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Evaluating Physiological Responses of Ten Alfalfa (Medicago sativa subsp. falcata) Germplasm to Drought Treatments

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ABSTRACT

Alfalfa is the most widely produced perennial forage legume in North America. However, its use in the semiarid northern Great Plains is limited due to poor stand establishment and persistence under drought condition. The development of drought-tolerant alfalfa cultivars is of great need. Some *Medicago sativa* subsp. *falcata* populations have demonstrated promising drought resistance when compared to *M. sativa*. Morphological and physiological mechanisms play a critical role in drought tolerance by influencing seedling survival, stand establishment and drought recovery. Assessment of variability in physiological responses to drought among germplasm lines to drought is necessary for developing cultivars with improved drought tolerance. A greenhouse study was conducted to evaluate the variations of stomatal conductance, chlorophyll content, and root to shoot (R/S) ratio of eleven alfalfa populations under drought treatments. Eleven entries included one *sativa*-based commercial cultivar, six *falcata*-based populations, and four rangeland naturalized populations. Uniform seedlings from each population were watered to attain drought treatment regime of 100, 50, and 25% of field capacity. The 50 and 25% treatments created mild and severe drought-stress. Two *falcata*-based germplasms originated under annual precipitation of 250mm and 165mm natural environmental conditions demonstrated the drought tolerant associated traits. They showed either the lowest stomatal conductance or the greatest increased leaf chlorophyll content under severe drought among 11 populations. Both exhibited the highest R/S ratio under severe drought. The results

indicated reducing water loss through minimizing transpiration while increasing root water absorption, and the ability of delaying leaf senescence thus retaining photosynthesis are some key traits that may contribute to drought tolerance in these *falcata*-based alfalfa.

Keywords: alfalfa seedling, drought tolerance, stomatal conductance, root to shoot ratio, chlorophyll, *falcata,*

INTRODUCTION

Alfalfa is one of the most important forage legumes in North America and the world. It has been shown that alfalfa has the highest protein production potential among all legumes and grain. Along with its high nutritive value to livestock, legumes like alfalfa provide ecosystem service by fixating atmospheric nitrogen, which benefits other organisms that depend on them. However, lack of soil moisture and frequent drought are the primary limitation to alfalfa establishment and persistence (Misar et al, 2015), and production (Kang et al. 2011). This is especially prevalent in the semi-arid conditions in the Northern Great Plains (NGP) region.

Drought has been defined by the Society of Range Management as "…prolonged dry weather when precipitation is less than 75% of the average amount" (Society for Range Management, 1989). The studies have shown that drought can reduce crop yields by 69% (Boyer, 1982) and caused extensive economic loss for farmers and ranchers. Global climate change is a major driver of more frequent drought. Northern mixed-grass prairie is expected to exhibit greater precipitation variability, which potential result in more frequent and longer-term drought in the future (Kunkel et al., 2013). Development of alfalfa cultivars with improved drought-tolerant at seedling stage is of great need and crucial for successful stand establishment. *Medicago sativa* subsp. *falcata* is commonly referred to as yellow-flowered alfalfa (YFA). Several YFA populations have been observed to have promising drought resistance when compared to *M. sativa.* Assessment of physiological response variability among germplasm lines to drought stress is necessary for developing drought tolerant cultivars.

A major limitation of using YFA in the semi-arid region is due to relatively low seed production and seed shattering (Berdahl et al. ,1986). This has been solved with the finding of the ability to hybridize with purple-flowered alfalfa (PFA) (*M. sativa*) common around the NGP (Oakley and Garver, 1917). Recently, YFA was found naturalized in northwestern South Dakota in the Grand River National Grasslands. This phenomenon indicates that YFA cultivars could be developed for use in harsh environmental conditions (e.g. drought, cold, and grazing) (Boe et al., 2004).

Interest in development of YFA cultivars has existed since it was first introduced to the United States in 1897 from Siberia by Neils E. Hansen (Oakley and Garver, 1917). Oakley and Garver (1917) observed that PFA is predominantly taprooted with a main single large root propelling downward, whereas YFA is characterized by a deep crown with branched and lateral roots sprawling out horizontally. Fibrous roots found in YFA have been shown to be more tolerant of winterkill conditions and tolerate of injury from grazing and drought when compared to PFA (Garver, 1922).

