

# Differences in Seedling Growth of 23 Creeping Bentgrass Cultivars under Polyethylene Glycol-induced Drought Conditions

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**SUMMARY.** Drought is the most important abiotic stress in crop production including turfgrass management. Using drought tolerant plants can help minimize stress damage. In this study, 23 commercially available cultivars of creeping bentgrass (*Agrostis stolonifera*) were evaluated for their responses to drought stress that was induced by polyethylene glycol (PEG) 6000 in a hydroponic system during the seed germination and seedling growth stage. In such a system, water potential was adjusted to 0.0 (the control), -0.3, and -0.6 MPa to mimic the drought condition. The absolute water content (AWC), shoot dry weight (SDW), root dry weight (RDW), longest root length (LRL), specific root length (SRL), and root-to-shoot dry weight ratio (RSR) in the plants grown for 4 weeks in the treatment were determined. Results showed that SDW and LRL were unaffected by drought; however, RDW and RSR increased, whereas SRL and AWC were reduced under drought. Among the 23 creeping bentgrass cultivars evaluated, Independence and Crystal Bluelinks had a higher turfgrass performance index (TPI), which represented the number of times a cultivar ranked in the top statistical group across all parameters. The results suggest that 'Independence' and 'Crystal Bluelinks' may be more adapted to drought than the other cultivars at the seedling stage.

Creeping bentgrass is widely used on golf course putting greens and fairways because of its fine leaf texture, high density, and excellent tolerance of low mowing height (Christians, 2011). However, frequent irrigation is required to retain its playability and aesthetical qualities under intensive use. Limited water resources and rapid suburban expansion have led to restricted irrigation in many communities. Deficient irrigation causes reduced turfgrass quality and even plant death when drought stress is severe. In addition to plant damage, prolonged drought can cause a severe economic impact on the turfgrass industry. For example, during a 2-month major drought in conjunction with watering restrictions in New Jersey in 1999, the local turfgrass industry lost \$150 million in revenue (Govindasamy et al., 2007).

It has been reported that inter- and intraspecific differences in drought tolerance in turfgrass exist. Fry and Huang (2004) reported that

tall fescue (*Festuca arundinaceae*) showed very good overall drought tolerance, whereas kentucky bluegrass (*Poa pratensis*) was rated good, and creeping bentgrass and perennial ryegrass (*Lolium perenne*) were rated fair in drought tolerance. Salaiz et al. (1991) indicated that genetic variations led to the differences in evapotranspiration (ET) rates and rooting depths in 10 creeping bentgrass cultivars. Of the 10 cultivars, Penncross showed the highest potential for drought tolerance because of its deep rooting habit and the highest ability to conserve water (i.e., low ET), whereas others only possessed one of the two aforementioned characteristics. Another study of drought tolerance in bentgrass cultivars showed that Penn A-4, Independence, and L-93 were more tolerant than Penncross, Putter, and Declaration (McCann and Huang, 2008).

In the last decade, a few improved creeping bentgrass cultivars have been released with high competitiveness

over annual bluegrass (*Poa annua*), such as T-1 and Alpha (Brede, 2007) and high overall quality, such as 007 and Tyece (National Turfgrass Evaluation Program, 2008), but very limited information on their drought tolerance is available. Turfgrass is more likely to experience drought stress at the seedling stage than the vegetative growth stage because of limited root system during the initial growth (i.e., seedling stage) (Reicher et al., 2006). Low drought tolerance at the seedling stage may easily result in failure of turfgrass establishment (Chai et al., 2006). The objective of this study was to compare the drought tolerance of 23 commonly used creeping bentgrass cultivars at the seedling growth stage.

