

## **Establishment of a Standard Screening Method for Drought Tolerance in Creeping Bentgrass**

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The goal of this project is to determine the reliability of selecting drought-tolerant creeping bentgrass in a polyethylene glycol (PEG) - hydroponic culture based on root characteristics. It includes two specific objectives:

- (1) Evaluating drought tolerance of 25 creeping bentgrass cultivars under putting green and fairway conditions in field plots;
- (2) Quantifying the differences in rooting depth and root to shoot ratio of 23 creeping bentgrass cultivars in a PEG-hydroponic system.

Creeping bentgrass is the dominant turfgrass species for golf course putting greens and fairways. Frequent irrigation, however, is required to remain its playability and aesthetical qualities under intense use. Limited water resources and rapid suburban expansion have led to restricted irrigation in many communities. Deficient irrigation (i.e. drought) causes reduced turfgrass quality, and plant death when the stress is severe. Use of drought tolerant plants can help reduce water use and minimize stress damage. Large progress has been made to breed drought-tolerant plants, including creeping bentgrass, generating a large number of new plant materials. Accurate phenotyping (i.e. screening) is critical for a successful breeding program. An ideal phenotyping technique includes use of reliable (heritable) selection criteria and a simple, rapid, and cost effective screening process. Various traits contributing to drought tolerance have been identified in plants, including two important morphological characteristics, deep rooting and high root to shoot ratio. These morphological characteristics are not only reliable but also easier and less costly to measure than physiological and molecular indices; however, sampling roots from field plugs or potted plants (most commonly with soil-based rooting mixtures) is labor intensive. Polyethylene ethylene - hydroponic culture has been successfully used in selecting drought-tolerant field crops; however, its potential application as a screen technique in turfgrass breeding has not been determined.

### **Field evaluation**

Two creeping bentgrass field trials were established at the Turfgrass Research Center, Fargo, ND in fall, 2008. The field trials have been maintained under golf putting green (soil-based pushup-type) or fairway conditions since spring, 2009. Both trials included 25 commercially available creeping bentgrass cultivars (Tables 1 and 2). The experimental setup was randomized complete block design with three replicates. In 2015, Irrigation was applied during spring green-up, but withheld since July 7 to stimulate drought conditions. Canopy reflectance was measured in the 340 - 1023 nm range using a spectrometer (Model: JAZ-COMBO-2; OceanOptics, Inc., Dunedin, FL). Data were collected every 7 – 14 days during cloud-free days during midday (10:45 a.m. - 12:00 p.m). The spectrometer was held at 3.1 ft. above the canopy. Three water indices, water index (WI) =  $R_{970} / R_{900}$ , normalized water index (NWI) =  $(R_{970} - R_{880}) / (R_{970} + R_{880})$ , and floating-position water band index (fWBI) =  $R_{900} / \min(R_{930-980})$  were estimated, in which R and the subscript numbers indicate the light reflectance at the specific wavelength (nm). Soil moisture content was recorded with a time-domain reflectometry (Model: Trime-FM; IMKO Micromodultechnik GmbH, Germany) in four randomly selected locations within each trial, after the canopy reflectance was measured. Turfgrass visual quality was recorded with a 1 – 9 scale, where 1 = dead grass, 6 = acceptable quality for putting green or fairway, and 9 = optimal quality. Air temperature and rainfall were recorded daily by a weather station located 200 ft away from the research trials.

Frequent rainfall occurred in 2015 (Fig. 1). The longest duration without precipitation was 12-day, lasting from Aug. 24 to Sept. 4. Monthly accumulated rainfall for July, Aug., and Sept. was 2.80, 2.14, and 1.6 inch, respectively. Soil water content decreased from 35.1% in early July to 15.4% in late Sept. in the putting green trail and from 43.7% to 21.2% in the fairway trail (Fig. 2). No significant differences were detected between the cultivars in any water index during the growing season of 2015 (Tables 1 and 2). It is probably due to a combination of frequent precipitation, high water holding capacity of clay soil at the research site, and cool climate of the upper Northern region. No visual drought symptoms were observed on any evaluation date in either trial.