Plants have evolved diverse physiological and morphological mechanisms to adapt to drought-stress conditions (Blum, 1996). One of these mechanisms is altering resource allocation between root and shoot system, such as increase root-to-shoot biomass ratio (R/S) (Harris, 1992). Under drought conditions, plants reduced shoot production to minimize transpiration, but plants often increase the relative proportion of root biomass for exploiting water in soil (Erice et al., 2010). Another mechanism developed in combating drought is through regulating stomatal conductance. Stomatal conductance regulates CO_2 uptake and water loss of plants. Drought tolerant plants can regulate stomatal conductance under drought conditions to limit water loss (Montague et al., 2008). The ability of plants to sustain chlorophyll content under drought conditions is an important adaptation to continue conducting photosynthesis to support growth, particularly root growth. Chlorophyll content deterioration is an early indicator of leaf senescence (Kang et al., 2011). Leaf senescence causes a loss of photosynthetic capacity and ultimately can lead to plant death.

The objective of this study was to determine physiological responses associated with drought tolerance among alfalfa populations under different drought-stress treatments. The hypothesis is that these physiological traits associated with drought tolerance can be used to develop alfalfa with improved seedling survival, stand establishment, and drought recovery in the future.

METHODS

Seed Source

Table 1: Description and marketer/origin of 11 alfalfa populations used to evaluate physiological traits for drought resistance at a greenhouse of South Dakota State University

* indicates the information of PIs from USDA-ARS National Genetic Resources Program

Entry	Description	Marketer/Origin*
PI631677	*M. sativa* subsp. *falcata, NPGS*	Mongolia, Lat. 49°49’32” N, Long. 92°3’48”E Elev. 1,141m
PI631678	*M. sativa* subsp. *falcata, NPGS*	Mongolia, Lat. 49°46’40” N, Long. 91°53’52”E Elev. 1,463m
PI631682	*M. sativa* subsp. *falcata, NPGS*	Mongolia, Lat. 48°10’33” N, Long. 91°45’29”E Elev. 1,232m
PI502441	*M. sativa* subsp. *falcata, NPGS*	Russian Federation, Lat. 46°11’24” N, Long. 43°53’24”E Elev. 55m
PI538984	*M. sativa* subsp. *falcata, NPGS*	Kazakhstan, Lat. 46°11’24” N, Long. 43°53’24”E
NE1010	*M. sativa* subsp. *falcata, NPGS*	Brookings, SD, Lat. 44°18’41” N, Long. 96°47’53”W
SD201	*M. sativa* subsp. *falcata, NPGS*	South Dakota State University experimental for forage and wildlife habitat, Brookings, SD, Lat. 44°18’41” N, Long. 96°47’53”W
SD202 (Coiled)	Predominantly *M. sativa* subsp. *falcata*	South Dakota State University experimental with coil-shaped seed pods collected from a feral population in native rangeland in northwest SD
SD203 (Sickle)	Predominantly *M. sativa* subsp. *falcata*	South Dakota State University experimental with sickle-shaped seed pods collected from a feral population in native rangeland in northwest SD
Wind River (WR)	Predominantly *M. sativa* subsp. *falcata*	Wind River Seed, Manderson, Wyoming, developed by Norman G. Smith, Lodgepole, South Dakota

Persist II	*M. sativa,* Cultivar, Conventional Hay-Type	Millborn Seeds Inc.

Eleven alfalfa populations were evaluated in this study, including 'Persist II', 'SD201', 'SD202', 'SD203', 'Wind River', 'NE1010', and six plant introductions (PIs) (PI631677, PI631678, PI631682, PI502441, and PI538984). The cultivar Persist II is a *M. sativa* type and was purchased from Millborn Seed Company in Brookings, SD. Seeds of SD201, SD202, SD203, and NE1010 were collected from the experimental plots at the Felt Family Research Farm of the South Dakota State University Agricultural Experiment Station. SD201 is a pure *M. falcata* and SD202 and SD203 were derived from naturalized populations in northwestern South Dakota with predominantly *M. falcata* backgrounds. Seeds of Wind River were purchased from Wind River Seed in Manderson , WY with predominantly *M. falcata* base. All PIs were acquired from National Germplasm System and the selection was based on their local distribution latitudes and climate similarity to South Dakota (Table 1).

