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Seed Germination of Five *Poa* Species at Negative Water Potentials

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Abstract

Under field conditions water is often inadequate for satisfactory seed germination. An experiment was conducted to determine the effects of simulated dry conditions on germination and seedling growth of five bluegrass (*Poa*) species, including: Texas, *P. arachnifera* Torr.; annual, *P. annua* L.; mutton, P. *fendleriana* Vasey; Kentucky, *P. pratensis* L., and Sandberg, *P. secunda* J. Presl. bluegrasses. Fifty-seed samples of each species were germinated at water potentials of −1.6, −0.8, −0.4, −0.2, and 0 MPa for a 42-d period. Annual and Sandberg bluegrass tolerated moisture stress at germination better than the other species, requiring 14 d to reach 40% germination in water of −0.8 MPa potential. Kentucky bluegrass required 21 d to reach 40% germination under similar conditions. Mutton and Texas bluegrass were the least tolerant of low water potentials, requiring 42 d to reach 20% germination. Selection for increased seed germination at low water potentials should be possible in *Poa* species assuming the trait has moderate heritability.

Keywords

1. Introduction

The genus *Poa* consists of more than 300 species that occur in temperate regions throughout the world [1] [2]. Commonly known as "bluegrass" in North America and "meadow-grass" in Europe and Asia, many of the species are important pasture grasses which are palatable and nutritious to livestock [2]. Barkworth *et al*. [3] recognized 75 bluegrass species for North America. Of the 75 species listed, Kentucky bluegrass (*P. pratensis* L.) is undoubtedly the most common and occurs in all 50 states of the USA [4]. Kentucky bluegrass has world importance where it is used for turf and for livestock forage [2]. Annual bluegrass (*P. annua* L.) is also of world importance as a weed and as a turfgrass and has been reviewed by Vargas and Turgeon [5]. Of regional importance,

mutton bluegrass (*P. fendleriana* Vasey) is adapted to cool temperatures and a variety of rangeland soils in the Rocky Mountains in the USA, making it excellent spring forage [4]. Texas bluegrass (*P. arachnifera* Torr.) is also adapted to a variety of soils in the central and southern Great Plains of the USA where it makes excellent winter forage for livestock [2]. Sandberg bluegrass (*P. secunda* J. Presl) occurs throughout western North America providing spring and fall grazing to all classes of livestock and wildlife [6].

The germination characteristics of Kentucky bluegrass have been researched extensively. Hite [7] found that direct sunlight or diffuse light a few hours each day in alternating temperatures of 20˚C - 30˚C gave a complete viability test for Kentucky bluegrass. The seed germination testing requirements for many *Poa* spp., including Kentucky bluegrass, are to germinate seeds in petri dishes or germination boxes on paper towel substrates moistened with distilled water incubated at 15˚C - 25˚C for 16 h dark and 30˚C for 8 h light for 21 to 35 d; counting seedlings every 7 to 10 d [8]. The effects of salinity on germination of Kentucky bluegrass cultivars and other turfgrass species cultivars have been examined by Harivandi *et al.* [9] and Horst and Taylor [10]. Harivandi *et al.* [9] compared the germination of "Merion" Kentucky bluegrass; "Penfine" perennial ryegrass (*Lolium perenne* L.), "Seaside" creeping bentgrass (*Agrostis palustris* Huds.), "Dawson" slender creeping red fescue (*Festuca rubra* var. *trichophylla* Ducros ex Gaud.), "Fults" weeping alkaligrass [*Puccinellia distans* (L.) Parl.], and "common" Lemmon alkaligrass [*Puccinellia lemmoni* (Vasey) Scribn.] in soils with differing degrees of salinity and found that Kentucky bluegrass had the lowest germination percentage compared with the other five species. Horst and Taylor [10] reported differences among 44 cultivars of Kentucky bluegrass for their tolerance to germinate in saline (equal portions of NaCl and CaCl$_2$) solutions. They concluded that enough variation existed among the 44 cultivars to breed and select for salt tolerance, but that apomictic reproduction may limit genetic improvement through recurrent selection. Larsen *et al.* [11] studied the interactions of water potential and temperature on the germination of Kentucky bluegrass, red fescue, and perennial ryegrass. They found that seed germination of all species decreased as water potential increased and that the interacting effects of temperature and water potential affected their ability to predict germination performance across all temperatures and water potential conditions.