## Materials and methods

Twenty-three creeping bentgrass cultivars used in this study are listed in Table 1. Seeds were germinated in a hydroponic system, following the method of Zhang et al. (2011). Briefly, 23 cells (6 cm diameter) were created on a 50 × 30 × 2-cm foam plate (one cell for each cultivar). Two layers of plastic screen were glued on the bottom of the plate. Two hundred fifty milligrams of seeds of one cultivar were placed in one cell. Each plate was floated in PEG-6000 (VWR International, Batavia, IL) solutions at 0.0 MPa [0% PEG-6000 (control, nonstressed)], -0.3 MPa (15% PEG-6000), or -0.6 MPa (22.5% PEG-6000) (Michel and Kaufmann, 1973). As seeds germinated, roots penetrated through the plastic screen into the PEG-6000 solution. No Hoagland's solution (Hoagland and Arnon, 1950) was added to the PEG-6000 solutions because nutrients are not required for turfgrass germination for up to 5 weeks (Horst and Taylor, 1983). The hydroponic system was constructed in a controlled culture room with an air temperature of 25 ± 2 °C under fluorescent light (36 μmol·s<sup>-1</sup>·m<sup>-2</sup>) with a 12-h photoperiod. Seeds were maintained in the hydroponic system for 4 weeks, during which

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## Units

| To convert U.S. to SI, multiply by | U.S. unit | SI unit | To convert SI to U.S., multiply by |
|------------------------------------|-----------|---------|------------------------------------|
| 0.1                                | bar       | MPa     | 10                                 |
| 2.54                               | inch(es)  | cm      | 0.3937                             |
| 28,350                             | oz        | mg      | 3.5274 × 10 <sup>-5</sup>          |
| (°F - 32) ÷ 1.8                    | °F        | °C      | (°C × 1.8) + 32                    |

the hydroponic system was constantly aerated to maintain the solubility and availability of oxygen (Mexal et al., 1975).

The experiment was arranged in a split-plot design with the drought level (i.e., PEG-6000 solution) being the whole-plot factor and cultivar being the subplot factor. The whole-plot factor was arranged in a randomized complete block design with three replications. Shoots and roots were harvested separately when the experiment was ended at week 4. Shoot fresh weight and the LRL were recorded immediately after the harvest. SDW and RDW were determined after tissues were oven-dried at 65 °C for 24 h. RSR was then calculated. AWC was determined as the difference between shoot fresh and dry weights (Hughes et al.,

1970). SRL was the ratio between LRL and RDW (Pérez-Harguindeguy et al., 2013). To avoid genetic differences in seed size, seedling vigor, and growth habit in different cultivars, data of the control treatment were standardized as 100% of each cultivar and the data at -0.3 and -0.6 MPa were presented as percentage of the control within each cultivar (Horst and Dunning, 1989). Data were then subjected to PROC MIXED (SAS version 9.4; SAS Institute, Cary, NC) with drought and cultivar being the fixed effects and replicate and replicate × drought being the random effects. Means were separated with the PDIFF statement of the least square means at  $P \leq 0.05$  (SAS version 9.4). Correlation coefficient analysis ( $r$ ) was performed using PROC CORR (SAS version 9.4) to

examine the relationships among the growth parameters. TPI was generated for each cultivar, representing the number of times a cultivar occurred in the top statistical group (Wherley et al., 2011).

**Results**

SDW was unaffected by the drought condition (Tables 2 and 3); however, significant differences in SDW among bentgrass cultivars were observed (Table 2). ‘Bengal’ showed the highest SDW (170.9% of the control), whereas ‘007’ had the lowest SDW (67.1% of the control) (Table 4). The average SDW of ‘Kingpin’, ‘Crystal Bluelinks’, ‘Independence’, ‘Declaration’, and ‘Pin-up’ was 132.0% of the control, which was 86.2% higher than that of ‘Penncross’, ‘Memorial’, and ‘007’ (average = 70.9% of the control).

**Table 1. Seed source, cultivars name, and year of release of the 23 commercially available creeping bentgrass cultivars used in the study.**

| Seed source                           | Cultivars name and yr of release  |
|---------------------------------------|---|
| Barenbrug USA (Tangent, OR)           | Bengal (2002)   |
| J.R. Simplot (Liberty Lake, WA)       | Putter (1989), South Shore (1992), L-93 (1995), Alpha (2004), T-1 (2004), V8 (2010)               |
| Lebanon Seaboard Co. (Lebanon, PA)    | Independence (2002), Declaration (2005)   |
| DLF Pickseed USA (Halsey, OR)         | Cobra 2 (2008)  |
| ProSeeds Marketing (Jefferson, OR)    | Kingpin (2006), Pin-up (2010)   |
| Seed Research of Oregon (Tangent, OR) | 007 (2006), MacKenzie (2006), SR 1150 (2006), Tyee (2006), Focus (2012)                           |
| Tee-2-Green (Hubbard, OR)             | Penn A-1 (1995), Penn A-4 (1995), Penncross (1955), Pennlinks II (2004), Crystal Bluelinks (2007) |
| The Scotts Co. (Marysville, OH)       | Memorial (2004)   |