Experimental Design

The experiment consisted of the eleven populations with seven replicates under three drought intensity regimes (100, 50 & 25% of field capacity) and two drought durations (15 days and 30 days) after imposed to drought regimes initiated. The 50 and 25% of field capacity treatments created mild and severe drought-stress with soil volumetric water content of 12 and 2.2% (Kang et al., 2011). In pre-experiment, field capacity for 100% was determined by watering soil until saturated and letting drain 24 hours while covering the top to prevent evaporation. Then 50 and 25% field capacity was obtained by measuring changes in cone container (Ray Leach "Cone-containers"; Stuewe and Sons, Inc., Tangent, OR) weight daily from 100% of field capacity to 50 and 25% of field capacity without top cover. The amount and frequencies of water added to cone container to maintain relative consistent of 100, 50, and 25% of field capacity were calculated and determined with 10 replicates for each field capacity regime. All experimental units were maintained in greenhouse conditions under 24±3°C, 16-hour light and 8-hour dark photoperiod for the duration of the experiment.

Experimental Procedure and Treatments

For each population, 150 uniform seeds were selected, scarified with sandpaper, and grown in a growth chamber for 7 days at 20°C (Fig. 1A). Uniform seedlings were taken after 7 days and transplanted into 164ml plastic cone containers filled uniformly with ~46g of Sunshine Mix #3 potting soil (Sun Gro Horticulture Canada Ltd. Seba Beach, AB, Canada with two seedlings per container.

Seedlings were first double planted into cone-containers (Fig. 1B) and allowed to grow for 10 days at 100% of field capacity, then thinned to one seedling per cone-container to ensure plants were relatively uniform in size. Seedlings were then grown for 11 more days under 100% of field capacity to reach trifoliate seedling stage with concomitant root development prior to treatment application.

For 100% of field capacity treatments, 21ml of water was given to every other day at the 9 a.m. to noon CT of the day from the beginning of experiment, 50% field capacity was allowed to dry out for 7 days until it just past 50% and watered back to 50% with 11ml of water every other day, 25% field capacity was allowed to dry out for 12 days just past 25% and watered back to 25% with 7ml of water every other day. Water was applied using 10ml pipette mixed with commercial Miracle-Gro® from the Scotts Miracle-Gro Company.

Data Collection

Root and shoot biomass was harvested at completion of each drought duration regime of 15 and 30 days. Soil was removed from belowground tissues by rinsing with water. Root and shoot biomass were separated by cutting at crown position, and dried in an oven at 60°C for 72 hours. Weight of root and shoot biomass was weighed using a 0.0001 gram scale. Stomatal conductance (SC) was measured using a SC-1 Leaf Porometer (Decagon Devices, Inc., Pullman, WA) during 9 a.m. to coon CT on day 15 after drought treatments were imposed with three repetitions per population per treatment (Fig. 1D). Chlorophyll (C) measurements were obtained using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta, Inc., New Jersey) during 9 a.m. to noon CT on day 16 after drought treatments were initiated with 7 repetitions per population per treatment (Fig. 1C).

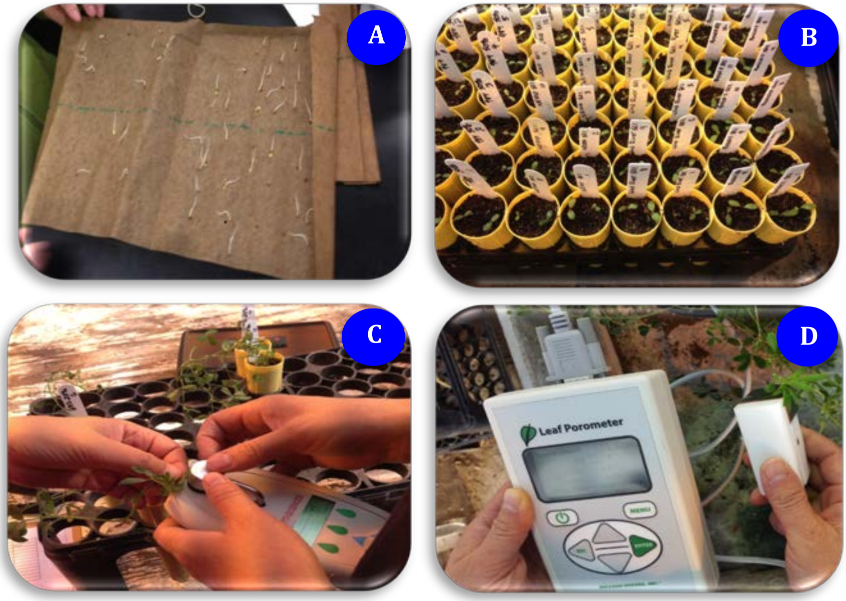

Figure 1: Alfalfa radical growth after 7 days in a growth chamber (A); two emerged alfalfa cotyledon seedlings per container (B); measuring chlorophyll of alfalfa on 16th day after drought treatment initiated (C); and measuring alfalfa stomatal conductance on 15th day after drought treatment initiated (D).

