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DETERMINATION OF *PROSOPIS TAMARUGO* Phil. GROWTH RESPONSE TO WATER STRESS

Thesis for the Degree of Master of Science in Agricultural Sciences

ALSON TIME

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SANTIAGO - CHILE
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I. MONOGRAPHY

1. Tamarugo

Prosopis tamarugo Phil. (tamarugo) is a native legume tree that grows in the Pampa del Tamarugal, Atacama Desert, region of Tarapaca, Chile (Acevedo *et al.*, 1985a). It belongs to the family Leguminosae, subfamily Mimosoideae. *Prosopis* is a genus with 44 species, of which three are native to Southeast Asia, one to tropical Africa (Galera, 2000) and 40 species are native to America (MINAGRI, 2006). The plants in this genus occupy large areas of soil and diverse climates, from humid subtropical to cold xeric, and from sea level to over 3.000 m altitude (Galera, 2000)**.** In Chile, the genus is represented by 6 species. *Prosopis tamarugo* is an endemic species that is under threat of extinction, according to the IUCN (International Union for Conservation of Nature) and it is listed as vulnerable in the red book of the conservation status of the Chilean flora (MMA, 2012).

Tamarugo grows in salt flats of arid areas where the water table depth is between 2 and 18 m (Acevedo and Pastenes, 1983; Habit, 1985). The species is very tolerant to salinity and has nitrogen fixing nodules on its roots (Acevedo *et al.* 2007).

The tamarugo is a species described as halophyte, tolerant to high salt levels; it is a deciduous tree, prickly, with open cup, with two growth habits in the same tree, a cup formed by rigid and erect branches, and a growth habit in which the branches tend to bend.

Tree height reaches between 8 and 20 m under favorable conditions, 15-20 m of cup diameter and trunks 0.5 to 0.8 m in diameter (Sudzuki, 1985). The foliage consists of compound leaves which have glands that allow movement of the leaflets to cope with radiation in the environmental conditions in which they live (Benavente, 2005; Chávez et al., 2013a).

It has a capacity to maintain high stomata conductance when subjected to increasing temperature and atmospheric water demand (Lehner *et al.*, 2001; Delatorre *et al.*, 2008). Chávez *et al*. (2013a) indicated that tamarugo is a plant capable of changing the angle of its leaflets in order to avoid high levels of radiation in the afternoon. This ability would be associated with the level of turgor in pulvinar structures in the leaf, and the authors note that individuals undergoing certain threshold of water stress would be less capable of carrying out this process, therefore being more susceptible to damage by photoinhibition (Chávez *et al.*, 2013b).

Tamarugo blooms all year around, with a typical spring bloom that peaks in October and one or two flowering peaks in winter, between late April and July, varying in number and intensity (Acevedo *et al*., 2007).

Tamarugo has a double root system: one pivoting, deep and made of three to four thick roots lignified and unbranched, and the other consisting of a large mass of shallow lateral roots, covering a perimeter approximately equal to the tree canopy diameter (Sudzuki, 1985). The superficial lateral roots are responsible for the accumulation of moisture in the soil in the area under the canopy of the tree, phenomenon described as the theory of "water elevator" (Richards and Cladwell, 1987) which states that during the night when the stomata are closed the deep roots absorb water from the water table or from wetter areas of soil, and transport it to the drier surface layers where they release the water to the soil.

Tamarugo is subjected to water stress due to ground water extraction for urban areas, mining industry and agriculture supply, that is generating depletion of the water table of the aquifer (Rojas and Desargues, 2007), and generates an unbalance in the tamarugo water budget.

2. Hydraulic drivers of plant growth

Growth is the result of a complex network of processes that occurs at multiple interconnected organizational levels, from the molecules, molecular complexes, cells, organs, to the whole plant and is characterized by successive stages of cellular differentiation (Massonnet *et al*., 2010). Growth is accomplished because new cells are continually produced by cell division in a special tissue called meristem. Growth in shoots and roots is localized in regions at the tips of these organs. Cell growth implicates numerous metabolic aspects and can be defined as an irreversible expansion of cells. Cell expansion is possible because of synthesis of membranes, organelles, proteins and cell-wall materials always associated with differentiation at the subcellular level (Hsiao and Acevedo, 1974). Additionally, turgor pressure has an important role in the expansive growth. A hydrostatic pressure, acting as the push from inside, is always necessary for the final expansion process, after the cell division and/or when the cell is metabolically prepared to expand. A positive turgor pressure (Ψ_p) is important for two principal reasons. First, growth of plant cells requires turgor pressure to stretch the cell walls. The second reason positive turgor is important is that turgor pressure increases the mechanical rigidity of cells and tissues. During periods of water deficit, growth is often the first process to diminish, given its acute sensitivity to cell turgor and its effect on cell division, enlargement and differentiation (Mitchell *et al.*, 2014).
In this way, Green (1968) in experiment with *Nitella* found that any change in turgor pressure causes immediate changes in growth rate.
A simple equation (Eq. 1) of Green (1968), that is always used to describe growth of cells, can explain in a best way the extreme sensitivity of growth to water stress:

$$\boldsymbol{GR = m(\Psi_p - \Psi_{th})} \qquad [\boldsymbol{Eq.1}]$$

where **GR** is growth rate, $\boldsymbol{m}$ is the cell extensibility, $\boldsymbol{\Psi_p}$ is tugor pressure and $\boldsymbol{\Psi_{th}}$ is turgor threshold bellow which growth will not occur.
According to this equation, growth is proportional to cell extensibility that includes metabolic events that soften the wall and provide building blocks for expanding the cell, and to turgor pressure above a threshold level. The threshold turgor values can be high, as 6 or 8 bar (Boyer, 1968). Growth tends to stop before $\boldsymbol{\Psi_p}$ falls to zero, as consequence of a finite threshold turgor in developing water stress situation (Hsaio and Acevedo, 1974).
However, Green *et al* 1971 evidenced that the cell extensibility and the turgor threshold are not constants, they may change with changes in water status in a way to facilitate growth under water stress, and the stress may increase the extensibility and decrease the turgor threshold. If a plant has the ability to raise its extensibility and lower its threshold turgor, it could be able to maintain better its growth under water deficit conditions. It has been evidenced that (Green *et al.*, 1971), under water stress situations, any amount of adjustments in these two parameters in the equation could allow growth restitution when the turgor pressure falls to zero. Nevertheless, some plants have the ability to maintain some growth through osmoregulation, which is a solutes accumulation in the cells and allow a positive turgor pressure development in spite of the low water potential. Acevedo *et al*. (1985)

evidenced, through pressure-volume curves, that *Prosopis tamarugo* has the ability to performs osmotic adjustment and maintains a high relative water content in the leaves at low values of water potential, favoring a positive pressure potential and keeping active its metabolism. They found turgor pressure at about 0.8 MPa when the soil water potential varied from -0.06 to -3.0 MPa.