Although periodic rainfall sometimes occurs during drought, it is virtually impossible for germinated seeds to become established and survive [12]. Moisture stress has been shown to decrease seed germination and seedling growth in several plant species [11] [13]-[16], and several experiments have simulated the effects of moisture stress on seed germination and growth of plants [13] [15]-[20]. These studies used a number of chemical compounds to simulate water deficits, such as carbowax 6000, D-mannitol, glucose, NaCl, sucrose, polyethylene glycol (PEG), and polyvinylpyrrollidone. The most commonly used compounds are mannitol and polyethylene glycol because they are considered chemically inert and nontoxic to plant growth [15] [21].

Knowledge of seed germination can help plant breeders develop lines and/or cultivars with increased seed germination and seedling growth at low water potentials. For example, Springer [22] determined that it was possible to select for increased seed germination in sand bluestem (*Andropogon hallii* Hack.) using recurrent selection. He found after two selection cycles that 7-d seed germination was increased by 16.9% over that of the base population at a water potential of −0.8 MPa and under field conditions emergence was increased by 8.6% after two selection cycles [23]. Hodgson-Kratky [24] reported that three cycles of recurrent selection increased seed germination of Russian dandelion (*Taraxacum kok-saghyz* L. E. Rodin) breeding lines 34.5% and 42.5%, and reduced germination time 3 and 5 d, respectively, for seeds incubated at low water potentials. Thus, the objective of this experiment was to determine the effects of moisture stress on the seed germination, rate of germination, and seedling growth of five *Poa* species.

2. Materials and Methods

Plant Materials

Seeds of mutton bluegrass, Kentucky bluegrass and Sandberg bluegrass used in this experiment were obtained from a commercial seed source. Seeds of annual bluegrass (PI 659870) were obtained from the National Genetic Resource Program, USDA-ARS [25], and seeds of Texas bluegrass were grown at the Southern Plains Range Research Station, USDA-ARS, Woodward, Oklahoma, USA (36˚25'N, 99˚24'W, elevation 586 m). Seeds of Texas bluegrass were harvested and processed according to the procedures of Goldman [26]. Species were selected based on modes of reproduction. In the laboratory seeds of all species were further processed using a South Dakota style seed blower (Seedburo, Chicago, IL, USA). Each seed lot was blown at an air value opening

of 20 mm in 3 g increments to remove light and empty seed units as well as other inert materials. This blowing technique produced a heavy seed fraction that was approximately 98% pure seed for each species. The seeds were further examined using an illuminated magnifier to remove the remaining inert materials.

Forty, 50-seed samples of each species were counted from the processed seeds, weighed, and retained for two germination experiments. For each germination experiment, 20 of the 50-seed samples of each species were randomly assigned to one of five water potential treatments. Water potential treatments of −1.6, −0.8, −0.4, −0.2 and 0 MPa were prepared by mixing 58.3, 29.2, 14.6, 7.3, and 0 g of D-mannitol in 0.5 kg of deionized water, respectively [16]. Deionized water was used as a control, water potential = 0 MPa. D-mannitol was chosen because it acts as an inert osmotic medium [15] [21]. Fifty non-sterilized seeds of each species were placed in sterile, clear plastic boxes (7.0 × 7.0 × 2.5 cm) on two layers of absorbent paper towel substrates moistened with 7 mL of each water potential solution. Germination was conducted in a seed germinator (Seedburo Equipment Company, Chicago, IL, USA) set for 8 h·d^{-1} of fluorescent light at 30°C and 16 h·d^{-1} of darkness at 20°C. These setting are the same as those used by Hite [7]. Cumulative "normal" germination counts were made at 7 d, 14 d, 21 d, 28 d, 35 d, and 42 d [22]. After 42 d, the number of firm (non-germinated, apparently dormant) seed was recorded. In addition, a seedling classified as "normal" would possess both a root and a shoot each 2 mm or more in length. Seedlings with only a root and no shoot or a shoot and no root, but were otherwise normal in appearance, were retained in the germination box for an additional 7 d germination period. After 14 d, if these seedlings had not grown the missing root or shoot, they were classified as "abnormal" [27]. Germination data were converted to percentages before analysis. The experimental design was a factorial arrangement of treatments (species and water potential) in a randomized block design with four replications. The experiment was repeated twice.