Data Analysis

Root-to-shoot ratio (R/S) was calculated by using the root dry weight divided by the shoot dry. Percent change in chlorophyll content was calculated by finding the difference between the mean chlorophyll content of 100 and 25% of field capacity divided by 100% of field capacity and changing to percent. Differences of physiological traits among populations evaluated with one-way analysis of variance ($p<0.05$) using procedures in SAS statistical analysis software (Institute, 2008) for each drought duration treatment (15 days *vs*. 30 days).

RESULTS

Stomatal Conductance

Stomatal conductance differed among the 11 populations measured at day 15 drought duration treatment at each field capacity (Fig 2). The SC for 25% of field capacity ranged from 11.3 to 36.2 mmol H_2O m^{-2} s^{-1}, 50% of field capacity ranged from 56.5 to 109.3 mmol H_2O m^{-2} s^{-1}, and 100% of field capacity ranged from 71.9 to 135.0 mmol H_2O m^{-2} s^{-1}. The SC of all 11 populations showed the similar response pattern to field capacity treatments, which decreased as field capacity decreased. In general, YFA populations demonstrated lower SC values compared to PFA populations, particularly in 25% of field capacity. PI502441 had the lowest SC and Persist II had the highest SC values (Fig. 2).

Chlorophyll Content

The relative change in chlorophyll content from 100% field capacity to 25% field capacity treatments decreased 25.9% in Persist II and increased 8.7% in PI502441 (Fig. 3). Chlorophyll content increased under severe drought (25% of field capacity) at 15 days drought duration treatment for all PIs (*falcata*-based populations) except PI631682. This indicated that these *falcata*-based populations have the ability of delaying leaf senescence and thus better maintain photosynthesis.

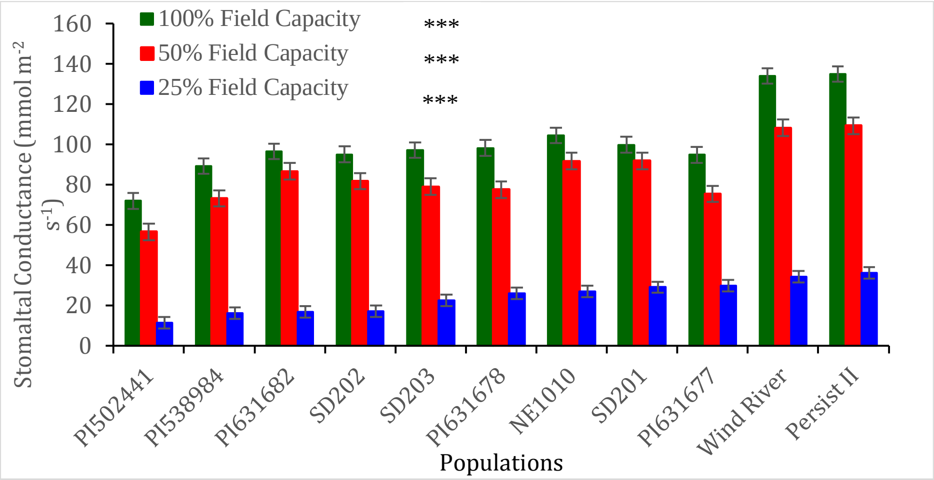

Figure 2: Stomatal conductance for 25, 50, and 100% of field capacity and 15 days drought duration treatment (n = 3) (*** indicates $P < 0.0001$ within each field capacity). Bars indicate standard errors.