A plant is under stress when it is subjected to a condition significantly different to the optimal for development, the optimum requirement condition is different among different species and varieties and therefore they are susceptible to a particular stress (Valladares 2004). Besides the process as cell growth, inhibition of cell division, inhibition of wall and protein synthesis, accumulation of solutes, closing of stomata, and inhibition of photosynthesis; water stress induced by water deficit also affects leaf water potential, osmotic potential, the relative water content (RWC) and transpiration rate of species, and then twig growth rate. However, the tolerance range of a species to an unfavorable factor is unique. If a stress factor exceeds the threshold resistance, the survival of the plant will depend on the activation of physiological and biochemical mechanisms of resistance, on the flexibility of these mechanisms, on the compensatory abilities and on the intensity and duration of the stress (Mandre, 2002). In water deficit situations, each species has the ability to develop its own physiological responses of adaptation to the environment and the degree of adaptation to drought may vary considerably within genera or species. In experiences reported by Sanchez-Blanco *et al.* (2002), it is shown how resistance mechanisms developed by two species to confront a water deficit situation are different. For example, in *Cistus albidus* Phil. the main limiting factor of growth was cell expansion, whereas in *Cistus monspeliensis* Phil. photosynthesis was the limiting factor, but both species responded to water deficit by developing avoidance mechanisms based on stomatal closure, a reduction in leaf area and root hydraulic conductivity, and epinasty, which can be considered as complementary mechanisms for regulating transpiration. Phreatophyte plants can regulate their water demand via partial stomatal closure and essentially through foliage loss (Cooper *et al.*, 2003, 2006)

How plants respond generally to water stress? Chapin (1991) evidenced that all plants respond to stress of many types in the same way, besides plants also have a centralized system of stress response that enables them to respond to any physiological stress, regardless of the nature of that stress. However, ecologist and physiologist characterize plants from low-resource environment responses to stress in a different way. According to ecologist view, slow growth, low photosynthetic rate, and low capacity for nutrient uptake (Chapin 1980, Grime 1977, Parsons 1968) are suites of traits that characterize plants from all low-resource environments like deserts, tundra, shaded understory, and infertile soils. For physiologist view, in addition, change in hormonal balance, high frequency production of acid abscisic and less cytokinins are considered as responses of individual plants to most environmental stresses (Chapin *et al*. 1988). These hormonal changes are considered by the author as the basis of direct reduced growth in response to environmental stress and low availability of a resource simply activates the stress-response system of the plants. Plants always develop specific mechanisms to respond to specific stresses; some plants adjust osmotically in response to salt and water stress (Morgan 1984), some others increase their potential to absorb nutrients in response to nutrient stress (Lee 1982), and other plants modify the quantity and balance of photosynthetic enzymes in response to shade or light stress (Evans 1989). Slow growth is considered, as well, as a trait common to plants in low-resource environments in response to stresses. Chapin (1991) associates that slow growth to low capacity of these kind of plants to acquire certain resources. According to him, this slow

growth of low-resource-adapted plants could be explained by three physiological mechanisms; low physiological capacity to acquire resources per gram of tissue; few allocations of resources to growth because of proportionally greater allocation to functions that improve survivorship in harsh environments; internal constraint on growth as less production of growth hormones or less sensitivity to growth hormones.

Water stress, furthermore, affects photosynthesis. Therefore, photosynthetic enzymes and photosynthetic rate decrease in response to drought has been reported by Kluge (1976).

Additionally, it has been reported that water stress affects plant growth, by decreasing cytokinin transport from roots to shoots and increasing leaf ABA, and these changes in hormonal balance provoking changes in cell-wall extensibility (Blackman and Davies 1985).

However, there are observations that suggest the decline in photosynthesis is not directly responsible for drought-induced growth declines. According to Hsiao *et al* (1976), the increase of carbohydrate concentration is product of mild drought stress. Munns *et al*. (1982) and Wardlaw (1969), on the other hand, attributed the decline in leaf growth as a precedent of the decline in dry weight accumulations. According to these observations, drought may cause a reduction in growth most directly by altering hormonal balance, but this decline in growth are more associated and interconnected with changes in plant nutrition, carbon balance, and water relations. Referring to the carbon balance, changes in carbohydrate demand from growth and respiration and supply from photosynthesis in woody plants is a reflect of fluctuations in plant carbon balance (Mitchell *et al*., 2014). A positive carbon balance occurs in plants when the photosynthesis produces more carbohydrates than is required by growth, respiration and defense; subsequently, photosynthesis may decline in response to these carbon sink limitations (Pinkard *et al*., 2011). And when there is excess of carbohydrate, plants store these excess of carbohydrate as non-structural carbohydrates (NSC), primarily starch, and later use it during periods of stress and recovery (Chapin *et al.* 1991).

Growth measurements

Plant growth can be considered as a multi-level process, operating from the cellular to the whole-plant and plant community level. The choice of the level at which growth is measured depends heavily on the reason for measuring (Nathalie *et al*., 2015).

Parameters that are usually used to quantify plant growth are size, cell number, height, dry weight, fresh weight, leaf area, leaf length and carbon fixation.

2.1. Stomatal control over plant growth

Plant strategies to cope with water deficits are the product of adaptive traits that enable resistance of plant functioning to changes in water supply and recovery when water deficit is relieved (Mitchell *et al.*, 2014). Gas exchange, growth, water transport and carbon (C) metabolism reduce during drought according to their respective sensitivities to declining water status. However, the plants generally have the capacity to regulate their carbon and water balance under drought conditions of differing intensities and duration (McDowell *et al*., 2008; Allen *et al*., 2010). When a plant is exposed to low-intensity but long duration drought, it may maintain water status above critical water potential thresholds but deplete stored carbohydrates to lethal limits (i.e. carbon starvation). In addition, under high-intensity drought, incapacity to regulate plant water status above critical thresholds will promote xylem cavitation and death through dehydration (i.e. hydraulic failure) (Patrick *et al*., 2013). Therefore, the timing of the sequence of declining physiological functions under water deficit may determine how water and carbon relations compromise plant survival.
Theoretical relationships, based on the hydraulic framework, between the temporal length of drought (duration) and the relative decrease in water availability (intensity) have been approached by McDowell *et al*. (2008) and Allen *et al.* (2010) in studies on the mechanisms of drought-related mortality in plants. McDowell *et al*. (2008), hypothesized in a general framework that plant mortality is due to biotic agents, demographics, hydraulic failure, and carbon starvation. And the relevance of these different mechanisms is related to the intensity and duration of water stress. According to McDowell *et al*. (2008), the hydraulic-failure hypothesis predicts that reduced soil water supply coupled with high evaporative demand causes xylem conduits and the rhizosphere to cavitate (become air-filled), stopping the flow of water and desiccating plant tissues. Hydraulic failure may be particularly likely if drought is sufficiently intense that plants run out of water before they run out of carbon. The carbon-starvation hypothesis predicts that stomatal closure to prevent hydraulic failure causes photosynthetic uptake of carbon to diminish and the plant starves because of continued metabolic demand for carbohydrates. This process may be exacerbated by photoinhibition or increased respiratory demands associated with elevated temperatures during drought. Carbon starvation may be particularly likely if drought is not intense enough to cause hydraulic failure, but lasts longer than the equivalent amount of plant carbon reserves.