The rate (speed of germination) was determined using a modified procedure of Maguire [28]. Maguire [28] stated that, "The germination rate is calculated by dividing the number of normal seedlings per 100 seeds obtained at each counting in the standard germination test by the number of days the seeds have been in the germinator. The values obtained at each count are then summed at the end of the germination test to obtain the germination rate". Since only 50 seeds were used in each experimental unit, we modified the procedure by multiplying the number of seedling counted at each germination count by 2. In addition at each germination count, the root and shoot length of an average normal seedling was determined by using a millimeter ruler.

Data for percentage cumulative normal germination, percentage firm seed remaining and seedling root and shoot lengths were analyzed separately by species as a randomized complete block experiment using PROC GLIMMIX [29]. Water potential treatment was a fixed effect and blocks within experiments were a random effect. Mean separations were made using a least significant difference (LSD) test at $P \leq 0.05$. Linear, quadratic, and cubic effects were obtained for the water potential treatment for each bluegrass species using orthogonal polynomials [29].

3. Results

3.1. Seed Germination

Comparisons among bluegrass species were not performed because seeds were grown in different environments and the age of seeds at germination time were unknown. Therefore, data were analyzed by bluegrass species only making comparisons among water potential treatments. Water potential treatments for 7 - 42 d cumulative "normal" seed germination counts varied within species ($P < 0.05$). In general, as water potential decreased from −1.6 MPa to 0 MPa the percentage germination increased. After a 42 d germination period, very little (< 1%) or no germination occurred for bluegrass seeds germinated in water of −1.6 MPa potential. The effects of water potential treatment were linear ($P < 0.01$) and quadratic ($P < 0.01$) for annual bluegrass and germination was nearly complete in 28 d (**Figure 1**). In deionized water (0 MPa) more than 60% of the seed of annual bluegrass germinated in 7 d. Annual bluegrass had the lowest seed weights, but had high rates of germination (**Table 1**). Similarly, the effects of water potential treatment were linear ($P < 0.01$) and quadratic ($P < 0.01$) for Sandberg bluegrass, and germination was complete in 21 d at all water potential treatments (**Figure 2**). Approximately 60% of the germinable seeds of Sandberg bluestem germinated within 7 d in deionized water (0 Mpa, **Figure 2**). Sandberg bluegrass had the second highest rates of germination at all water potential treatments and the second highest seed weight (**Table 1**). The effects of water potential treatment were linear ($P < 0.01$) and quadratic ($P < 0.01$) for Kentucky bluegrass, and germination was complete in 21 d (**Figure 3**). Less than 20%

Table 1. Mean ± SE for 50-seed weight for five *Poa* species and germination rates of the species at four water potentials.

| *Poa* species | 50-seed weight (mg) | Germination rate at water potential treatment (MPa) | | | |
		−0.8	−0.4	−0.2	0
annua	15.0 ± 0.22	4.2 ± 0.28 a[†]	7.9 ± 0.28 b	9.1 ± 0.28 c	10.8 ± 0.28 d
arachnifera	16.8 ± 0.22	0.5 ± 1.02 a	1.9 ± 1.02 b	2.3 ± 1.02 b	3.6 ± 1.02 c
fendleriana	32.5 ± 0.22	0.8 ± 0.43 a	2.8 ± 0.43 b	3.1 ± 0.43 b	4.2 ± 0.43 c
pratensis	18.3 ± 0.22	2.9 ± 0.57 a	4.7 ± 0.57 b	5.1 ± 0.57 b	6.7 ± 0.57 c
secunda	29.1 ± 0.22	3.9 ±0.89 a	6.6 ± 0.89 b	7.1 ± 0.89 b	9.5 ± 0.89 c

[†]Means of germination rates at different water potential treatment followed by the same letter within species (row) are not significantly different at $P > 0.05$ (LSD test).
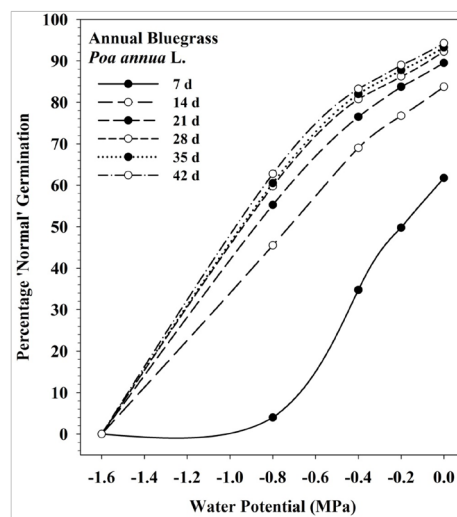