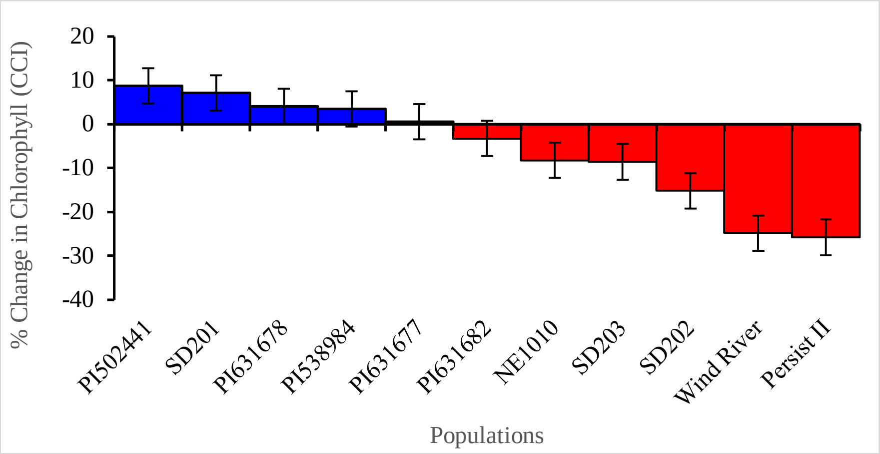

Figure 3: Mean percentage change of chlorophyll content index (based on SPAD readings) from 100% to 25% field capacity (n = 7) at 15 days drought duration treatment ($P < 0.0001$). Bars indicate standard errors.

Root and Shoot Dry Weights

No significance differences in root or shoot dry weight were observed for the 15 days drought duration treatment, whereas for the 30 days drought duration treatment shoot biomass significantly differed among 11 populations for all 3 field capacity treatments. Shoot dry weight for the 25% of field capacity treatment ranged from 0.13 g to 0.39g, for the 50% of field capacity ranged from 0.38 g to 0.66 g, and for 100% of field capacity ranged from 0.84 g to 1.90 g. Shoot biomass decreased as field capacity decreased from well watered to mild to severe drought. Root dry weight for 25% of field capacity ranged from 0.09 g to 0.12 g, no significant difference among 11 populations. For 50% of field capacity, it ranged from 0.18 g to 0.34 g, and 100% of field capacity ranged from 0.09 g to 0.38 g (Fig. 4). Root biomass showed significantly difference among 11 populations under 50% and 100% of field capacities.

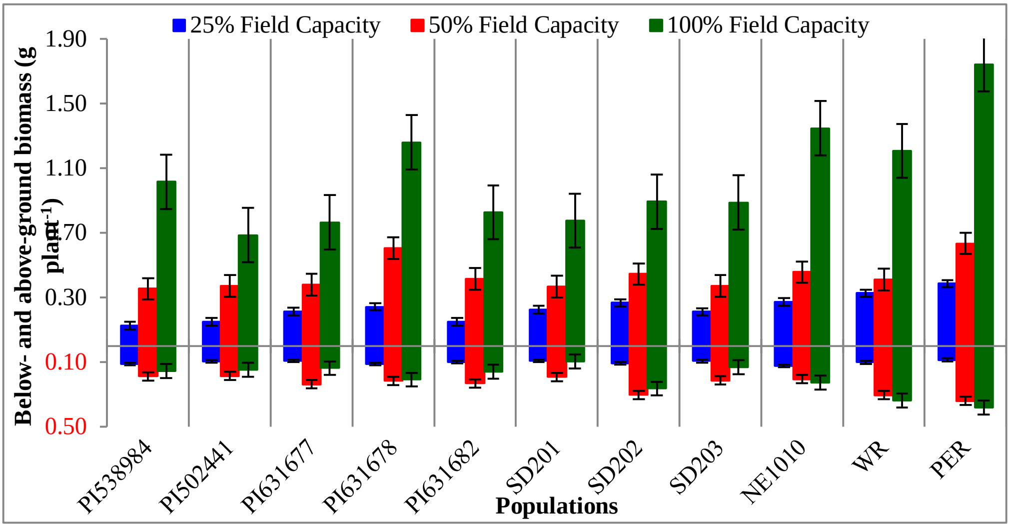

Figure 4: Above and below ground biomass comparison for 25, 50, and 100% field capacity in 30 days drought duration treatment (n = 7). Horizontal line separates aboveground and belowground. Bars indicate standard errors.

The R/S responses were not significantly different in 15 days drought duration treatment for all 3 field capacities. The R/S was significantly different for 30 days under 25% of field capacity, which ranged from 0.23 to 0.97, and PI538984 germplasm had the highest R/S and Persist II had the lowest. The R/S ranked from high to low in order from pure *falcata* lines, predominantly *falcata* lines to *M. sativa* lines, respectively. There was no obvious pattern among 11 populations for 50% field of capacity treatment with R/S ranging from 0.36 to 0.76. For 100% of field capacity treatment, R/S was lower compared to other two drought intensity treatments, but still differed among 11 populations with a range of 0.12 to 0.29 (Fig. 5).

