Root and aboveground traits expressed by landraces and interspecific hybrid of alfalfa (*Medicago sativa*. hybr. (alborea)) with putative drought tolerance in Mediterranean environments

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Abstract. Alfalfa's drought tolerance has not been a major breeding target until recently, and the extent of genetic variation for this trait and its contributing mechanisms have not been thoroughly elucidated. Eight populations, including four landraces (Alta Sierra, Aragon, APG6567, APG44669), two Australian cultivars (Venus and Genesis), and two interspecific hybrids (AF3448 and AF3347) of alfalfa were selected based on their outstanding breeding values for dry matter production and plant persistence in Mediterranean drought-prone environments. The objective of this work was to evaluate the below and aboveground phenotypic expression of these drought-tolerant alfalfa accessions, in order to identify morpho-physiological mechanisms conferring to alfalfa greater agronomical performance in drought-prone environments. Individual plants of each population were established on mesocosms of PVC tubes 11 cm in diameter and 100 cm in depth. Plants were grown at two water regimes: with water deficit (WD) and well-watered (WW). Both trials were organized in a complete block design with four replicates. Plant height, stem elongation rate, shoot dry matter, chlorophyll content, stomatal conductance, canopy temperature, leaf area, specific leaf area, crown diameter, relative length density, and root dry matter at 0-30, 30-60 y 60-100 cm were determined. The water regime affected significantly the phenotypic expression of all above and belowground morpho-physiological traits evaluated ($P<0.05$), which resulted in a 40% reduction in shoot dry matter and plant height in WD relative to WW. Alfalfa populations with putative drought tolerance in Mediterranean environments did not exhibit a unique phenotypic strategy for facing severe water stress. Populations APG44669 and Alta Sierra showed divergent phenotypic expression in terms of stomatal conductance, leaf traits, root architecture, and root biomass partitioning profile.

Introduction

Mediterranean drought-prone environments are one the most threatened by climate change. The increase in temperature along with the decline and larger interannual variability of rainfall, are affecting dryland farming systems and their profitability, due to lower water availability for forage yield and therefore livestock production. Alfalfa (*Medicago sativa* L.) is a perennial forage legume of high protein content and yield potential. It is originated from arid and cold regions of Transcaucasia and possibly also in Central Asia. Agronomical, physiological, and biochemical studies suggest that alfalfa is a plant species relatively tolerant to drought and a valuable genetic resource in the face of climate change in Mediterranean environments (Humphries et al., 2020). However, its use under water-stress conditions as well as low-input systems, demands further improvement of its drought tolerance. Drought tolerance for perennial legumes is defined as the ability to survive and produce forage after various water stress episodes (Inostroza et al., 2015). In alfalfa, drought tolerance has not been a major breeding target until recently, and the extent of genetic variation for this trait and its contributing mechanisms have not been thoroughly elucidated. In order to improve drought tolerance in alfalfa, the existence of genetic diversity among landraces and cultivars is essential (Humphries et al., 2020; Inostroza et al., 2021). In previous works, an alfalfa diversity panel was agronomically characterized in Mediterranean drought-prone environments of Chile and Australia (Inostroza et al., 2021). After four growing seasons, eight accessions, including landraces, cultivars, and interspecific hybrids of alfalfa were selected based on their outstanding breeding values for dry matter production and plant persistence in drought-prone environments. Therefore, the objective of this work was to evaluate the below and aboveground phenotypic expression of these drought-tolerant alfalfa accessions, in order to identify morpho-physiological mechanisms conferring to alfalfa greater agronomical performance in Mediterranean drought-prone environments.