Figure 1. Percentage 7-d through 42-d seed germination of annual bluegrass, *Poa annua* L., germinated at five water potentials. SE of means was 1.9% for 7 d germination, 4.0% for 14 d germination, 3.3% for 21 d germination, 2.7% for 28 d germination, 2.7% for 35 d germination, and 2.5% for 42 d germination.
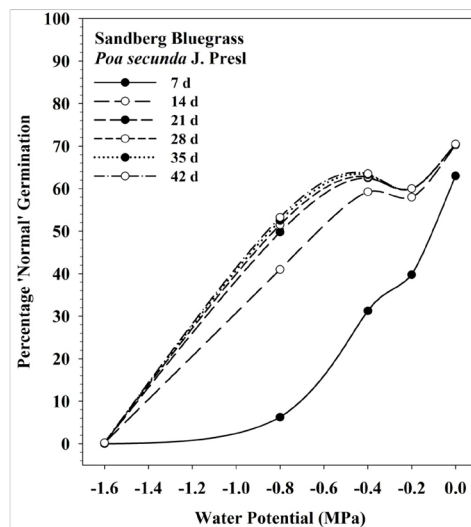

Figure 2. Percentage 7-d through 42-d seed germination of Sandberg bluegrass, *Poa secunda* J. Presl, germinated at five water potentials. SE of means were 4.3% for 7 d germination, 6.3% for 14 d germination, 6.6% for 21 d germination, 6.4% for 28 d germination, 6.1% for 35 d germination, and 5.9% for 42 d germination.
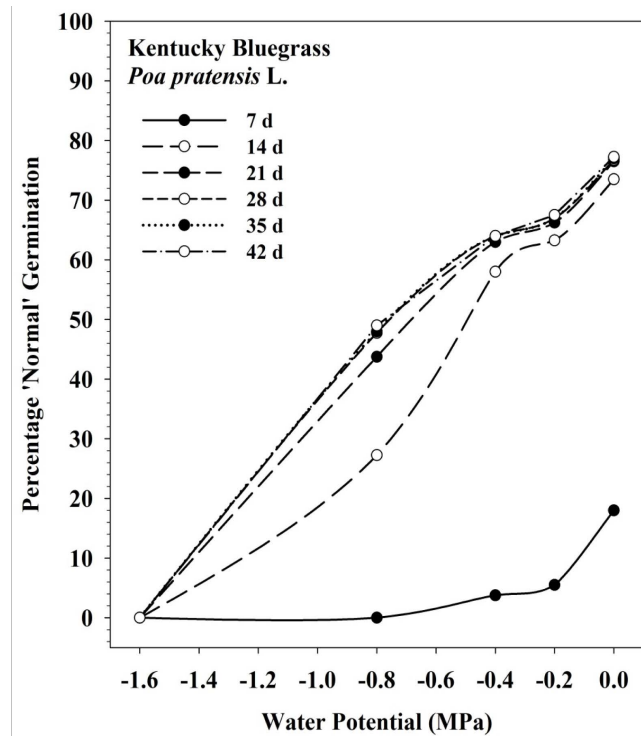
Figure 3. Percentage 7-d through 42-d seed germination of Kentucky bluegrass, *Poa pratensis* L., germinated at five water potentials. SE of means were 1.8% for 7 d germination, 5.9% for 14 d germination, 8.5% for 21 d germination, 9.2% for 28 d germination, 9.1% for 35 d germination, and 9.1% for 42 d germination.