**Table 2. Analysis of variance of creeping bentgrass cultivars grown under drought at the seedling stage. Data at -0.3 and -0.6 MPa were presented as percentage of the control (0.0 MPa) within each cultivar.<sup>2</sup>**

| Source of variation | df | Shoot dry wt | Absolute water content | Longest root length | Root dry wt | Specific root length | Root:shoot dry wt ratio |
|---------------------|----|--------------|------------------------|---------------------|-------------|----------------------|-------------------------|
| Drought (D)         | 2  | NS           | *                      | NS                  | *           | *                    | *                       |
| Cultivar (C)        | 22 | *            | *                      | *                   | *           | NS                   | *                       |
| D × C               | 44 | NS           | NS                     | NS                  | NS          | NS                   | NS                      |

<sup>2</sup>Drought was induced by polyethylene glycol 6000 at 0.0, -0.3, and -0.6 MPa; 1 MPa = 10 bar.

\*. NS:Significant and no significant differences at  $P \leq 0.05$ .

**Table 3. Relative seedling growth of 23 creeping bentgrass cultivars as affected by drought. Data at -0.3 and -0.6 MPa were standardized as percentage of the control (0.0 MPa) within each cultivar. Data were pooled across cultivars.**

| Drought (MPa) <sup>2</sup> | Shoot dry wt         | Absolute water content | Longest root length | Root dry wt | Specific root length | Root:shoot dry wt ratio |
|----------------------------|----------------------|------------------------|---------------------|-------------|----------------------|-------------------------|
| (% of the control)         |                      |                        |                     |             |                      |                         |
| 0.0                        | 100.0 a <sup>3</sup> | 100.0 a                | 100.0 a             | 100.0 b     | 100.0 a              | 100.0 b                 |
| -0.3                       | 115.1 a              | 91.7 a                 | 109.9 a             | 384.1 a     | 34.5 c               | 361.7 a                 |
| -0.6                       | 97.3 a               | 52.5 b                 | 73.7 a              | 204.7 b     | 58.2 b               | 262.5 a                 |

<sup>2</sup>Drought was induced by polyethylene glycol 6000 at 0.0, -0.3, and -0.6 MPa; 1 MPa = 10 bar.

<sup>3</sup>Means followed by the same letter are not significantly different from each other via PDIFF statement of the least square means at  $P \leq 0.05$  (SAS version 9.4; SAS Institute).

There were no differences in AWC in the plants grown at  $-0.3$  MPa compared with those at  $0.0$  MPa when data were pooled across cultivars (Table 3). As the drought severity increased to  $-0.6$  MPa, AWC decreased to 52.5% of the control. Among all cultivars, Crystal Blue-links, Declaration, Penn A-4, and Penn A-1 had a similar AWC that was higher than the other cultivars when data were pooled across PEG concentrations (Table 4). The average AWC of 'Pin-up', 'Alpha', 'Independence', and 'V8' was 91.8% of the control, significantly higher than 'L-93', '007', and 'Penncross'. Limited differences in AWC were observed among the other cultivars.

No significant difference between the nonstressed and drought-induced conditions was observed for LRL (Table 2). LRL was 94.5% of the control when data were pooled across drought conditions (Table 3). Significant differences in LRL were observed in the bentgrass cultivars, ranging from 122.9% of the control in Independence to 71.9% of the control in Memorial (Table 4). 'Penncross', 'Crystal Blue-links', and 'Tyee' had a higher LRL than that of 'L-93', 'South Shore', 'Pin-up', 'Bengal', 'Pennlinks II', and 'Memorial'. Although 'SR 1150' had a similar LRL as 'Penncross', 'Crystal Blue-links', and 'Tyee', but it performed better than 'Bengal', 'Pennlinks II', and 'Memorial'.

RDW was 384.1% of the control at  $-0.3$  MPa, 152.1% higher than that at  $0.0$  and  $-0.6$  MPa (average = 152.4% of the control) (Table 3). Among the cultivars, Independence and Memorial had the highest and lowest RDW, respectively, when data were pooled across drought conditions (Table 4). RDW of 'Alpha' and 'L-93' was similar to that of 'Independence'. Five bentgrass cultivars (Independence, Alpha, L-93, MacKenzie, and SR 1150) had a higher RDW than 'Memorial'.