Methods

The experiment was carried out under greenhouse conditions at the Instituto de Investigaciones Agropecuarias-INIA in Chillán (36°34´S; 72°06´W), Chile. Eight alfalfa populations, including four landraces, originated from Chile (Alta Sierra), Spain (Aragon and APG6567), and Perú (APG44669), two Australian cultivars (Venus and Genesis), and two interspecific hybrids (AF3448 and AF3347) were established on mesocosms of PVC tubes of 11 cm of diameter and 100 cm depth, containing as substrate a mixture (v/v) of fine sand (50%), vermiculite (35%), soil (10%) and perlite (5%). Previous to the sowing, the substrate was fertilized with 1L of a complete nutrient solution. Plants were grown at two water regimes: water-deficit (WD) at -0.5 MPa of soil water potential and well-watered (WW) at -0.01MPa. Substrate water potential was monitored with capacitance sensors (MPS1; Decagon Devices Inc., USA) at 1h intervals during the entire experimental period. A pressurized irrigation system with drip emitters (2 L h^{-1}) was implemented in both experiments. The irrigation frequency for each experiment was determined based on the water potential recorded by capacitance sensors. Each water regime was arranged in a randomized complete block design with three replicates. Each replicate included 5 mesocosms, each one containing a single plant. In total, 240 mesocosms were handled. Morpho-physiological traits were evaluated at 130 and 174 days after sowing. Plant height, stem elongation rate, shoot dry matter (ShootDM), chlorophyll content, stomatal conductance (gs), xylem water potential (Ψx), canopy temperature (Tcanopy), leaf area (Larea), leaf dry weight (LDW) and specific leaf area (SLA=Larea/LDW) were evaluated. After the second evaluation, mesocosms were divided into three sections (0-30, 30-60 y 60-100 cm) and the roots of each section were obtained by washing them with water. In a dark room, each root section was totally extended on a black background tray with a 2 cm layer of water. The tray was illuminated with two 160 LED panels. Then, the root system was digitalized with a digital-SLR camera (Eos Rebel T5i, Canon, Tokyo, Japan) located 1.5 m above the tray. All digital images were collected under a standardized light environment and camera setup. Average root diameter, total root volume (Vol), total root length (TrootL), root length density (RLD =TrootL/mesocosm volume), crown diameter, altitude (a), magnitude (µ), and external path length (Pe) were determined. All root traits were obtained with image analysis software (WinRhizo Pro, Régent Instruments, Québec, QC, Canada). The dichotomous branching index (DBI) was calculated in accordance with Inostroza et al. (2020). Finally, the root biomass of each section was determined by drying it in a force-air oven. Results were analyzed by ANOVA and means were separated by LSD test (p=5%). All statistical analyses were performed by using R software (http://www.r-project.org/).

Results and Discussion

Aboveground morpho-physiological traits

The water regime affected significantly the expression of all aboveground phenotypic traits (Table 1). Under the WD regime, the Ψx and gs were reduced more than three times, and the leaf traits Larea and SLA were reduced 25% in average, compared to the WW regime. Morpho-physiological changes induced by water stress resulted in 40 % reduction in ShootDM and plant height in WD relative to WW regime (Table 1). The APG44669 population achieved the highest ShootDM, plant height, SER and SLA, but the lowest gs and LA. Otherwise, Alta Sierra population exhibited the lowest ShootDM with the highest gs. These results are accounting for a divergent behavior in the gas exchange components.

Table 1. Mean values of xylem water potential (Ψx), stomal conductance (gs), chlorophyll content (Chl), leaf area (Larea), specific leaf area (SLA), canopy temperature (Tcanopy), shoot dry matter (ShootDM), plant height (Pheight) and stem elongation rate (SER) of eight alfalfa populations grown under water deficit (WD) and well-watered (WW) regimes. Measurements performed at 174 days after sowing.

Population	Ψx (bar)	gs (mmol $m^{-2}s^{-1}$)	Chl (µg cm^{-2})	Larea (cm^2)	SLA (cm^2gr^{-1})	Tcanopy (ºC)	ShootDM (g $plant^{-1}$)	Pheight (cm)	SER (cm day^{-1})
AF3448	-15.3	234.9	40.9	38.0	244.5	24.1	50.89	71.90	1.78
AF3347	-16.5	249.4	33.3	36.7	233.1	24.2	51.34	70.47	1.75
Venus	-12.7	244.5	39.7	30.8	207.2	23.7	51.01	63.13	1.55
Genesis	-16.0	267.1	40.2	38.0	212.2	23.9	54.79	69.07	1.67
Aragon	-15.8	138.6	38.3	41.8	210.4	23.8	44.93	68.67	1.55
Alta Sierra	-12.2	359.7	36.3	36.0	215.3	23.5	43.66	70.23	1.72
APG6567	-15.3	219.0	38.8	40.7	237.7	24.2	50.08	72.27	1.80
APG44669	-15.3	188.7	40.3	30.3	252.8	23.9	59.57	79.03	1.93
LSD	ns	113.1*	ns	8.4*	40.1*	ns	7.85*	8.1*	0.30*
Water effect									
WW	-6.5	376.5	34.7	50.0	262.4	23.6	63.00	87.9	2.21
WD	-23.3	99.0	42.2	23.1	190.9	24.3	38.57	53.27	1.21
LSD	2.78 ***	56.0 ***	4.0 ***	4.7 ***	23.8 ***	0.50 *	3.9 ***	5.3 ***	0.17 ***