of the seeds of Kentucky bluegrass germinated in 7 d at a water potential of 0 MPa. Kentucky bluegrass had an intermediate rate of germination and seed weight (**Table 1**). For mutton bluegrass, the effects of water potential treatment were linear ($P < 0.01$) and cubic ($P < 0.01$, **Figure 4**). In deionized water, less than 50% of the mutton bluegrass seeds germinated in 14 d. Mutton bluegrass had slow but steady germination, but when water stressed, germination was below 50%. Mutton bluegrass had the heaviest seed and the second lowest rate of germination at all water potential treatments (**Table 1**). The effect of water potential treatment was linear ($P < 0.01$) for Texas bluegrass. Texas bluegrass had the slowest rate of germination at all water potential treatments with slightly over 50% of seeds germinating in 21 d (**Table 1**, **Figure 5**). Like mutton bluegrass, Texas bluegrass had slow but steady increases in germination for water potentials between −0.8 MPa through 0 MPa. Texas bluegrass had the second lowest seed weight (**Table 1**).

3.2. Non-Germinated Seeds

As water potential decreased from −1.6 MPa to 0 MPa the percentage of firm (non-germinated) seed after 42 d in the germinator decreased (**Figure 6**), and the modeling of water potential data as to linear, quadratic, and/or cubic was the same as that for cumulative "normal" germination data as outlined above. Except for mutton bluegrass, the percentage of firm seeds remaining after 42 d in the germinator ranged from 78% - 90%. The percentage of firm seeds was high for mutton bluegrass and Texas bluegrass for water potential −0.8 MPa through 0 MPa indicating that germination was not complete for these species even after 42 d (**Figure 6**).

3.3. Seedling Roots and Shoots

The root lengths of 7-d old seedlings increased as water potential decreased from −1.6 MPa to 0 MPa (**Figure 7**). The five species exhibited linear effects ($P < 0.01$). Three species, Kentucky bluegrass, Sandberg bluegrass and Texas bluegrass, also displayed quadratic effects ($P < 0.01$). Sandberg bluegrass had the longest seedling root lengths at all water potential treatments (**Figure 7**).
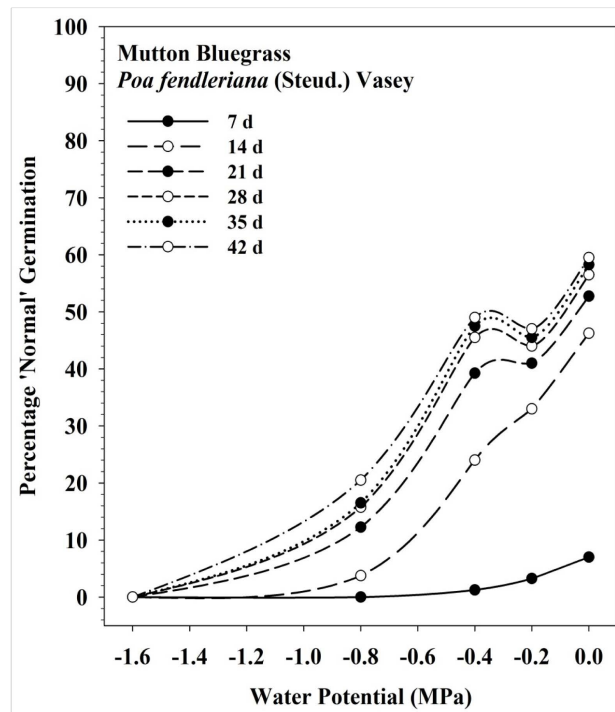
Figure 4. Percentage 7-d through 42-d seed germination of mutton bluegrass, *Poa fendleriana* (Steud.) Vasey, germinated at five water potentials. SE of means were 0.9% for 7 d germination, 3.7% for 14 d germination, 6.0% for 21 d germination, 5.5% for 28 d germination, 5.3% for 35 d germination and 5.4% for 42 d germination.
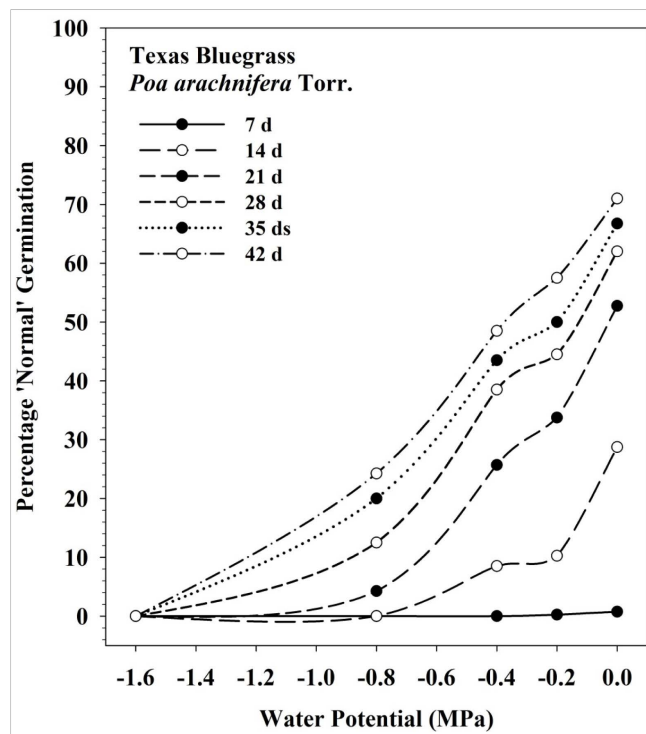