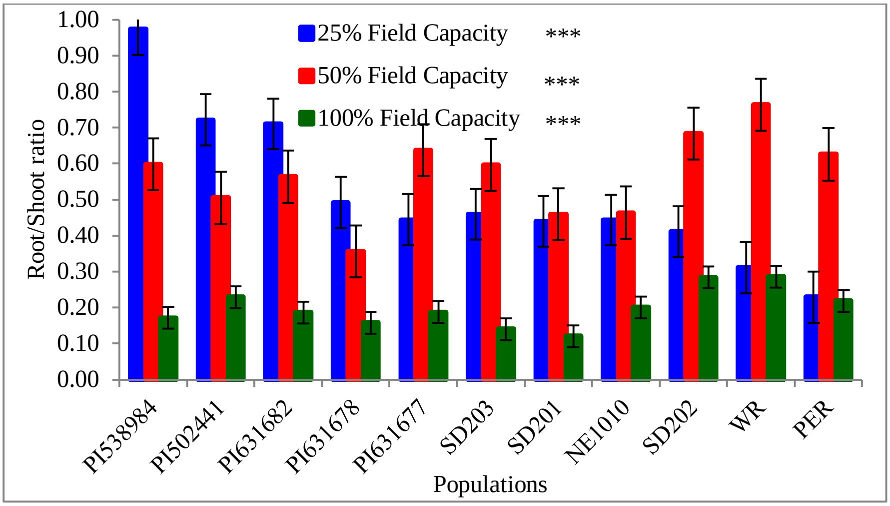

Figure 5**:** Root to shoot ratio for 25, 50, and 100% field capacity and 30 days drought duration treatment (n = 7) (*** indicates $P < 0.0001$ within each field capacity). Bars indicate standard errors.

DISCUSSION

Alfalfa plants responded to drought in a variety of different ways. The main physiological pathways for drought resistance included closing of stomata and reducing of shoot biomass to hinder water loss through transpiration, increasing allocation biomass to root system to facilitate exploiting water absorption, and delaying chlorophyll degradation to maximize resource utilization in needed processes.

Stomatal conductance declined under mild to severe drought and was lower for *falcata*- than *sativa*-based populations. PI502441 had the lowest SC under severe drought (Fig. 2). The results showed *falcata*-based populations are better adapted to regulating transpiration loss under unfavorable soil moisture. Similar results were found by Kang et al. (2011) that showed *falcata*-based populations having a greater SC response to drought. The mechanism for regulating stomatal conductance may be caused by levels of xylem abscissic acid (Tardieu et al., 1992).

Percentage of chlorophyll content change increased under severe drought for all PIs except PI631682. PI502441 had the greatest increase in chlorophyll content, indicating delayed leaf senescence and continued photosynthesis (Fig 3). In contrast, Persist II chlorophyll content decreased more than 25% under severe drought from 100% of field capacity, suggesting chlorophyll degradation, leaf senescence, and reduced photosynthetic capacity. All these physiological processes indicated *falcata*-based populations were more drought tolerant than *sativa*-based populations, which supports Kang, Han et al. (2011). In addition, the chlorophyll content changes under drought conditions showed a correlation with stomatal conductance (Fig 2) (r = -0.5439). With a better control in stomatal conductance the plants can better conserve water to continue photosynthesis.

Root to shoot ratio increased under severe drought for *falcata*-based populations. PI538984 had the greatest R/S, suggesting greater resource allocated to root growth to access water (Fig 5), because water is the most limited factor for their growth under drought condition (Berdahl, et al., 1989). This research found that *falcata*-based alfalfa had dormancy during midsummer drought and slowed down the growth compared to *sativa*-based alfalfa. This leads to better ability to persist through drought conditions to reserve energy and resource. The results of this study showed a slower growth in both above- and belowground biomass under mild to severe drought condition (Fig. 4), which was consistent with Wang et al. (2012).

Falcata-based alfalfa has been found to be tolerant of grazing Misar et al. (2015) and has the ability to develop adventitious shoots from roots (Kannenberg and Xu, 2014). Continued screening to identify germplasm lines will hasten development of better drought, cold, and grazing tolerant cultivars for the semi-arid and arid regions in the world.

LIMITATIONS

This project was conducted in a greenhouse instead of field conditions. Future field-based experiments are needed to better evaluate potential *falcata*-based populations. The implementation of re-watering after drought treatment will allow us to evaluate drought recovery capability among these *falcata*-based populations for resistance and persistence.

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