

Root Anaerobic Metabolisms and Gene Expressions of Kentucky Bluegrass in Response to Short-Term Waterlogging Alone and in Combination with High Temperatures

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Objective:

To characterize physiological responses and anaerobic metabolism of Kentucky bluegrass (*Poa pratensis* L.) to waterlogging alone and combined with high temperatures

Rationale:

Waterlogging, flooding, or over-irrigation followed by slow drainage reduces oxygen availability to roots and negatively affects plant growth. Moreover, low oxygen stress often occurs simultaneously with high temperatures during summer. High temperature increases plant demand for oxygen (e.g., respiration), and the increased respiration under high temperature and reduced oxygen availability in the saturated soil can be more injurious to plants.

Metabolic adjustments occur as an adaptive strategy to facilitate tolerance of the plants to waterlogged or flooded soils. Little is known about how they are affected by combined waterlogging and high temperatures in plant species. In particular, changes in anaerobic metabolisms of perennial grasses under warm temperatures and low oxygen environments are not well understood. Kentucky bluegrass adapts to well-drained soils and is used on athletic fields, golf course fairways, and home lawns. However, any of these sites may become partially or fully waterlogged or flooded, which may affect grass quality and plant persistence, especially when temperatures are high. A better understanding of the root metabolism of Kentucky bluegrass would provide a basis for managing this grass and for further revealing the mechanisms of grass adaptation to saturated or flooded soils under high temperatures.

Materials and methods:

Two cultivars (Unique and Serene) of five-year-old Kentucky bluegrass sod plugs were collected in the fall of 2005 from the William H. Daniel Research and Diagnostic Center at Purdue University in West Lafayette, IN. The 10-cm diameter plugs were cut to 2.5 cm deep and grown in a greenhouse for 75 d in plastic pots (15 cm diameter x 14.5 cm deep) containing mixture of topsoil (Seafield or Gilford fine sandy loam, Reynolds, Indiana) and coarse river sand (1:1). The top soil had a pH of 6.9. The grasses were irrigated as necessary to prevent drought stress, mowed three times a week at 4 cm with electric hand-held clippers, and fertilized weekly with water soluble fertilizer of 20-20-20 (N-P₂O₅-K₂O) (Scotts-Sierra Horticultural Products Co., Marysville, Ohio) to provide 244 kg • ha⁻¹ N, 107 kg • ha⁻¹ P and 202 kg • ha⁻¹ K per year. The grasses were allowed to be well-established before the waterlogging and high temperature treatments began.

Grass pots were placed into containers (80 cm length × 34 cm wide × 15 cm deep) and tap water was added to the containers. The water level in each pot was kept at the soil surface and the water was changed every 3 d during the experiment. The containers were placed in growth chambers under low and high temperature regimes of 20°C /15°C and 35°C /30°C (day/night), respectively. The photoperiod was 14 h with a light intensity of 600 μmol m⁻²s⁻¹ photosynthetic photon flux during the treatments. The treatments included:

- 1) Well-drained, low temperature (Control)
- 2) Waterlogging, low temperature (WL)

- 3) Well-drained, high temperature (HT)
- 4) Waterlogging, high temperature (WL+HT)

Grasses were harvested at 3 and 5 days of treatments. Photochemical efficiency (Fv/Fm) was measured on 4 to 5 arbitrarily selected leaves from each pot after 15-min dark adaptation using a fluorescence meter (OS-30P, OPTI-Sciences, Hudson, NH). The measurement was repeated 5 times on each pot, and a total of 20 to 25 leaves were selected for Fv/Fm determination. Water-soluble protein extraction, root ethanol concentration, and activities of pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and lactate dehydrogenase (LDH) in the roots were determined. Native polyacrylamide gel electrophoresis (PAGE) for ADH isoenzyme detection and activity stain were performed. Gene expressions of ADH, LDH and PDC of roots were examined using RT-PCR.

The experiment was a split-split-plot design with temperature treatments as the whole-plot, waterlogging treatments as the sub-plot, and two cultivars as the sub-sub-plot. Two growth chambers were randomly assigned to low and high temperatures, respectively, and waterlogging treatments were arranged randomly with each chamber. Cultivars were arranged randomly with each treatment. Four pots representing the four replicates were used in this study. The data from 3 and 5 d of treatments were analyzed separately. The analysis of variance and Fisher's protected LSD mean separation tests were performed using PROC MIXED (SAS Institute, Version 9.1, Cary, NC).