Under water deficit, the plant stomatal regulation strategy also determines the carbon gain and water consumption of plants and affects the ability to survive under conditions of water stress. In a water stress condition, the soil and plant water content affects the opening of the stomata. Some data demonstrated that stomata remain unaffected until the leaf water potential drops to some critical threshold value (Hsiao and Acevedo, 1974). However, Tardieu and Simonneau (1988) indicated that the stomatal behavior is regulated by the concentration of abscisic acid (ABA) and that its response may or may not be regulated by the leaf water potential (Ψ_L), which in turn is associated to the atmospheric demand (vpd). The stomatal opening is also dependent on the relationship among hormonal balance and other factors under good water availability. Thus, partial closure of stomata has been reported (Garcia, 2006) when the proportion of ABA was greater than that of cytokinins. Hence, when water loss by transpiration cannot be compensated by the water absorption, a hormonal imbalance is produced, increasing the proportion of abscisic acid, the potassium concentration in guard cells varies, they lose turgor and stomata are partially closed. Thus, by closing the stomata,

stomatal conductance (gs) decreases (increases stomatal resistance rs) limiting the evaporation of water and reducing the CO_2 fixation.
Relationship between CO_2 assimilation and stomata opening affected by water stress has been reported by many old and recent data. Hsiao (1973) established that there are nonstomatal effects of stress in suppressing photosynthesis in addition to stomatal effect in some species when stress is sufficiently severe. He lied the basis for these effects to altered transport parameters for CO_2 from the inter-cellular space to the chloroplast or the altered ability of chloroplast to photosynthesize, and to an increase in respiration in the leaf.
However, stomatal closure is considered as one of the earliest plants responses to water stress and the highest factor of limitation of photosynthesis (Flexas *et al*. 2014). And this may depend of the plant stomatal regulation behavior (McDowell *et al.*, 2008); Stomatal earliest closure is typical of isohydric plants and anisohydric plants, by contrast, delay closing their stomata in water shortage condition, they keep on transpiring and let the leaf water potential decrease as the soil water potential is declining.
Under water stress, the tamarugo tends to decrease water loss by partially closing their stomata (Calderon *et al.* 2015) in search of a water balance, and a translocation of assimilates to the root zone may occur (Lambers *et al.*, 1998).

2.2. Assessing of plant growth through isotopic composition analysis.

In nature, there are two stable isotopes of carbon, ^{12}C (98.982%) and ^{13}C (1.108%), and three stable isotopes of oxygen, ^{16}O (99.759%), ^{17}O (0.037%) and ^{18}O (0.204%) (Dawson *et al.* 2002), being the ^{12}C and ^{16}O the most abundant source of stable isotope of carbon and oxygen respectively. The total abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric carbon dioxide Farquhar *et al* (1989). Plants have less ^{13}C than the atmospheric CO_2, which is the source of photosynthetic carbon, due to the isotopic discrimination ($\Delta^{13}C$) that occur in the physical and chemical process during the incorporation of CO_2 in the plant biomass. This discrimination occurs in the isotopic composition of plant tissue and generally plants show a positive discrimination (Δ) against ^{13}C. Naturally C_3 plants have a discrimination of ~ 20 x 10^{-3} or 20‰.

$\Delta^{13}C$ gives an integrated photosynthetic activity since the leaf tissue formation (Farquhar *et al.* 1982; Dawson *et al.* 2002) and through it, it is possible to trace the photosynthetic activity of the plants. When the plants are subjected to water stress and close their stomata partially or totally, the partial pressure of CO_2 into the leaf (ci) decreases and decreases discrimination of ^{13}C. Therefore, by measuring the isotopic composition of leaves formed during a period it is possible to deduce the behavior of photosynthesis and stomatal conductance, the higher the value of Δ implies high rate of photosynthesis and stomatal conductance. Nevertheless, variation in isotopic composition of ^{13}C may depend on several other factors than water availability. Warren *et al.* (2001), in study on the availability of water and the isotopic composition of ^{13}C in leaves and wood of conifers, documented that the isotopic composition of ^{13}C is more affected by factors such as; interception of radiation and concentration of nutrients; than by water availability.

Evaporation and transpiration processes cause a clear isotopic enrichment in soil and leaves, in the soil-plant system. Aravena and Acevedo (1985), linked this isotopic enrichment to the behavior of different isotopic species in the liquid-vapor system. The isotopic composition of oxygen in the tissues of the plant ($\delta^{18}O$) reflects the variation in $\delta^{18}O$ of the source caused by evaporative enrichment, leaf transpiration water and biochemical fractionation occurring during the synthesis of organic matter (Yakir 1992; Farquhar and Lloyd 1993). According to Barbour (2007), high stomatal conductance of plants is associated with low $\delta^{18}O$ in the same environmental conditions. On the other hand, in water deficit situations, low stomatal conductance tends to be associated with a high $\delta\ ^{18}O$ (Barbour, 2007). $\Delta^{13}C$ and $\delta^{18}O$ are negatively correlated if $\Delta^{13}C$ varies due to changes in the stomatal conductance (Cernusak *et al*. 2005). However, there is no relationship between $\Delta^{13}C$ and $\delta^{18}O$ if the variation in $\Delta^{13}C$ is produced by a variation in photosynthetic capacity. Low ^{13}C discrimination and more enriched ^{18}O was found by Garrido *et al*. (2016) in leaf tissue of *prosopis tamarugo* subjected to stress due to increased ground water table depth in the Pampa of Tamarugal.

2.3.Water potential and *P. tamarugo* twig elongation rate

Water makes up most of the mass of plant cells and each cell contains a large water-filled vacuole. In such cells the cytoplasm makes up only 5 to 10% of the cell volume; the remainder is vacuole. Water is the most abundant resource that plants need to grow and function; it typically constitutes 80 to 95% of the mass of growing plant tissues. Cell growth is strongly influenced by water potential and its components, the water potential governs transport across cell membranes, and it is often used as a measure of the water status of a plant (Taiz and Zeiger, 1998).

The water absorbed by the roots and transported by the xylem of the tree is transpired by the leaves. However, during the night when the stomata are closed the water status of the tree is recovered, showing an increase in water potential during the night until dawn (Caldwell & Richards, 1986). The water potential at pre-dawn is then the maximum water potential obtained by the tree in a day-night cycle (León, 2002). Water potential decrease has been observed in conditions of water stress, both at noon and at pre-dawn in individuals of *Populus* spp. (Rood *et al*., 2003). Other authors confirm this decline in water potential in water-deficit situations, reducing the pre-dawn leaf water potential (Rood *et al*., 2000; Horton *et al*., 2001 and Cooper *et al*., 2003), and the midday water potential (Smith *et al*., 1991; Busch and Smith, 1995; Sparks and Black, 1999; Rood *et al*, 2000; Horton *et al*., 2001; Amlin and Rood 2003 and Cooper *et al*., 2003).

Water potential (Ψ_w) is also a measure of how hydrated a plant is and thus provides a relative index of the water stress the plant is experiencing. In leaves of well-watered plants, Ψ_w ranges from –0.2 to about –1.0 MPa, but the leaves of plants in arid climates can have much lower values, perhaps –2 to –5 MPa under extreme conditions (Taiz and Zeiger, 1998).

Twig growth is directly related to the water status required for cell elongation and development. The growth of twigs is defined by Wilson (2000)**,** as the production of new biomass from assimilates originated from the same main branch on photosynthetic organisms.

