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Water relations and use-efficiency, plant survival and productivity of nine alfalfa (*Medicago sativa* L.) cultivars in dryland Mediterranean conditions

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ABSTRACT

The potential for alfalfa to be a valuable rainfed pasture option that can be used to increase livestock and crop production in central-south Chile is demonstrated for the first time. The persistence, productivity and water use efficiency of alfalfa (*Medicago sativa*) cultivars was evaluated at four sites in dryland Mediterranean environments of Chile between 2012 and 2015. Nine alfalfa cultivars were evaluated for plant persistence, winter growth, forage yield, shoot water potential (Ψ), stomatal conductance (gs), and carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) compositions. The percentage of plant survival after four years varied between 69 and 97% of the established plants. Alfalfa forage yield was >9 t ha^{-1}yr^{-1} at Yungay (Andes foothills) in year two, and at Cauquenes (interior dryland site) in years three and four. The highest forage yield was obtained in cultivars SARDI Ten and Aquarius and the lowest in WL326HQ. In 2015, the forage yield during late spring and early summer (November 2015–January 2016) ranged from 3170 to 3570 kg ha^{-1} (average of the nine cultivars), which represented between 29% and 38% of the total production. The response of gs to Ψ was different among cultivars but all cultivars exhibited a strong decline when Ψ was <−1.0 MP. Shoot water potential evaluated in October–November was negatively correlated with $\delta^{13}C$ and $\delta^{18}O$ in shoot biomass. The apparent water use efficiency (kg DM/ha/mm of rainfall) across the nine cultivars was highest in Cauquenes in the three years evaluated (>16 kg mm^{-1}). It is concluded that alfalfa is a high yielding forage that can extend the growing season into early summer and autumn in dryland Mediterranean environments.

1. Introduction

In Chilean Mediterranean farming systems, mixed cropping and livestock production are dominated by intensive cropping using conventional tillage, which has resulted in a deterioration of soil physical-chemical characteristics. In many areas production is constrained as a result of soil compaction, reduced infiltration, low nutrient content and water holding capacity, and high runoff and soil erosion (Martínez et al., 2011, 2012). Livestock production is based on annual pastures and is heavily constrained by the lack of available forage from summer through to autumn.

Deep-rooted perennial legumes with the capacity to extend the growing season of the feed base have emerged as new alternatives for dryland production systems (Dear et al., 2003; Dear and Ewing, 2008; Davies 2005; Li et al., 2008, 2010). Perennial herbs usually show greater root: shoot ratios and a wider lateral root spread than annuals (Cocks, 2001; Schenk and Jackson, 2002). Moreover, plants with deep roots can uptake nutrients from deeper soil horizons in nutrient deficient soils (McCulley et al., 2004) and are able to improve soil physicochemical characteristics (Holford et al., 1998; Cocks 2001; Dear et al., 2003). Other studies have demonstrated that phases of alfalfa in cropping sequences can improve soil aggregation and water infiltration (McCallum et al., 2004; Masri and John Ryan, 2006) and restore soil carbon and nitrogen content and microbial biomass (Li et al., 2012). In addition, perennial legumes have better water use compared to annual legumes (Ward et al.,

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2006) and are probably more resilient to changing climate conditions (Howieson et al., 2008; Ghahramani and Moore 2013). Thus, the inclusion of deep rooted perennial legumes into Chilean dryland farming systems could fulfill the demand for crop cultivars with higher drought tolerance and improved water use efficiency, and could help to rehabilitate deteriorated soils of the area.

Plant adaptations to water deficit in perennial plants comprise different strategies: (a) improving water uptake, (b) reducing water consumption and (c) improving water use efficiency (WUE, Moreno et al., 2008). When soil water availability is low or absent alfalfa (*Medicago sativa* L.) is able to remain dormant and to survive long dry periods due to its deep root system (Ward et al., 2006; Fillery and Poulter, 2006); when the early autumn rains start it resumes growth (Humphries and Auricht, 2001). In dryland Mediterranean environments alfalfa is able to achieve high yield and persists long dry periods (Dear et al., 2003; Benabderrahim et al., 2015). However, genetic improvement has allowed alfalfa to grow in areas where it was previously considered unsuited (Dear et al., 2003). In particular, Australian breeding programs have developed winter-active cultivars specifically targeting Mediterranean environments and the broad acre grazing systems employed in these environments (Humphries and Auricht, 2001; Humphries et al., 2006).

Traits related to tolerance to drought, high temperature and soil water deficit include leaf water potential (ψ) and stomatal conductance (gs). Under moderate or severe drought stress, relative water content (RWC) and gas exchange decrease strongly in alfalfa but they recover when water supply is re-established (Erice et al., 2010, 2011). Stomatal conductance is closely related to the water status of the plant (Medrano et al., 2002). When water deficits start to build up, gs usually decreases faster than carbon assimilation, leading to increased instantaneous water use efficiency (WUE; Chaves and Oliviera, 2004). In alfalfa and *Dorycnium hirsutum*, gs and photosynthesis were substantially reduced once leaf water potential declined below −1.8 MPa (Bell et al., 2007).