SRL was reduced at  $-0.3$  and  $-0.6$  MPa compared with the control, with the highest reduction at  $-0.3$  MPa (Table 3). All bentgrass cultivars showed a similar level of SRL with an average of 64.3% of the control (Table 4).

Plants grown at  $-0.3$  and  $-0.6$  MPa had a similar level of RSR (average = 312.1% of the control) (Table 3). 'L-93' and 'Pin-up' had the highest

and lowest RSR, respectively (Table 4). Bentgrass cultivars ranked closely in RSR, with 11 cultivars performed similarly as L-93 and 13 cultivars performed similarly as Pin-up.

All growth parameters were significantly correlated with each other, except SRL and AWC (Table 5). The highest correlation coefficient was observed between RDW and SRL ( $r = -0.69$ ), whereas the lowest correlation coefficient was between RSR and SDW ( $r = -0.19$ ) and between SDW and RSR ( $r = -0.19$ ). 'Independence' appeared four times in the top ranking group, followed by 'Crystal Blue-links' (Table 4). By contrast, 'Cobra 2', 'V8', 'Pennlinks II', 'South shore', and 'Memorial' performed poorly (TPI = 0).

## Discussion

Creeping bentgrass is one of the most common turfgrass species used on golf putting greens and fairways and requires the most intensive management input (including irrigation) of all golf course turfgrass species. Rooting characteristics, including RDW and LRL, are closely related to water uptake. SRL is the ratio between water acquisition (i.e., LRL) and carbohydrate investment (i.e., RDW) (Pérez-Harguindeguy et al., 2013). Plants with a high SRL are generally considered more efficient in water uptake per unit dry weight (Pérez-Harguindeguy et al., 2013). Huang and Fry (1998) reported a higher SRL in the drought tolerant tall fescue cultivar, K-31, than the sensitive one, MIC18. Similar results were also reported by Huang (2001). However, drought tolerance of centipedegrass (*Eremochloa ophiuroides*), seashore paspalum (*Paspalum vaginatum*), common bermudagrass (*Cynodon dactylon*), and zoysiagrass (*Zoysia* sp.) was negatively associated with SRL under both well-watered and drought conditions (Huang et al., 1997). Pérez-Harguindeguy et al. (2013) suggested that LRL and RDW both need to be taken into consideration when evaluating SRL, as high SRL may be a result of LRL, RDW, or both. Deep rooting is one of the major morphological traits for drought avoidance, indicating a high potential to acquire water from deep soil layers (McCann and Huang, 2008). Plants with deep roots often exhibit low root diameter;

therefore, having increased root surface area for high water and nutrient absorption. Plants with low RDW, in general, indicate that they have low penetration to exploit deep soil layers and poor root longevity (Pérez-Harguindeguy et al., 2013). In this study, drought stress enhanced RDW, but did not affect root elongation which may explain the reduction of the SRL (Table 4). Furthermore, SRL was more closely correlated with RDW ( $r = -0.69$ ) than LRL ( $r = -0.19$ ) in the present study (Table 5).

Our results indicated that SDW was unaffected by drought. However, AWC, an indicator of leaf area, was decreased at  $-0.6$  MPa (Table 3), which is in agreement with previous research findings that cell expansion is more sensitive to drought than photosynthetic traits, such as chlorophyll content, stomatal regulation, and carbon dioxide assimilation (Pugnaire et al., 1999). This study suggested that the bentgrass leaf was smaller but thicker at  $-0.6$  MPa. Plants often have a reduced leaf number and a smaller leaf surface area when exposed to drought to minimize water requirement and water loss caused by transpiration. Such morphological changes in return contribute to low carbohydrate assimilation, especially under prolonged drought conditions, resulting a deficient energy supply for survival, poststress recovery or both. Zhang et al. (2015) reported thickened cuticles in drought-stressed sugarcane (*Saccharum officinarum*). Furthermore, the drought resistant sugarcane cultivar, F172, showed higher thickness of the lower epidermal cuticle than the sensitive YL6.