In a study conducted by Scott *et al*. (1999), on *Populus* subjected to water stress by lowering the water table, it was not possible to find differences in mortality or volume of live crown when the water table declined to 5 m, however a decrease in annual twig growth was

recorded. In studies done by Squella (2013) on the water status of some individuals in the natural tamarugo woods of the "Salar de Llamara" it was shown that as ground water table depth increased, negatively affected the tamarugo twig growth. He found twig growth value of cero cm at 11.07 m of ground water table depth and -2.04MPa of water potential.
Zobel (1983) indicates that twig growth rate increases during the day along with the temperature in winter. The increase in the growth average was correlated with the increase in the daily temperature average during the monitoring period of growth; the daily temperature average was more correlated with increased growth than night temperature average.

2.4. Leaf Relative Water Content on the plants water status

Living cells need to be more or less saturated with water to function normally, but they are usually incomplete in this desirable condition (Turner, 1981). The water content and the energy status of the water in the cell are two basic parameters that describe the degree of unsaturation or the plant water deficit. The water content is usually expressed as relative to that at full saturation, i.e. the relative water content or water saturation deficit, and the energy status of the water is usually expressed as the total water potential (Turner, 1981). The two parameters are linked in such a way that the total water potential decreases as the water content decreases, the relationship between the two, known as the moisture release curve. Relative water content (RWC) is used extensively to determine the water status of plants relative to their fully turgid condition (Boyer *et al.*, 2008).
RWC provides a measurement of the water deficit of the leaf, and may indicate a degree of stress expressed under drought and heat stress. RWC integrates leaf water potential with the effect of osmotic adjustment (a powerful mechanism of conserving cellular hydration) as a measurement of plant water status. A genotype with the ability to minimize stress by maintaining turgid leaves in stressed environments will have physiological advantages maintaining turgor dependent processes such as growth and stomatal activity, and to protect and maintain the photosystem complex (Mullan and Pietragalla, 2012).

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CHAPTER II

I. DETERMINATION OF *PROSOPIS TAMARUGO* PHIL. GROWTH RESPONSE TO WATER STRESS.

1. Abstract

The aim of this study was to determine the growth response of *P. tamarugo* to the intensity and duration of water stress. We hypothesized that *Prosopis tamarugo* Phil., being a desert plant, is able to grow at medium to low leaf water status. The effect of the intensity and duration of water stress on the growth factors and the water status of *P. tamarugo* was studied at the Antumapu Experimental Station. The experimental design was a CRD. The water condition factor had three levels; well-watered, medium low-watered and non-watered; and the sampling date factor had levels that varied from five to nine measurement date. Branching architecture, specific leaf area and twig growth rate were evaluated. The results of this study demonstrate that the water stress generally affects the growth of *P. tamarugo*. In the intense water stress, tamarugo twig growth decreased along with twig water potential. The growth rate values were 0.72, 0.51, 0.00 and 0.71, 0.22, 0.00 cm/day for well-watered, low-watered and non-watered respectively at the 20th and 40th day of measurement. Tamarugo had the capacity to growth at low leaf water potential, its growth rate was practically nil at a low leaf water potentials of -3,16 MPa.

Additional keywords: Water potential, Drought, Water depletion, Twig elongation.

2. Introduction

One of the most limiting factor of plant growth is water. Consequently, plants have developed mechanisms to fight stress caused by water limitation. When an environmental stress factor exceeds the threshold resistance, the survival of the woody plant may depend on the activation of physiological and biochemical mechanisms of resistance, on the flexibility of these mechanisms, on the compensatory abilities and on the intensity and duration of the stressor (Mandre, 2002). At present the mortality rate in most woodland community biomes has experienced an increase associated to increases in temperature and a higher incidence of drought (Williams *et al*., 2013), along with human intervention that can generate stresses in the future or significantly affect an already stressed environment (Frelich, 2002). This is the case of Pampa del Tamarugal (Atacama Desert, Chile), a hyper-arid desert dominated by *Prosopis tamarugo*, a strict phreatophyte growing under water table depletion condition due to groundwater extraction for urban areas, mining industry and agriculture supply (Rojas and Desargues, 2007).

Water deficit affects many variables and functions in plants, such as stomata functioning (Tardieu and Simonneau, 1988), hydric traits as pre-dawn, mid-day water potential and xylem hydraulic conductivity (Rood *et al*., 2000; Horton *et al.*, 2001 and Cooper *et al*., 2003) and growth traits like twig elongation rate, leaf shoot ratio and specific leaf area (Lambers, 1998, Guan *et al.* 2003), and the performance of these and other traits and process, may determinate growth capacity and plant survival (McDowell *et al.*, 2008).

The stomatal regulation determines water consumption of plants and affects the ability to survive under conditions of water stress. In a water stress condition, the soil and plant water content affects the opening of the stomata. Some data demonstrated that stomata remain unaffected until the leaf water potential drops to some critical threshold value (Hsiao and Acevedo, 1974). The stomatal closure is considered as one of the highest factor of photosynthesis limitation (Flexas *et al.* 2014). It depends on the plant stomatal behavior (McDowell *et al.*, 2008); under water stress stomatal earliest closure is typical of isohydric plants. By contrast, anisohydric plants delay closing their stomata in water shortage condition; enhancing gas exchange and let the leaf water potential decrease as the soil water potential is declining (Tardieu and Simonneau, 1988). Isohydric plants tend to experience a negative carbon balance, while anisohydric plant experiment low water potential wich induce hydraulic failure by xylem cavitation and embolism.

Being desert plant, tamarugo has developed some mechanism of adaptation to survive its environmental conditions. Such mechanism as capacity to maintain high stomata conductance when subjected to increasing temperature and atmospheric water demand (Lehner *et al.*, 2001; Delatorre *et al.*, 2008) by partially closing its stomata to decrease water loss and change the angle of its leaflets to avoid high levels of radiation in the afternoon (Chávez *et al*., 2013a); it has osmoregulation, which is a solute accumulation in the cells allowing a positive turgor pressure development in spite of the low water potential.

Studying the aboveground growth response of tamarugo to the intensity and duration of water stress is of interest, it may lead to guarantee the survival of tamarugo and save it from extermination provoked by induced groundwater depletion.

Growth of tamarugo was studied in this study, under semi-controlled condition in the Antumapu Experiment Station of the Faculty of Agronomy of the University of Chile during the spring 2016, with the objective to determine effects of the water stress on the growth, the water status and the stomata functioning of *P. tamarugo*.

3. Hypothesis

3.1. *Prosopis tamarugo* Phil., being a desert plant, is able to grow at medium to low leaf water status.

4. Objectives

4.1. Main objective

Determine the growth response of *P. tamarugo* to the intensity and duration of water stress.

4.2. Specifics objectives

4.2.1. Determine the growth of *P. tamarugo* trees under various water stress levels.

4.2.2. Evaluate the water status and stomata functioning of *P. tamarugo* trees under various water stress levels.

5. Materials and Methods

5.1. Experiment location

The experiment was performed at the Antumapu Experiment Station, Faculty of Agronomy, University of Chile (Metropolitan Region, 33 º 40'S and 70 º 38' W, 605 m altitude).

The experimental place belongs to a temperate climate zone and a Mediterranean semiarid climate. The annual temperature is between 29ºC as the maximum mean in January and 2,8ºC as the minimum mean in July. The long term mean precipitation is 369.5 mm. (INIA, 1989).