Results :

Significant waterlogging \times temperature interactions showed that WL or HT alone did not affect Fv/Fm and WSC, but WL+HT significantly reduced Fv/Fm and WSC at both 3 and 5 d (Table 1). WSC under WL+HT was only 55% and 54% of the control at 3 d and 5 d, respectively. Root ethanol concentration under WL alone was higher than the control and WL+HT at 3 d. At 5 d, no differences in root ethanol concentration were observed among the control, WL, or HT alone; but WL+HT had lower root ethanol concentration than the other three treatments. The ADH activities under WL and WL+HT were 14- and 10-fold of the control, respectively. There were no differences in ADH activities between WL and WL+HT or between the control and HT. Compared to control, the LDH activities were increased 14- and 40-fold at 3 d and 19- and 54-fold at 5 d under WL and WL+HT, respectively.

Gene expressions of *ADH1* and *ADH2* and *PDC* showed the similar patterns. Expressions of *ADH1* and *ADH2* genes were not shown under the well-drained control and HT alone but were induced under WL or WL+HT (Figure 1A). The gene expression of *PDC* was also enhanced under WL or WL+HT.

Six isoforms of ADH were detected in root tissues of Unique at both 3 and 5 d (Figure 1B). Compared to the control, the staining intensity of ADH increased under WL and WL+HT, which was consistent with the total activity assay. The isoform with the lowest molecular weight (ADH1) was strongly induced under WL and WL+HT conditions at both 3 and 5 d.

Acknowledgements

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Table 1. Interactive effects of waterlogging and temperature on photochemical efficiency (Fv/Fm), root water-soluble carbohydrates (WSC), root ethanol concentration (Ethanol), and activities of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) in Kentucky bluegrass

Day	Effect		Fv/Fm	WSC	Ethanol	ADH	LDH
	Treatment	Temperature		-mg g ⁻¹ DW-	- μmol g ⁻¹ FW -	- μmolmin ⁻¹ mg ⁻¹ Protein -	
3	Well-drained	Low	0.770 a [†]	119.4 a	1.00 b	0.06 [‡]	0.01 [‡] b
	Waterlogging	Low	0.769 a	108.3 a	1.38 a	0.92	0.15 a
	Well-drained	High	0.768 a	113.9 a	1.08 ab	0.15	0.01 b
	Waterlogging	High	0.746 b	65.4 b	0.94 b	1.50	0.41 a
5	Well-drained	Low	0.773 a	100.9 a	1.79 a	0.06 b	0.01 b
	Waterlogging	Low	0.763 a	111.1 a	2.06 a	0.85 a	0.20 a
	Well-drained	High	0.770 a	104.9 a	1.76 a	0.16 b	0.01 b
	Waterlogging	High	0.727 b	54.4 b	1.21 b	0.63 a	0.55 a

[†]Means followed by the same letters within a column were not significantly different based on protected Fisher's LSD ($p=0.05$). [‡]Data were presented using back transformed means (n=8)

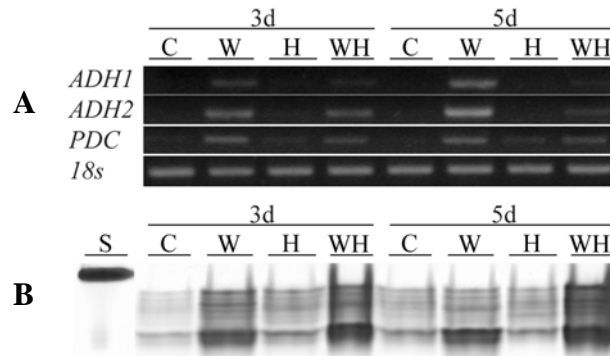


Figure 1. Gene expressions of alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) (A) and native gel stained for the activity of ADH isoforms (B) in roots of Kentucky bluegrass after 3 and 5 d of treatments. C, W, H, and WH represent well-drained control, waterlogging, high temperature, and waterlogging combined with high temperature for Unique, respectively. 18s was internal loading control. Lane S represents the standard ADH. Equal amounts of protein (7 μg) were loaded to each lane for B.