Figure 5. Percentage 7-d through 42-d seed germination of Texas bluegrass, *Poa arachnifera* Torr., germinated at five water potentials. SE of means were 0.3% for 7 d germination, 6.6% for 14 d germination, 13.3% for 21 d germination, 15.0% for 28 d germination, 15.8% for 35 d germination, and 14.7% for 42 d germination.
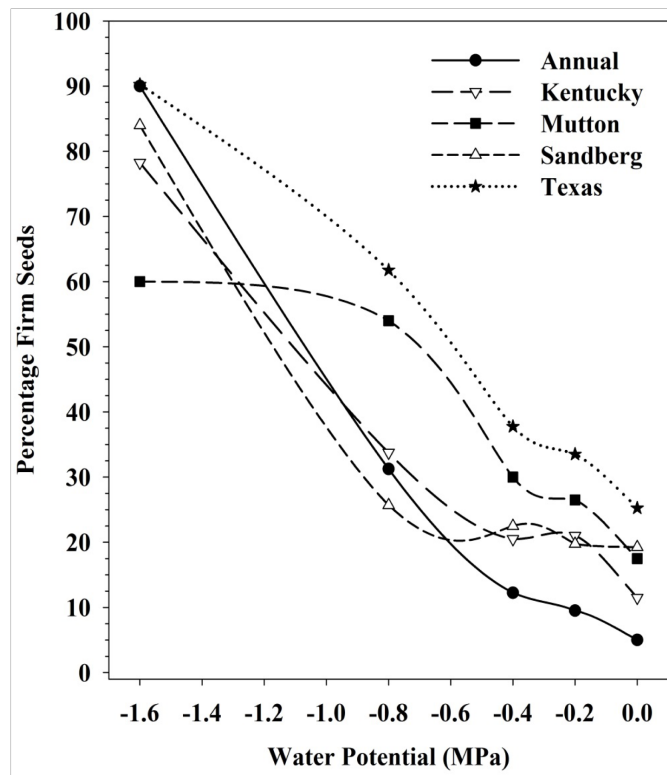
Figure 6. Percentage non-germinated (firm) seed remaining after a42 d germination period for the five bluegrass species germinated at five water potentials. Annual, *Poa annua* L.; Kentucky, *P. pratensis* L.; Mutton, *P. fendleriana* (Steud.) Vasey; Sandberg, *P. secunda* J. Presl; and Texas, *P. arachnifera* Torr. SE of means is 4.8%.
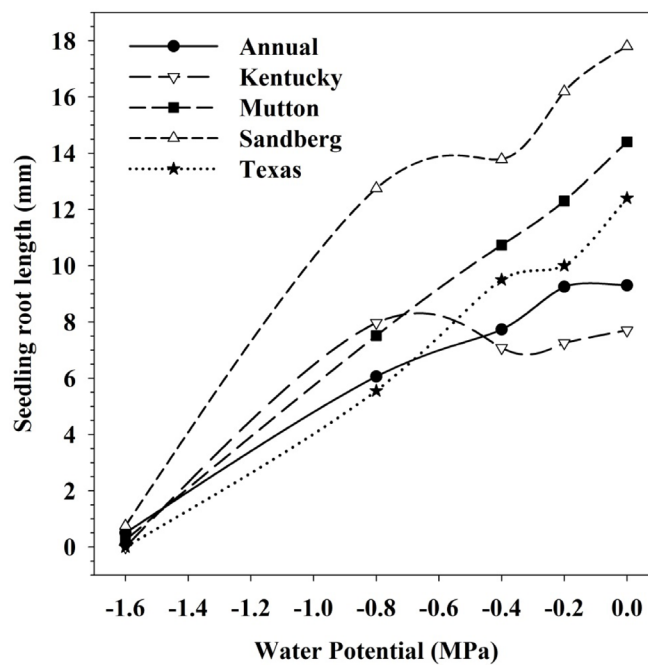