5.2. Experimental design.

The experimental design was a completely random design (CRD). The water condition factor had three levels, (i) well-watered, (ii) medium stress intensity (low watered) and (iii) intense stress (non-watered), with 10 replicates each level. The well-watered was maintained between pot capacity and 60% available water; the medium stress was maintained between pot capacity and 20 % available soil moisture and the intense stress received only a liter of water during the experiment period. The sampling date factor was variable, depending of the frequency of the variables measurement. The experimental unit was a pot with 16.1 liters approximately (Polypropylenes white tubs of 0.80 m height and 0.16 m diameter) containing homogeneous substrate made of 1/3 of sand and 2/3 compost.

Table 1 shows the water condition and the sampling date factors including their respective levels.

Table 1. Water condition factor and sampling date factors and their respective levels.

Factors	Levels
Water condition	Well-watered Low-watered Non-watered
Sampling date	1 2 3 4 5 6 7 8 9

Plants of tamarugo 2-3 years old were planted in these pots in the field. They were acclimated to the site for a month period, the plants being at their optimal condition. Trees with similar morphology were selected for the experiment after the acclimation period. The acclimated plants were subjected to three water stress levels during a period of two months.

The establishment of the water treatment was January 25, where all water levels were irrigated. January 29, February 1, 5, 9, 12, 16, 19, 23, 26 and March 1rst, 4 are the irrigation dates of the WW. February 9 and 26 are the irrigation date of the LW. And the NW received a liter of water after being recorded growth nil for this water level on February 15.

A nutrients application with dose of 13 g per plant was done. The commercial formula of the fertilizer was (ANASAC trees and shrubs: N-P-K 11-10-15) (Expressed as N, PO and KO respectively). This was done within a few days of transplanting.
A control of pests, with an approximate frequency of 15 days, was made during the period with 0.1% Dimethoate (ANASAC). The water used for irrigation was the drinking water. The water had pH: 7.10 and electrical conductivity: 0.24 mmhos/cm, low in sodium and carbonates, the detail is presented in Appendix IX.

5.3. Measurements

5.3.1. Growth

The growth of tamarugo was evaluated in terms of branching architecture, specific leaf area and twig length.

5.3.1.1. Branching architecture

The branching architecture was measured at a frequency of one week. A twig that best represented the branching architecture reaching the outer part of the canopy was elected. The base of this branch was the starting point for measuring (1) the total length of the branch, which is the distance from the starting point to the tip of its longest-living terminal and (2) the number of ramification points that lead to living branches. The indicator of the branching architecture, called apical dominance index (ADI), was obtained by dividing the number of ramifications by the total length of the branch in centimeters (Perez *et al*., 2013).

$$\mathbf{ADI = NR/TLB\ (cm^{-1})} \qquad \mathbf{[Eq.\ 2]}$$

Where, ADI is the apical dominance index, NR is the number of ramifications and TLB is the total length of the branch, as shown in figure 1.

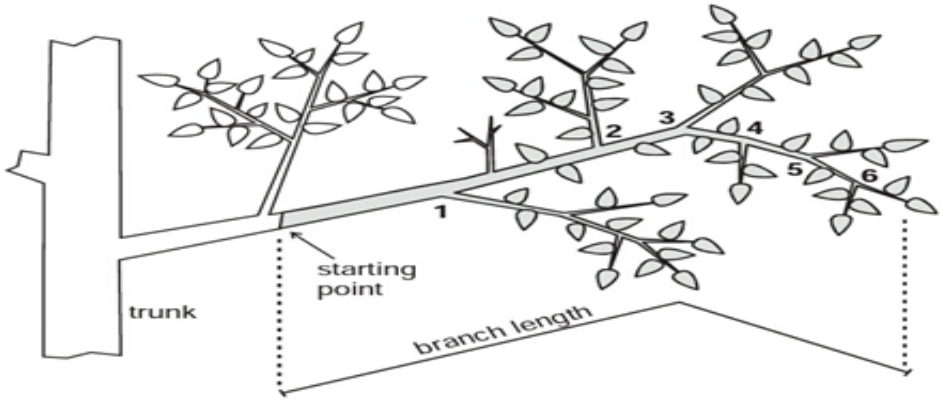

Figure 1. Measurement of branching architecture. From Perez *et al.*, 2013.

5.3.1.2. Specific leaf area

A sample of five leaves per experimental unit was used for the determination of specific leaf area (SLA) at a frequency of one week. Leaf area was determined using photography and software (Image J) as described by Patrick *et al.* (2013). Immediately after taking the picture for leaf area determination, the sample was put into an oven at 60^{o} for 48 hours to determinate the dry weight.

The specific leaf area was calculated using the following model:

$$\mathbf{SLA = LA/LDW\ (cm^2.g^{-1})} \qquad \mathbf{[Eq.\ 3]}$$

Where SLA is the specific leaf area, LA is the leaf area and LDW is the leaf dry weight.

5.3.1.3. Twig growth rate (TGR)

The length of two twigs of each pot were measured at a frequency of 3 and 4 days using a ruler. The twigs were measured from the starting point to the tip. The first measurement was considered as reference or time zero. The first twig (TL_1) was calculated by subtracting the twig length of the first-time measurement to the twig length of the second measurement, to guarantee a uniform twig length data $(TL_j - TL_{j-1}) / (T_j - T_{j-1})$.

Twig growth rate was calculated with the data obtained during the experiment period, as shown below:

$$\mathbf{(TL_j - TL_{j-1}) / (T_j - T_{j-1})\ (cm/day)} \qquad \mathbf{[Eq.\ 4]},$$

Where TL_j is twig length in T_j, TL_{j-1} is the twig length in T_{j-1}, T_j is the last measurement time and T_{j-1} is the first measurement time.

5.3.2. Water Status

5.3.2.1. Relative water content (RWC)

The RWC was calculated as described by Mullan and Pietragalla (2012), the leaf material was weighed immediately after sampling to take a fresh weight. Later, the measurement of the fully turgid weight of the tissue was taken by weighting the samples after placing the sample in petri dishes containing distilled water in the laboratory at ambient light and temperature for 24h and removing any excess water on their surface using an absorbent tissue. Later the samples were dried at 70^{o}C for 24h before reweighing for the dry weight. The RWC was calculated at a frequency of one week according to the following equation,

RWC= (FW-DW) / (TW-DW) x 100 **[Eq. 5]**

Where FW is the fresh weight, DW is the dry weight and TW is the fully turgid weight.

5.3.2.2. Predawn leaf (Ψpd) and midday Leaf Water Potential (Ψmd)

The leaf water potential was measured with a Wescor C-52 sample cyclometric chamber in leaflets taken at pre-dawn and at mid-day. The predawn measurements were done at the end of the night, before dawn, between 3h and 6h am. And the mid-day leaf water potential was measured in samples taken close to solar noon, from 14:40 to 15:30 hours.

5.3.3. Stomata functioning

5.3.3.1. Stomata conductance

Stomata conductance was recorded during six weeks with a weekly frequency, between 8h and 10h am; and between 14h and 16h pm, using a leaf porometer Decagon, Model SC-1 (Decagon Devices, Inc. 2365 NE Hopkins Ct. Pullman, WA 99163 USA). A diurnal cycle of stomatal conductance was run for each water level. For the measurements, the leaflets of the composite leaf were positioned in the porometer chamber taking four measurements on four leaves of different branches of each plant.