### **Laboratory experiments**

Twenty-three creeping bentgrass cultivars, including 19 cultivars from the field trials (Table 1) and ‘Focus’, ‘Cobra 2’, ‘V8’, and ‘Pinup’ creeping bentgrass, will be exposed to a PEG-

hydroponic system to determine rooting characteristics during the germination and seedling growth stage (Experiment 1) and the vegetative growth stage (Experiment 2). The grasses were germinated under the control (i.e. non-stress, 0 MPa) and PEG at -0.4, -0.8, and -1.2 MPa in a preliminary study (data not shown). All grasses showed adequate germination (> 90%) under the non-stress condition and at -0.4 MPa. Seedling dry weight ranged from 13.9 to 62.9 mg and 40.9 to 104.5 mg at 0 and -0.4 MPa, respectively. Germination rate decreased to less than 18% at -0.8 MPa with little seedling biomass to be collected. Grasses at -0.4 and -0.8 MPa appeared in darker green color than the control plants (visual observation). No grasses germinated as the drought level increased to -1.2 MPa.

Based on the preliminary results, two PEG levels, -0.3 and -0.6 MPa, have been selected to be used in to determine rooting depth and root to shoot ratio during germination and seedling growth (Experiment 1) and vegetative growth (Experiment 2). Data will be collected on seedling biomass (shoot and root) and the longest root length in Experiment 1 and visual quality, tissue biomass (shoot and root), and the longest root length in Experiment 2. Experiment 1 is currently ongoing.

Table 1. Water index (WI), normalized water index (NWI), and floating-position water band index (fWBI) of 25 creeping bentgrass cultivars managed under golf putting green condition on three representative dates in 2015.

Cultivar	July 14			Aug. 12			Sept. 1		
	WI <sup>‡</sup>	NWI <sup>‡</sup>	fWBI <sup>‡</sup>	WI	NWI	fWBI	WI	NWI	fWBI
L-93	1.020	-0.006	1.543	1.078	-0.035	1.290	1.099	-0.051	1.836
T-1	1.022	-0.008	1.580	1.035	-0.016	1.161	1.094	-0.049	1.773
Alpha	1.011	-0.002	1.515	1.045	-0.019	1.130	1.086	-0.046	1.790
Putter	1.010	-0.001	1.551	1.045	-0.021	1.151	1.083	-0.043	1.662
South shore	1.014	-0.003	1.529	1.053	-0.025	1.189	1.077	-0.040	1.756
Kingpin	1.023	-0.007	1.578	1.050	-0.020	1.197	1.103	-0.051	1.792
Crenshaw <sup>†</sup>	1.013	-0.002	1.529	1.039	-0.0169	1.180	1.083	-0.043	1.771
Imperial <sup>†</sup>	1.017	-0.005	1.627	1.043	-0.0199	1.171	1.083	-0.043	1.795
Century <sup>†</sup>	1.014	-0.003	1.500	1.039	-0.0150	1.151	1.087	-0.045	1.781
Penncross	1.010	-0.001	1.573	1.033	-0.0133	1.143	1.072	-0.037	1.731
Penn A-4	1.003	0.001	1.626	1.038	-0.0151	1.153	1.089	-0.045	1.822
Crystal bluelinks	1.017	-0.004	1.546	1.060	-0.0278	1.228	1.090	-0.047	1.814
Alister <sup>†</sup>	1.015	-0.004	1.479	1.046	-0.0195	1.134	1.080	-0.040	1.689
Pennlinks II	1.004	0.002	1.487	1.040	-0.0184	1.180	1.082	-0.042	1.660
Penn A-1	1.006	0.002	1.580	1.041	-0.0178	1.159	1.092	-0.047	1.780
Penn G-6 <sup>†</sup>	1.008	-0.001	1.534	1.039	-0.0158	1.121	1.071	-0.037	1.702
007	1.005	0.002	1.494	1.042	-0.0169	1.148	F1.086	-0.045	1.800
MacKenzie	1.022	-0.007	1.532	1.050	-0.0226	1.156	1.095	-0.049	1.834
Tyee	1.006	0.001	1.606	1.038	-0.0137	1.120	1.095	-0.049	1.952
SR 1150	1.010	-0.002	1.552	1.050	-0.0204	1.134	1.103	-0.052	1.812
Memorial <sup>†</sup>	1.016	-0.004	1.481	1.045	-0.0194	1.161	1.109	-0.055	1.774
Independence	1.029	-0.010	1.590	1.065	-0.0294	1.204	1.093	-0.047	1.744
Declaration	1.013	-0.003	1.600	1.036	-0.0155	1.139	1.082	-0.041	1.764
LS - 44 <sup>†</sup>	1.012	-0.002	1.554	1.043	-0.0189	1.161	1.092	-0.048	1.787
Bengal	1.023	-0.008	1.571	1.050	-0.0225	1.158	1.095	-0.048	1.794
	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns means not significantly different at  $P \leq 0.05$  level.