Drought may also influence carbon allocation in plants. For example, RSR was higher in drought-stressed rice (*Oryza sativa*) compared with the well-watered plants (Xu et al., 2015). Similar results were reported in other crops, including oleander [*Nerium oleander* (Niu and Rodriguez, 2008)], cotton [*Gossypium hirsutum* (Pace et al., 1999)], and arabidopsis [*Arabidopsis thaliana* (Durand et al., 2016)]. Huang and Gao (2000) reported an increased proportion of  $^{14}\text{C}$  allocated to the root than shoot in tall fescue cultivars under the soil drying condition, with a greater increase in tolerant cultivars, and a higher proportion in the deeper root. Drought could inhibit or deactivate metabolic activities in shoot tissue

**Table 4. Relative shoot and root growth of 23 creeping bentgrass cultivars grown under drought at the seedling growth stage. Data at  $-0.3$  and  $-0.6$  MPa were standardized as percentage of the control (0.0 MPa) within each cultivar. Data were pooled across drought levels (0.0,  $-0.3$ , and  $-0.6$  MPa). Cultivars are ordered according to their turf performance index.<sup>z</sup>**

| Cultivar          | Shoot dry wt           | Absolute water content | Longest root length | Root dry wt | Specific root length | Root:shoot dry wt ratio | Turf performance index <sup>y</sup> |
|-------------------|------------------------|------------------------|---------------------|-------------|----------------------|-------------------------|-------------------------------------|
|                   | (% of the control)     |                        |                     |             |                      |                         |                                     |
| Independence      | 130.5 a-d <sup>x</sup> | 92.0 c-g               | 122.9 a             | 454.5 a     | 51.5 a               | 341.2 ab                | 4                                   |
| Crystal Bluelinks | 136.7 a-c              | 127.0 a                | 108.8 ab            | 253.2 b-f   | 60.1 a               | 163.8 ef                | 3                                   |
| Declaration       | 128.8 a-d              | 123.0 ab               | 97.3 b-e            | 250.3 b-f   | 54.8 a               | 185.2 d-f               | 2                                   |
| Alpha             | 108.6 b-f              | 93.3 c-g               | 91.1 b-f            | 328.1 ab    | 65.9 a               | 303.9 a-d               | 2                                   |
| SR 1150           | 98.3 b-f               | 64.3 hi                | 105.9 a-c           | 283.7 b-e   | 57.4 a               | 286.8 a-e               | 2                                   |
| L-93              | 85.0 d-f               | 59.2 i                 | 85.6 c-f            | 319.3 a-c   | 62.0 a               | 364.2 a                 | 2                                   |
| Penncross         | 74.5 ef                | 58.4 i                 | 110.9 ab            | 212.8 b-f   | 81.5 a               | 305.5 a-d               | 2                                   |
| Kingpin           | 138.5 ab               | 95.6 b-e               | 93.0 b-e            | 225.9 b-f   | 54.7 a               | 175.6 d-f               | 1                                   |
| Pin-up            | 125.5 a-d              | 94.8 c-f               | 85.2 c-f            | 150.7 ef    | 65.2 a               | 126.5 f                 | 1                                   |
| Penn A-4          | 121.0 b-e              | 108.7 a-c              | 90.5 b-f            | 201.2 b-f   | 58.3 a               | 171.8 d-f               | 1                                   |
| Penn A-1          | 109.5 b-f              | 102.2 a-d              | 94.6 b-e            | 221.3 b-f   | 84.0 a               | 187.0 d-f               | 1                                   |
| Mackenzie         | 98.1 b-f               | 77.7 d-i               | 92.8 b-f            | 298.1 b-d   | 53.7 a               | 294.6 a-e               | 1                                   |
| Focus             | 95.1 b-f               | 74.0 e-i               | 95.4 b-e            | 250.6 b-f   | 57.7 a               | 296.4 a-d               | 1                                   |
| 007               | 67.1 f                 | 58.4 i                 | 101.0 b-d           | 173.4 d-f   | 75.1 a               | 329.7 a-c               | 1                                   |
| Bengal            | 170.9 a                | 70.1 e-i               | 83.3 d-f            | 197.8 b-f   | 58.3 a               | 181.2 d-f               | 1                                   |
| Putter            | 86.2 d-f               | 67.9 f-i               | 92.1 b-f            | 215.5 b-f   | 55.5 a               | 288.7 a-e               | 1                                   |
| Tyee              | 84.0 d-f               | 68.2 e-i               | 106.9 ab            | 188.2 c-f   | 75.3 a               | 245.6 a-f               | 1                                   |
| T-1               | 85.7 d-f               | 75.3 d-i               | 93.1 b-e            | 188.3 c-f   | 61.8 a               | 237.6 a-f               | 1                                   |
| V8                | 103.9 b-f              | 86.9 c-h               | 93.0 b-e            | 194.9 b-f   | 70.5 a               | 199.9 c-f               | 0                                   |
| Cobra 2           | 90.7 c-f               | 67.9 f-i               | 94.9 b-e            | 217.6 b-f   | 57.3 a               | 250.5 a-f               | 0                                   |
| South Shore       | 96.5 b-f               | 72.0 e-i               | 85.4 c-f            | 169.1 d-f   | 64.9 a               | 197.7 c-f               | 0                                   |
| Pennlinks II      | 88.9 d-f               | 68.8 e-i               | 78.5 ef             | 154.3 ef    | 74.6 a               | 187.1 d-f               | 0                                   |
| Memorial          | 71.1 ef                | 66.6 g-i               | 71.9 f              | 131.4 f     | 77.7 a               | 231.8 b-f               | 0                                   |