5.3.3.2. Isotopic discrimination of ^{13}C and enrichment of ^{18}O

Leaf samples were taken three times during the period of evaluation between January and March 2016. In each sampling date, a leaf sample of 20 g approximately was taken from two pots (plants) of the same water level (n=5 per water level). Each sample was dried at 60 °C for 48 hours in a forced air oven (Venticell, MMM Group, Germany) until constant weight. The samples were then crinkled to a homogeneous powder with a power mill. Two sub-samples were taken from each sample, weighted with an analytical balance (Precisa 125A, Switzerland) and put in tin (0.002-0.003 g) and silver (0.0008-0.001 g) capsules to measure isotopic composition of ^{13}C ($\delta^{13}C$) and ^{18}O ($\delta^{18}O$) respectively. Analyses were performed by the Stable Isotope Laboratory at the Agricultural Sciences Faculty of the University of Chile with a Isotope-ratio mass spectrometer (IRMS) model INTEGRA2 (SERCON Ltd. Cheshire, UK) based on the high performance 20-20 stable isotope analyzer and ANCA-SL, sample preparation module with a precision of 0.3‰ y 0.5‰ for ^{13}C y ^{18}O respectively. As reference, wheat flour OAS (SC0464, SERCON Ltd. Cheshire, UK) of known isotopic composition ($\delta^{13}C$ = -28.01 ± 0.12 ‰ and $\delta^{18}O$ = 32.27 ± 1.2 ‰) was used and ($\delta^{13}C$ = -25.64 ± 0.17 ‰ and $\delta^{18}0$ = 28.51 ± 0.2 ‰) was used as the internal control of *P. tamarugo.*

5.3.4. Pot water content (PWC)

The pot volumetric water content was recorded every two days during the experimental period, using a Decagon soil humidity sensor, Model CS4 (Decagon Devices, Inc. 2365 NE Hopkins Court, Pullman WA 99163, USA) along with a data logger ProCheck Decagon, Model PC-1 (Decagon Devices, Inc. 2365 NE Hopkins Court, Pullman WA 99163, USA). The pots had five access points at a 13,5 cm of distance from the top of the pot down covered with scotch tape. The volumetric water content was measured at 10-15, 23-28, 37-42, 50-55, and 64-69 cm depth.

5.4. Data Analysis

The data were analyzed trough analysis of variance using a mixed model with measurements repeated in time by considering water condition and sampling date.

The model used was:

$$Yijk = \boldsymbol{\mu} + \boldsymbol{H}_i + \mathbf{t}_j + (\boldsymbol{H}*\mathbf{t})\,ij + \boldsymbol{P}_{ik} + \boldsymbol{\varepsilon}ijk \quad [\mathbf{Eq.\ 6}]$$

Where **Yijk** is the response variable, **μ** is mean, ***H*i** is the watering effects, **tj** is the times effects, (***H**t**)ij is the interaction between watering and time effects, $\boldsymbol{P}_{ik}$ is the plot effects and also main plot errors and ***εijk*** is the split plots errors.

The analysis of variance (ANOVA) was performed by running a General Linear Model (GLM) method and the traditional ANOVA process. A $\alpha \leq 0.05$ was chosen for all the ANOVAs performed in this study.

6. RESULTS

6.1. *P. tamarugo* water relations under three water condition levels.

6.1.1. Leaf Water Potential of *P. tamarugo* observed under three water levels.

Table 2 presents the mean and standard error (±S.E.) for predawn leaf water potential (PD Ψ) (Table 2.B) and midday leaf water potential (MD Ψ) (Table 2.A) of *P. tamarugo* under the three water levels. The interaction sampling date-water condition was significant for pre-dawn water potential (Ψpd; $P < 0.0001$) and midday water potential (Ψmd; $P = 0.0001$) (Appendix I).

Table 2. Midday leaf water potential (MD Ψ) and Predawn leaf water potential (PD Ψ) (MPa) of *P. tamarugo* observed under three water condition levels.

A)	**Water conditions**		
Sampling date	**WW**	**LW**	**NW**
02-05-2016	-1.90 ±0.15 a	-2.85 ±0.15 b	-3.26 ±0.15 b
02-11-2016	-2.06 ±0.15 a	-2.37 ±0.15 a	-3.92 ±0.15 c
02-18-2016	-1.86 ±0.15 a	-2.95 ±0.15 b	-2.92 ±0.15 b
02-25-2016	-2.01 ±0.15 a	-3.89 ±0.15 c	-3.80 ±0.15 c
03-03-2016	-2.37 ±0.15 a	-3.08 ±0.15 b	-4.02 ±0.15 c
03-10-2016	-2.32 ±0.15 a	-3.85 ±0.15 c	-4.99 ±0.15 d
Mean between Jan and Mar-16	**-2.09 ±0.09 a**	**-3.17 ±0.09 b**	**-3.82 ±0.09 c**

B)	**Water conditions**		
Sampling date	**WW**	**LW**	**NW**
02-05-2016	-1.70 ±0.12 a	-2.27 ±0.12 b	-2.50 ±0.12 b
02-11-2016	-1.48 ±0.12 a	-2.00 ±0.12 b	-2.56 ±0.12 b
02-18-2016	-1.67 ±0.12 a	-2.28 ±0.12 b	-2.63 ±0.12 b
02-25-2016	-1.62 ±0.12 a	-3.66 ±0.12 d	-3.29 ±0.12 c
03-03-2016	-1.73 ±0.12 a	-2.27 ±0.12 b	-4.15 ±0.12 e
03-10-2016	-2.27 ±0.12 b	-3.14 ±0.12 c	-3.90 ±0.12 d
Mean between Jan and Mar-16	**-1.75 ±0.07 a**	**-2.60 ±0.07 b**	**-3.17 ±0.07 c**

Means and standard errors (±S.E.) of Midday leaf water potential (MD Ψ) (A) and Predawn leaf water potential (PD Ψ) (B) (MPa) of *P. tamarugo* under three water levels. Different letters indicate significate difference for water condition, according to the DGC test (α=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

Figure 2 shows the behaviors of the predawn leaf water potential (PD Ψ) and midday leaf water potential (MD Ψ) of *P. tamarugo* under the three water levels.

