding of cell wall synthesis under both optimum and stress conditions. However, fundamental physiological and molecular characterizations of switchgrass ecotypes under water deficit conditions are lacking. Our long-term goal is to identify genes and gene products of cell wall components that will be useful as selection criteria in switchgrass improvement programs and lead to sustainable and more profitable management of perennial grasses used for biofuels.

Materials and methods:

The experiment was conducted from March to May in October in the greenhouse at Purdue University. The cultivar of Cave-in-Rock was seeded in pots (20 cm diameter x 40 cm deep) containing top soil with a pH of 6.9. Each pot contained the same soil volume and plant population, and was initially watered to field capacity in order to achieve uniformity of experimental materials. The grasses were fertilized with water-soluble fertilizer of 24-8-16 (N-P₂O₅-K₂O) (Scotts-Sierra Horticultural Products Co., Marysville, Ohio) to supply 120 kg N ha⁻¹ during the growing season. This fertilizer rate provides the optimum biomass yield of Cave-in-Rock in the Midwest. Irrigation was supplied as necessary for seedling establishment and for preventing drought stress. The temperatures in the greenhouse were 75 to 85 F/55 to 65F (day/night) during the experiment period.

The drought treatment started when grass were well-established. Irrigation was shut down during drought treatment. Leaf, leaf sheath and stem samples were collected after 4 days of drought stress. Grasses were then allowed to recover after drought stress. Cell wall components such as cellulose, hemicellulose, and lignin content were measured. For gene expressions, leaf and stem plus leaf sheath samples were collected after 2 and 4d of stress treatments. Gene expression of cell cellulose synthase, hemicellulose synthase, and enzymes involved lignin biosynthesis were detected using RT-PCR techniques.

The experiment was a randomized complete block design with four replicates. The well-watered and drought-stressed pots were randomly assigned within each block. The data was analyzed using SAS program.

Results to date:

Leaf relative water content and dry weight were decreased 34 % and 17 % under drought stress, respectively; but recovered back to the control level after 1 day of re-watering (Table 1). Leaf hemicellulose content was decreased 18 % under drought stress but did not recover after re-watering. Drought stress increased leaf cellulose content and did not change lignin content.

Drought stress decreased water content and dry weight of leaf sheath and stem but did not change cellulose and hemicellulose content (Table 2). Lignin content in leaf sheath and stem was increased 31 % under drought stress.

Expression of *ZmCesA1* gene (maize cellulose synthesis) was clearly and consistently suppressed by drought stress in leaf blades, sheath and stems, and the suppression of this gene was reversed by re-watering to the plants (Figure 1). The results indicated that *ZmCesA1* gene changed rapidly in response to drought stress and recovery from stress, and it can be a candidate gene used for further studies in switchgrass.

Acknowledgements:

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Table 1. Effects of drought stress on leaf relative water content (RWC), leaf dry weight, cellulose, hemicellulose and lignin content in switchgrass.

Treatment	RWC (%)	Dry weight (g)	Cellulose(%)	Hemicellulose(%)	Lignin(%)
Control	96.0 a [†]	0.53 a	27.0 b	38.3 a	5.70 a
Drought	63.2 b	0.44 b	28.9 a	31.5 b	5.95 a
Recovery	96.4 a	0.57 a	26.3 b	29.9 b	4.95 a

[†]Means followed by the same letters within a column were not significantly different based on LSD ($p=0.05$).

Table 2. Effects of drought stress on leaf sheath and stem water content (WC), dry weight, cellulose, hemicellulose and lignin content in switchgrass.

Treatment	RWC (%)	Dry weight (g)	Cellulose(%)	Hemicellulose(%)	Lignin(%)
Control	74.9 a [†]	0.71 ab	35.7 a	32.0 a	7.45 b
Drought	62.8 c	0.59 b	34.0 a	31.2 a	9.75 a
Recovery	71.4 b	0.80 a	36.3 a	30.6 a	8.18 b

[†]Means followed by the same letters within a column were not significantly different based on LSD ($p=0.05$).

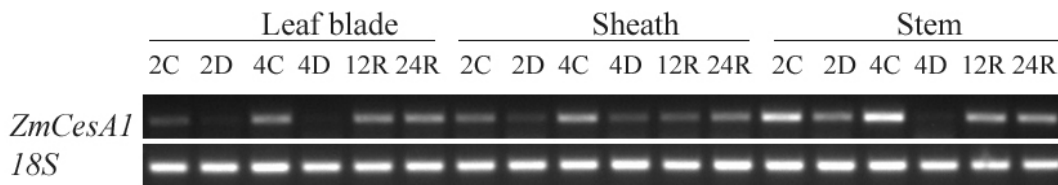


Figure 1. Gene expressions of *ZmCesA1* in switchgrass. 2C, 2D, 4C, 4D, 12R, and 24R represent 2 day of the well-watered control, 2 day of drought, 4 day of the well-watered control, 4 day of drought, 12 hour of recovery, and 24 hour of recovery, respectively. *18S*, a constitutively expressed gene was used as a marker gene.