<sup>†</sup>Creeping bentgrass cultivar not included in the laboratory experiments as it is no longer commercially produced.

<sup>‡</sup>water index (WI) =  $R_{970} / R_{900}$ , normalized water index (NWI) =  $(R_{970} - R_{880}) / (R_{970} + R_{880})$ , and floating-position water band index (fWBI) =  $R_{900} / [\min(R_{930-980})]$ , in which R and the subscript numbers indicate the light reflectance at the specific wavelength (nm).

Table 2. Water index (WI), normalized water index (NWI), and floating-position water band index (fWBI) of 25 creeping bentgrass cultivars managed under golf fairway condition on three representative dates in 2015.

Cultivar	July 14			Aug. 12			Sept. 1		
	WI	NWI	fWBI	WI	NWI	fWBI	WI	NWI	fWBI
L-93	1.022	0.000	1.146	1.037	-0.012	1.177	0.924	0.052	0.994
T-1	1.027	-0.005	1.119	1.045	-0.018	1.196	0.932	0.045	1.026
Alpha	1.048	-0.030	1.555	1.063	-0.027	1.198	0.967	0.025	1.136
Putter	1.026	-0.003	1.163	1.042	-0.015	1.193	0.920	0.053	1.012
South shore	1.026	-0.006	1.157	1.045	-0.016	1.186	0.925	0.049	0.994
Kingpin	1.030	-0.013	1.186	1.046	-0.017	1.190	0.958	0.030	1.060
Crenshaw <sup>†</sup>	1.026	-0.003	1.160	1.043	-0.016	1.185	0.912	0.057	0.980
Imperial <sup>†</sup>	1.034	-0.009	1.119	1.056	-0.022	1.205	0.926	0.050	1.010
Century <sup>†</sup>	1.044	-0.012	1.142	1.058	-0.023	1.209	0.921	0.051	1.020
Penncross	1.031	-0.006	1.147	1.047	-0.018	1.208	0.917	0.054	0.989
Penn A-4	1.033	-0.008	1.160	1.046	-0.017	1.192	0.920	0.051	0.986
Crystal bluelinks	1.023	-0.002	1.156	1.044	-0.016	1.193	0.920	0.052	0.981
Alister <sup>†</sup>	1.029	-0.005	1.152	1.057	-0.023	1.194	0.926	0.050	0.996
Pennlinks II	1.022	-0.003	1.160	1.040	-0.014	1.190	0.917	0.053	1.013
Penn A-1	1.042	-0.013	1.160	1.050	-0.019	1.181	0.924	0.050	1.017
Penn G-6 <sup>†</sup>	1.018	-0.013	1.149	1.045	-0.017	1.222	0.960	0.031	1.109
007	1.051	-0.018	1.180	1.055	-0.022	1.192	0.928	0.047	0.999
MacKenzie	1.023	-0.001	1.110	1.042	-0.015	1.183	0.926	0.049	0.991
Tyee	1.036	-0.010	1.176	1.046	-0.017	1.207	0.958	0.030	1.112
SR 1150	1.038	-0.012	1.187	1.054	-0.021	1.188	0.925	0.051	1.002
Memorial <sup>†</sup>	1.025	-0.003	1.162	1.039	-0.013	1.183	0.923	0.050	1.008
Independence	1.041	-0.012	1.132	1.059	-0.024	1.209	0.929	0.048	1.005
Declaration	1.049	-0.017	1.199	1.037	-0.012	1.191	0.913	0.057	0.988
LS - 44 <sup>†</sup>	1.026	-0.021	1.224	1.045	-0.017	1.194	0.987	0.013	1.172
Bengal	1.036	-0.009	1.148	1.043	-0.015	1.189	0.929	0.053	1.013
	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns means not significantly different at  $P \leq 0.05$  level.