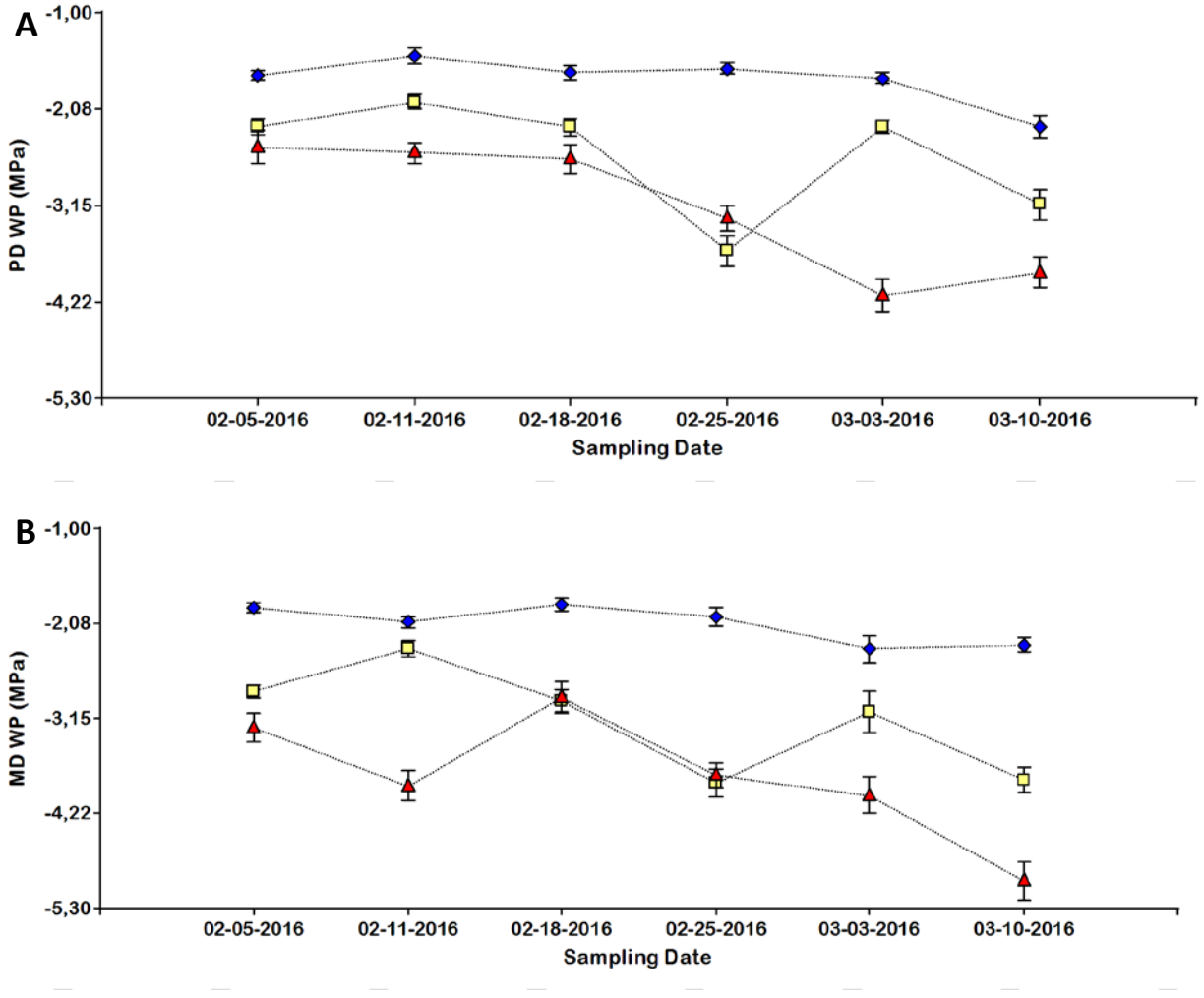

Figure 2. Means of (A) predawn leaf water potential, (B) midday leaf water potential of tamarugo observed under three water levels during 6 sampling dates. Bars indicate ±SE of the mean. Each curve within a graph represents a water level. Diamonds shapes represent the mean of well-watered plants; square shapes represent the mean of the low-watered plants and the triangle shapes represent the mean of the non-watered plants, between January and March 2016.

6.1.2. Relative water content

Significant difference between water levels was observed for RWC. The well-watered showed high RWC values compared to the others water levels. There was a high and constant RWC in all sampling date in the well-watered. A decrease in RWC was observed as water stress increased. A very low water content (60.02%) in the third sampling date of the medium stress level was observed.

Table 3. Leaf relative water content (RWC) (%) of *P. tamarugo* observed under three water levels.

	Water conditions		
Sampling date	**WW**	**LW**	**NW**
02-11-2016	78.88 ±3.69 a	72.70 ±3.69 a	63.18 ±3.69 b
02-18-2016	78.56 ±3.69 a	75.51 ±3.69 a	74.86 ±3.69 a
02-25-2016	79.67 ±3.69 a	60.02 ±3.69 b	70.11 ±3.69 a
03-03-2016	73.97 ±3.69 a	73.43 ±3.69 a	56.14 ±3.69 b
03-10-2016	74.12 ±3.69 a	71.04 ±3.69 a	59.20 ±3.69 b
Mean between Jan and Mar-16	**77.04 ±1.65 a**	**69.94 ±1.65 b**	**64.70 ±1.65 c**

Means and standard errors (±S.E.) of leaf relative water content (RWC) of *P. tamarugo* observed under three water levels. Different letters indicate significate difference for water condition, according to the DGC test (**α**=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

Figure 3 shows the relative water content vs total leaf water potential of *P. tamarugo*. We observed that tamarugo maintained more than fifty percent of its relative water content for a low leaf water potential.

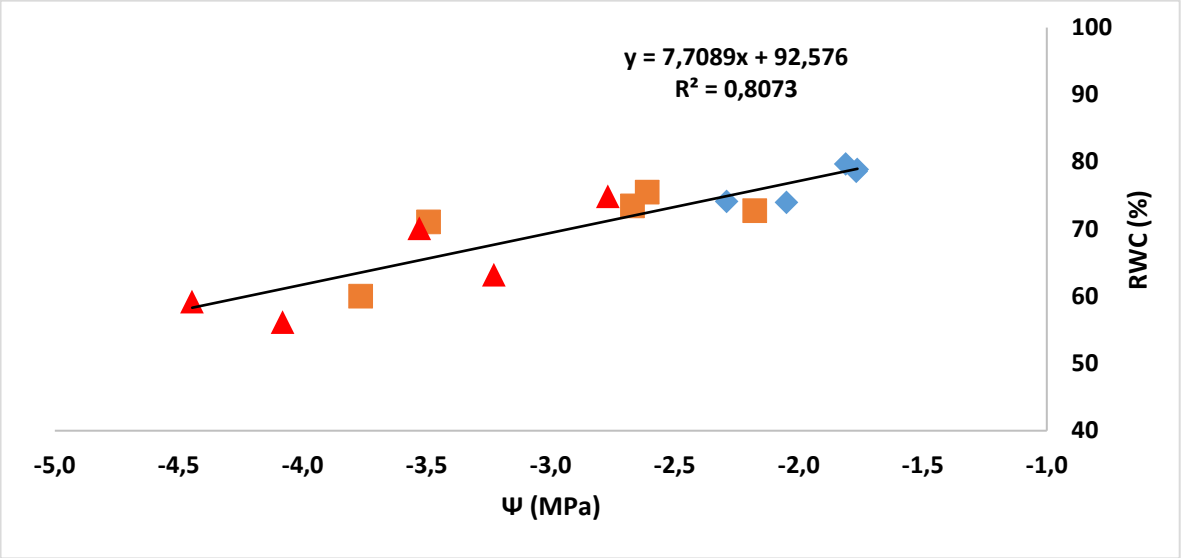

Figure 3. Relative water content (RWC) vs. total leaf water potential (Ψ) in *P. tamarugo* leaves. Antumapu, January-March 2016.
Diamonds shapes represent the mean of well-watered plants; square shapes represent the mean of the low-watered plants and the triangle shapes represent the mean of the non-watered plants.

6.1.3. Tamarugo stomata functioning under water stress

Table 4 presents the means and standard errors (±S.E.) for stomatal conductance (Gs) of the morning (AM) and stomatal conductance (Gs) of the afternoon (PM) of *P. tamarugo* under the three water levels. There was significant interaction for water condition-sampling date (**APPENDIX** II) ($P < 0.0001$) for AM and PM stomatal conductance (Gs).