<sup>x</sup>Drought was induced by polyethylene glycol 6000 at 0.0,  $-0.3$ , and  $-0.6$  MPa; 1 MPa = 10 bar.

<sup>y</sup>Turfgrass performance index represents the number of times a cultivar occurred in the top statistical group.

<sup>z</sup>Means followed by the same letter are not significantly different from each other via PDIFF statement of the least square means at  $P \leq 0.05$  (SAS version 9.4; SAS Institute).

**Table 5. Correlation coefficients ( $r$ ) among shoot dry weight (SDW), absolute water content (AWC), longest root length (LRL), root dry weight (RDW), specific root length (SRL), and root to shoot dry weight ratio (RSR) of 23 creeping bentgrass cultivars grown under drought at the seedling growth stage. Data at  $-0.3$  and  $-0.6$  MPa were standardized as percentage of the control (0.0 MPa) within each cultivar. Data were pooled across drought levels and cultivars.<sup>z</sup>**

|     | SDW  | AWC   | LRL   | RDW   | SRL                 | RSR    |
|-----|------|-------|-------|-------|---------------------|--------|
| SDW | 1.00 | 0.41* | 0.22* | 0.27* | -0.30*              | -0.19* |
| AWC |      | 1.00  | 0.40* | 0.24* | -0.05 <sup>ns</sup> | -0.22* |
| LRL |      |       | 1.00  | 0.53* | -0.19*              | 0.36*  |
| RDW |      |       |       | 1.00  | -0.69*              | 0.36*  |
| SRL |      |       |       |       | 1.00                | -0.62* |
| RSR |      |       |       |       |                     | 1.00   |

<sup>z</sup>Drought was induced by polyethylene glycol 6000 at 0.0,  $-0.3$ , and  $-0.6$  MPa; 1 MPa = 10 bar.

\*. <sup>ns</sup>Significant differences and no significant differences at  $P \leq 0.05$ .

to minimize the use of water and nutrients, whereas metabolic activities in roots are enhanced to improve water and nutrient uptake (Gargallo-Garriga et al., 2014). In this study, drought stress increased RSR, which was primarily the result of enhanced root growth (Table 4). Similarly, RSR was more closely related to RDW ( $r = 0.36$ ) than SDW ( $r = -0.19$ ) (Table 5). Xu and Zhou (2005) and Xu et al. (2010) suggested that such an asymmetric growth trend would diminish

or be reversed under severe drought because of a significant reduction in root demand for carbon allocation.

Although differences in the shoot and root growth among the 23 creeping bentgrass cultivars were observed in the present study, the ranking of their relative growth was close (Table 4). Under the drought condition, 'Independence' and 'Crystal Bluelinks' were more drought tolerant (i.e., higher TPI), whereas 'Cobra 2', 'V8', 'Pennlinks II', 'South shore', and 'Memorial'

were drought sensitive (TPI = 0), and the other 16 cultivars were intermediate between the two contrasting groups.

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