Plant water status is also closely related to isotope composition of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) (Farquhar et al., 1989; Barbour and Farquhar, 2000). Under water stress conditions plants reduce gs and the availability of internal CO_2, leading to a lower discrimination against C^{13} ($\Delta^{13}C$), and therefore higher $\delta^{13}C$ (Ehleringer, 1990; Cabrera-Bosquet et al., 2007). Theoretical and empirical approximations have shown that $\Delta^{13}C$ can provide an indirect determination of WUE in alfalfa (Johnson and Tieszen, 1994; Erice et al., 2011). Also, when water evaporates heavier $H_2{}^{18}O$ molecules diffuse more slowly and have lower vapour pressure than $H_2{}^{16}O$, leading to an enrichment in ^{18}O of leaves (Barbour and Farquhar, 2000). Negative correlations have thus been found between $\delta^{18}O$ of leaves and gs and transpiration rates in wheat (Barbour et al., 2000; Cabrera-Bosquet et al., 2009) and cotton (Barbour and Farquhar, 2000).

The objective of this study was to compare the plant survival, forage yield, plant water status and water use efficiency of seven alfalfa cultivars developed in Australia for rainfed conditions, with two W-L alfalfa cultivars that are commonly used in irrigated fields in Chile, in four dryland Mediterranean environments of central-south Chile across four years (2012–2015). It was hypothesized that Australian alfalfa cultivars show better physiological adaptation to cope with water deficit, and also have higher survival rates after the prolonged dry periods during summers of central-south Chile.

2. Materials and methods

2.1. Plant material, sites and growing conditions

Nine varieties of alfalfa from Australia and USA (Table 1) were evaluated in four sites in the Mediterranean climate region of central-south Chile, between 2012 and 2015. The characteristics of soils in the four sites are shown in Table 2. Cauquenes and Yungay were the extreme for average annual rainfall and growing season length. Temperature and precipitation data for each experimental site during the evaluation period were obtained from meteorological stations located nearby (Supplemental Table S1).

Table 1
Characteristics, rainfall requirements and origin of alfalfa cultivars evaluated at four locations in central-south Chile between 2012 and 2015.

Cultivar	Average annual rainfall (mm)	Winter activity class	Origin
Aquarius	≥600	8	Australia
Venus	≥350	5	Australia
Genesis	≥550	7	Australia
SARDI Five	>600	5	Australia
SARDI Seven	>350	7	Australia
SARDI Ten	>350	10	Australia
SARDI Grazer	>350	6	Australia
WL458HQ	>600	6	USA
WL326HQ	>350	4	USA

The soil was prepared one month before sowing using a chisel plow and disc harrows. Seedlings were grown in a glasshouse from seeds inoculated and lime pelleted with *Sinorhizobium meliloti* strain WSM2141 for two months. Seedlings of each genotype were planted in two 3 m long rows separated by 40 cm (60 plants per plot), in a randomized complete block design with four replicates, in June-July 2012. Fertilization free of nitrogen was applied at seedling establishment, using 90 kg ha^{-1} of P_2O_5, 2000 kg ha^{-1} of $CaCO_3$, 100 kg ha^{-1} of K_2SO_4 and 20 kg ha^{-1} of boron calcite.

Soil water content (% v/v) was evaluated using EC-5 sensors (Decagon Device, USA), installed at 20, 40, 80, and 100 cm depth and connected to a data logger EM50 (Decagon Device, USA). There were two sets of sensors at each site (in two replicate blocks). The data were collected throughout the evaluation period.

2.2. Plant survival, plant height and forage yield

Plant survival was evaluated at the end of the summer period (February 2013 and April 2014 and 2015) by counting the number of green plants per plot and expressing the result as a percentage of the establishment density. Plant height was evaluated in August (end of winter). Forage yield was evaluated at the end of the growing season (December) in the first year of the experiments, and in September, November and January in the second, third and fourth seasons, by cutting the whole plot at 5 cm height. Samples were oven-dried with forced air ventilation at 70 °C until reaching a constant weight for dry matter determination.

2.3. Plant water potential and stomatal conductance

Shoot water potential (Ψ) was measured on clear days from 11:00 to 16:00 using a pressure chamber (Soil moisture Equipment Corp., Santa Barbara, CA, USA). The stems (one per plot) were covered with a plastic sheet and aluminum foil, for at least one hour before measurement. Stomatal conductance (gs) was assessed simultaneously with water potential using a SC-1 porometer (Decagon Device, USA). Measurements were performed on 5 leaflets per plot and repetition. Evaluations were conducted on clear days between 11:00 and 16:00 h, in November 2012 (one measurement), October and December 2013 (two measurements), and November-December 2014 (one measurement spanning the four environments).