Roots traits

Root biomass accumulation (RootDM) and architecture were significantly affected by water environment (Figure 1; Table 2). The RootDM measured at three depths (0-30, 30-60 and 60-100 cm) showed significant population x water environment interaction ($P<0.05$). Water environment changed root biomass partitioning across the mesocosm profile. Three patterns were observed: populations APG44660 and Alta Sierra increased their total RootDM by 14 and 30%, respectively in WD relative to WW; cultivars Venus and Genesis decreased their RootDM by 18 and 26%, respectively in WD relative to WW and the RootDM of the other populations (AF3448, AF3347, Aragon, APG6567) was not affected by water environment ($P>0.05$; Figure 1). The effect of water environment on RootDM was mostly observed in the 0-30 cm depth. Similar to aboveground phenotypic expression, the root architecture of populations APG44669 and Alta Sierra showed a divergent pattern. Populations APG44669 and Alta Sierra showed the highest and the lowest dichotomous branching index (DBI) values, respectively, which describes the branching pattern of a root system. DBI values range between one and zero, indicating that the root system tends to herringbone or dichotomous pattern, respectively. In this work, low DBI values were observed (Table 2), which is associated with a highly branched root system (dichotomous). Dichotomous root systems are more efficient in soil exploration and water and nutrient capture (Lynch, 2019). Additionally, populations APG44669 and Alta Sierra exhibited the lowest and highest root length density (RLD). Higher RLD populations tend to have greater plasticity in root growth, and greater physiological capacity for water and nutrient uptake, but less root longevity than populations of low root length (Inostroza et al., 2021).

Figure 1. Root dry matter (RootDM) production at three mesocosms depths [0-30 cm (T1); 30-60 cm (T2); 60-100 cm (T3)] of eight alfalfa populations grown in two water environments under water-deficit (WD) and well-watered (WW) regimes.

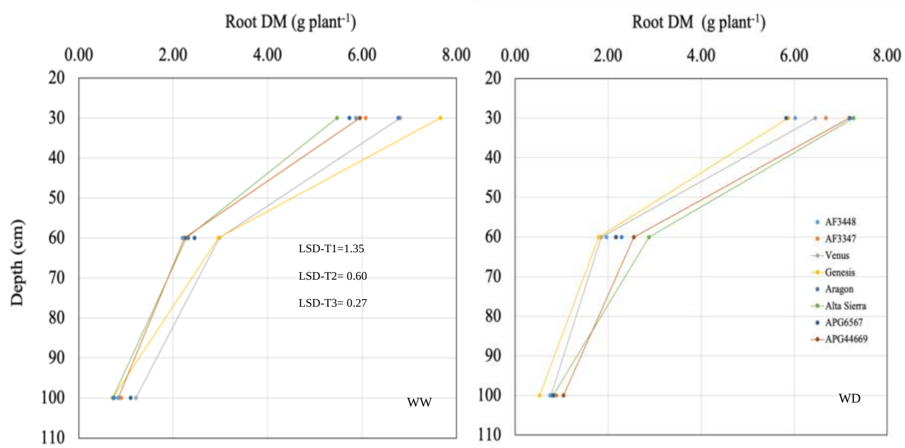

Table 2. Crown diameter (CrownD), relative length density (RLD), dichotomous branching index (DBI), and RootDM/ShootDM ratio of eight alfalfa populations grown under water-deficit (WD) and well-watered (WW) regimes.

Population	CrownD (mm)	RLD (cm cm^{-3})	DBI	RootDM/ ShootDM
AF3448	8.42	0.37	0.06	0.22
AF3347	7.99	0.44	0.05	0.20
Venus	8.03	0.41	0.05	0.21
Genesis	9.83	0.53	0.03	0.26
Aragon	9.08	0.48	0.06	0.27
Alta Sierra	8.01	0.52	0.01	0.26
APG6567	8.40	0.43	0.04	0.22
APG44669	8.37	0.34	0.09	0.21
LSD	1.2*	0.10**	0.03**	0.044*
Water effect				
WW	8.70	0.40	0.06	0.19
WD	8.34	0.48	0.04	0.27
LSD	ns	**	*	0.022***

Conclusions

Alfalfa populations with putative drought tolerance in Mediterranean environments did not exhibit a unique phenotypic strategy for facing severe water stress. Stomatal conductance, leaf traits, root architecture and root biomass partitioning in the mesocosms profile exhibited the most divergent phenotypic expression.

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