<sup>†</sup>Creeping bentgrass cultivar not included in the laboratory experiments as it is no longer commercially produced.

<sup>\*</sup>water index (WI) =  $R_{970} / R_{900}$ , normalized water index (NWI) =  $(R_{970} - R_{880}) / (R_{970} + R_{880})$ , and floating-position water band index (fWBI) =  $R_{900} / [\min(R_{930-980})]$ , in which R and the subscript numbers indicate the light reflectance at the specific wavelength (nm).

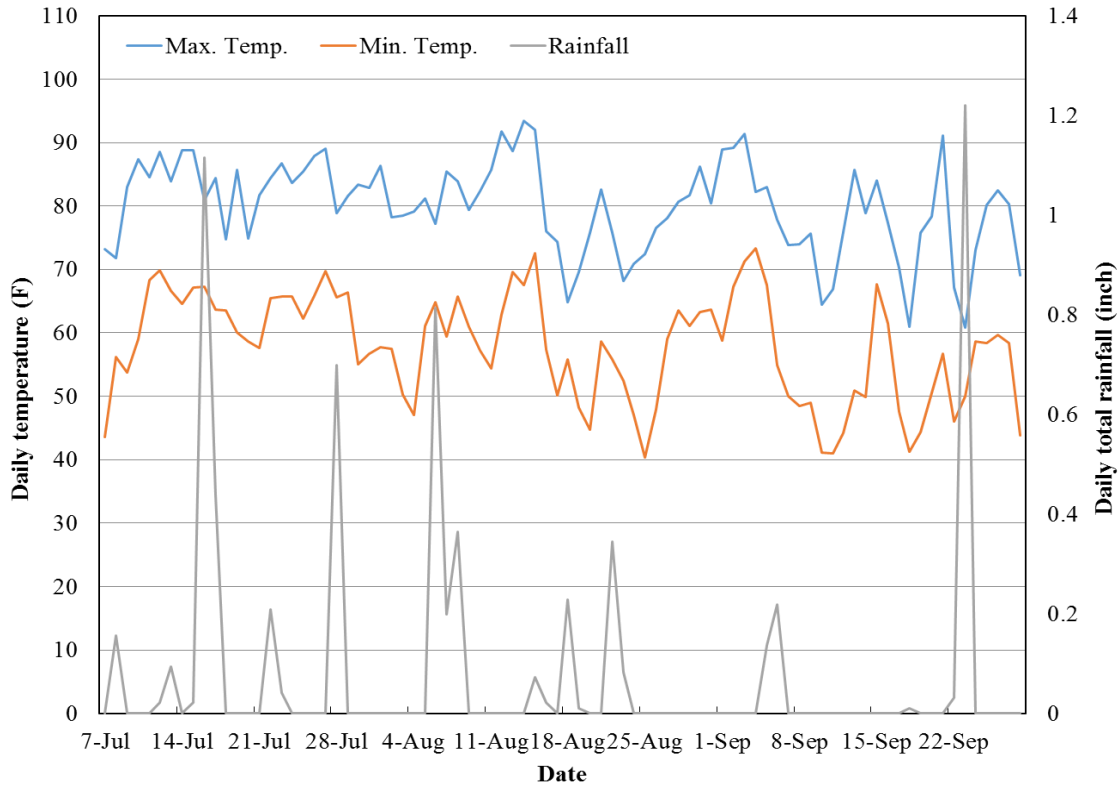


Fig. 1. Daily maximum and minimum temperature and rainfall from July 7 to Sept. 29, 2015 at the Turfgrass Research Center, Fargo, ND.

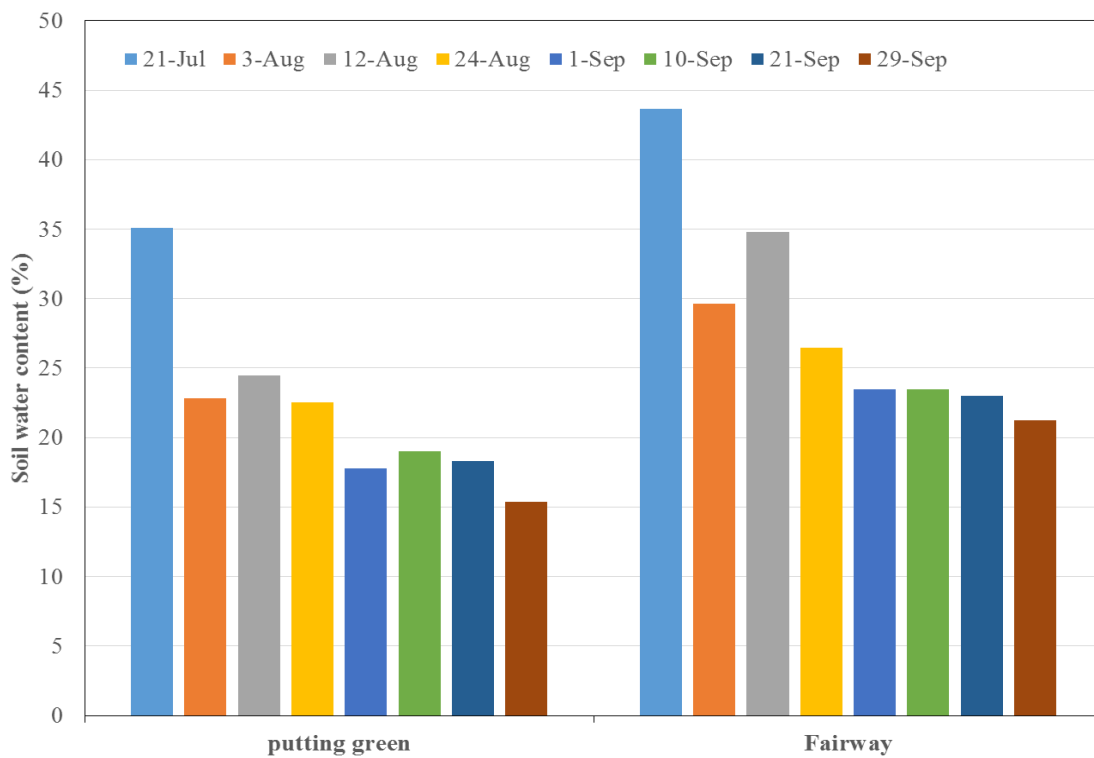


Fig. 2. Soil water content of creeping bentgrass putting green and fairway at Fargo, ND in 2015.