Figure 7. Seedling root length for the five bluegrass species germinated for 7 d at five water potentials. Annual, *Poa annua* L.; Kentucky, *P. pratensis* L.; Mutton, *P. fendleriana* (Steud.) Vasey; Sandberg, *P. secunda* J. Presl; and Texas, *P. arachnifera* Torr. SE of means is 1.3 mm.

The shoot lengths of 7-d old seedlings also increased as water potential decreased from −1.6 MPa to 0 MPa (**Figure 8**). All species exhibited linear effects ($P < 0.01$) and two species, Kentucky bluegrass and Texas bluegrass, also displayed quadratic effects ($P < 0.01$). Sandberg bluegrass also had the longest seedling shoot lengths at all water potential treatments, while the seedling shoot length for Kentucky bluegrass ranged from 6-to-8 mm for water potential treatments −0.8 MPa to 0 MPa (**Figure 8**).

4. Discussion

Annual bluegrass and Sandberg bluegrass appeared to tolerate moisture stress better than the other bluegrass species tested; still, it required 14 d for these species to reach 40% germination in water of −0.8 MPa potential (**Figure 1** and **Figure 2**). Likewise, it required 21 d for Kentucky bluegrass to do the same (**Figure 3**). Mutton bluegrass and Texas bluegrass were the least tolerant of low water potentials, requiring 42 d to reach approximately 20% germination (**Figure 4** and **Figure 5**). Rapid germination is an important factor influencing stand establishment of grasses [30]. In this experiment annual bluegrass and Sandberg bluegrass had the highest rates of germination at water potentials between −0.8 MPa through 0 MPa (**Table 1**).

Very little germination occurred in water of −1.6 MPa potential. Except for mutton bluegrass, the percentage of non-germinated firm seeds remaining after a 42 d germination period exceeded 75% (**Figure 6**). As expected, seedling root and shoot lengths declined as water potential increased. Root and shoot lengths of seedlings that germinated at −0.8 MPa in 7 d were 65% and 58% of the control (0 MPa). Parmar and Moore [15] reported root and shoot lengths of corn (*Zea mays* L.) seedlings that germinated at −1.01 MPa in 5 d were 55% and 32% of the control (0 MPa). Springer [16] reported root and shoot lengths of seedlings of chaffy-seeded grasses that germinated at −0.8 MPa in 7 d were 62% and 45% of the control (0 MPa).

It is possible to improve germination by using phenotypic recurrent selection in sand bluestem [22] [31]. Selecting seeds that germinated at a water potential of −0.8 MPa resulted in improved populations of sand bluestem that averaged 9.1% ± 1.8% increased germination each selection cycle. In addition to selecting for increased seed germination, Springer [22] simultaneously selected for increased spikelet weight, seedling root length, and increased field establishment [31]. Recurrent selection has been used successfully for population improvement
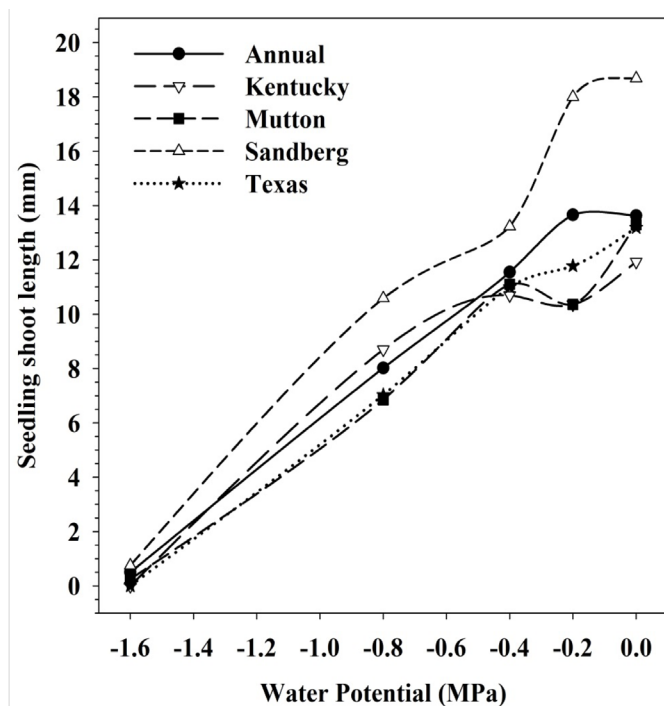

Figure 8. Seedling shoot length for the five bluegrass species germinated for 7 d at five water potentials. Annual, *Poa annua* L.; Kentucky, *P. pratensis* L.; Mutton, *P. fendleriana* (Steud.) Vasey; Sandberg, *P. secunda* J. Presl; and Texas, *P. arachnifera* Torr. SE of means is 1.1 mm.

and cultivar development in several cross-pollinated forage grasses [32]. The use of phenotypic recurrent selection in isolated polycross nurseries allows for the removal of less desirable plant genotypes, thereby increasing the frequency of superior genotypes in the next generation [33].

There are several breeding systems among bluegrass species each presenting challenges for genetic improvement [2] [34]. For example, annual bluegrass is gynomonoecious having both pistillate and hermaphroditic flowers on the same plant [2]. Although annual bluegrass possesses an outbreeding system of reproduction, it is self-compatible which may lead to a high degree of homozygosity. Self-compatibility is a positive pollination mechanism which insures optimal seed production and, it is a considerable advantage for annual plant species [35]. Breeding for improved germination would require testing and selection among many genotypes.

Except for the slowed germination response of Kentucky bluegrass at 7 d, Kentucky bluegrass and Sandberg bluegrass had similar germination patterns. Both of these species reproduce by aposporus apomixis, where an unreduced embryo sac is formed from a somatic cell of the nucellus or chalaza giving rise to an embryo that is formed without fertilization [2] [35] [36]. Obligate apomixis is considered a genetic dead end without the occurrence of sexual reproduction [35]. Kentucky bluegrass produces both apomictic and sexual reproducing flowers. Horst and Taylor [10] found that variation existed among the 44 cultivars of Kentucky bluegrass they tested to breed and select for salt tolerance, but that apomictic reproduction slowed the genetic improvement through recurrent selection. In highly apomictic bluegrass species, direct selection of genotypes for germination at low water potentials should produce superior genotypes for that trait. Several forage grass species that reproduce through apospory have been selected for superior traits and released as cultivars [37]-[39].

Mutton bluegrass is incompletely dioecious [40] with plants usually pistillate or occasionally hermaphroditic [41] and depending on the subspecies reproduces either by apospory apomixis and/or sexual reproduction [3] [41] [42]. Texas bluegrass is completely dioecious [3] and reproduces by sexual reproduction. For sexual reproducing forms of mutton bluegrass and for Texas bluegrass recurrent selection should be possible assuming the trait has moderate heritability. For the genotype of mutton bluegrass tested in this experiment, approximately 5% of the seed germinated in water of −0.8 MPa potential in 14 d. Similarly, for the line of Texas bluegrass tested, approximately 5% of the seed germinated in water of −0.6 MPa water potential in 14 d. By using the techniques of Springer [22], it should be possible to improve seed germination at a low water potential and possibly improve seedling vigor by increasing the rate of germination and size of the caryopsis and seedling. Thus, improving stand establishment.

5. Conclusion

Moisture stress affected the percentage of seed germination, rate of seed germination, and seedling growth of the *Poa* species tested. For these five bluegrass species very little germination occurred in water of −1.6 MPa potential and as water potential decreased from −1.6 to 0 MPa there was a curvilinear increase in seed germination overall. Annual bluegrass and Sandberg bluegrass had the highest rates of germination compared with the other bluegrass species. Seedling root and shoot lengths increased as water stress decreased. After determining a water potential where approximately 5% of the seeds germinate in a set period of time (e.g., 7d or 10d), selection for increased seed germination at a low water potential should be possible in *Poa* species.

Acknowledgements

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