Table 4. AM stomatal conductance (AM Gs) (Table 4. A) and PM stomatal conductance (PM Gs) (Table 4.B) (mol m^2 S^{-1}), and standard error (±SE) of *P. tamarugo* Phil. observed under three water levels.

A)	**Water conditions**		
Sampling date	**WW**	**LW**	**NW**
02-05-2016	0.36 ±0.02 b	0.29 ±0.02 b	0.24 ±0.02 c
02-11-2016	0.41 ±0.02 a	0.36 ±0.02 b	0.22 ±0.02 c
02-18-2016	0.38 ±0.02 b	0.29 ±0.02 b	0.34 ±0.02 b
02-25-2016	0.34 ±0.02 b	0.13 ±0.02 d	0.19 ±0.02 c
03-03-2016	0.45 ±0.02 a	0.29 ±0.02 b	0.14 ±0.02 d
03-10-2016	0.35 ±0.02 b	0.13 ±0.02 d	0.06 ±0.02 e
Mean between Jan and Mar-16	**0.38 ±0.01 a**	**0.25 ±0.01 b**	**0.20 ±0.01 c**

B)	**Water conditions**		
Sampling date	**WW**	**LW**	**NW**
02-05-2016	0.27 ±0.02 b	0.16 ±0.02 c	0.15 ±0.02 c
02-11-2016	0.32 ±0.02 a	0.35 ±0.02 a	0.13 ±0.02 c
02-18-2016	0.33 ±0.02 a	0.17 ±0.02 c	0.26 ±0.02 b
02-25-2016	0.31 ±0.02 a	0.08 ±0.02 d	0.09 ±0.02 d
03-03-2016	0.35 ±0.02 a	0.23 ±0.02 b	0.07 ±0.02 d
03-10-2016	0.35 ±0.02 a	0.05 ±0.02 d	0.03 ±0.02 d
Mean between Jan and Mar-16	**0.32 ±0.01 a**	**0.17 ±0.01 b**	**0.12 ±0.01 c**

Means and standard errors (±S.E.) of stomatal conductance of tamarugo under three water levels. Different letters indicate significate difference for water condition, according to the DGC test (**α**=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered. AM is the abbreviated **ante meridiem** and PM is the abbreviated of **post meridiem**, that's mean before noon and afternoon respectively.

Figure 4 shows the *P. tamarugo* stomatal conductance vs leaf water potential under the three water levels during the morning and the afternoon. Solid shapes represent the mean stomatal conductance on the afternoon vs midday leaf water potential and frame fill shapes represent the mean of stomatal conductance of the morning vs predawn leaf water potential.

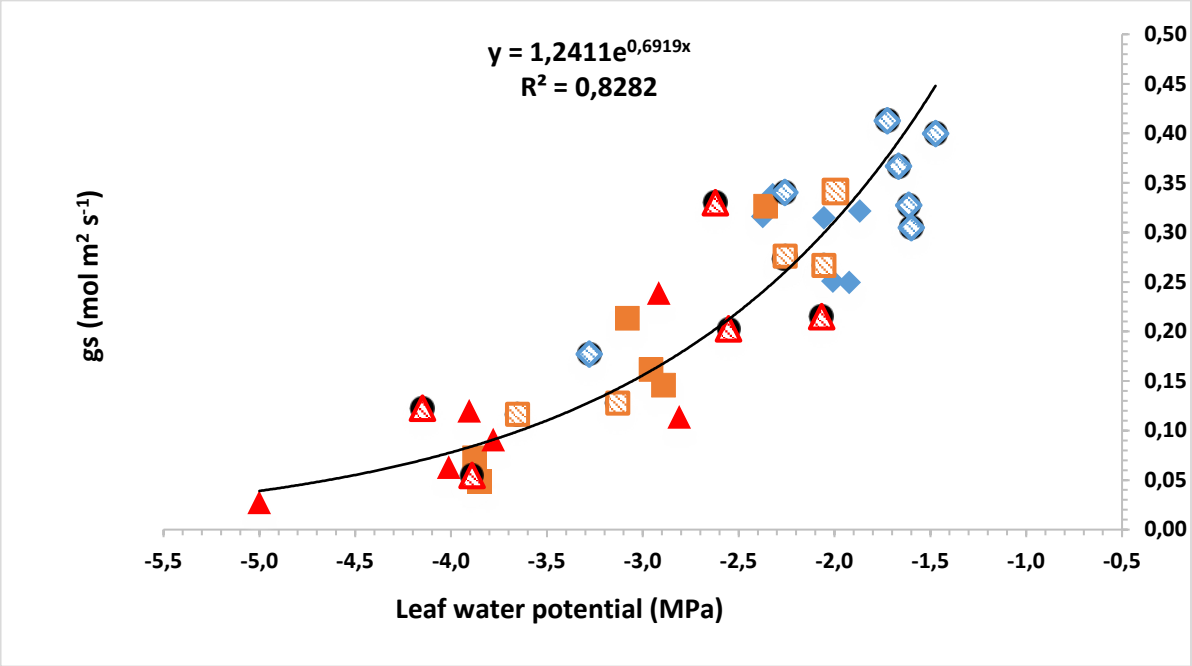

Figure 4. *P. tamarugo* Stomatal conductance vs leaf water potential.
Solid diamond shapes represent the mean of well-watered plants; solid square shapes represent the mean of the low-watered plants and the solid triangle shapes represent the mean of the non-watered plants. Frame fill diamond shapes represent the mean of well-watered plants; frame fill square shapes represent the mean of the low-watered plants and the frame fill triangle shapes represent the mean of the non-watered plants.

6.2. *P. Tamarugo* growth

6.2.1. Comparison of *P. tamarugo* twig growth rate under three water levels.

A clear significant difference was observed starting the second time of measurement for twig growth rate in all water levels. The well-watered had a high growth rate in all sampling dates. In the low-watered the twig growth maintained a constant rate from the second measurement date onwards. Tamarugo growth rate had a very high sensitivity to water deficit as shown in table 5 and figure 5. Growth detention was observed at the fourth measurement date or between 15 to 20 days in the non-watered. By re-watering the non-watered with a liter of water, after the growth rate had reached zero in the fourth sampling date, growth resumed.

Table 5. Twig growth rate (cm day^{-1}) of *P. tamarugo* observed under three water levels.

	Water conditions		
Sampling date	**WW**	**LW**	**NW**
01-31-2016	0.64 ±0.08 b	0.57 ±0.08 b	0.69 ±0.08 b
02-03-2016	0.65 ±0.08 b	0.36 ±0.08 c	0.44 ±0.08 c
02-08-2016	0.90 ±0.08 a	0.28 ±0.08 c	0.10 ±0.08 d
02-14-2016	0.72 ±0.08 b	0.51 ±0.08 c	0.00 ±0.08 d
02-17-2016	0.74 ±0.08 b	0.34 ±0.08 c	0.05 ±0.08 d
02-21-2016	1.03 ±0.08 a	0.20 ±0.08 c	0.25 ±0.08 c
02-26-2016	0.90 ±0.08 a	0.23 ±0.08 c	0.04 ±0.08 d
03-02-2016	0.90 ±0.08 a	0.39 ±0.08 c	0.01 ±0.08 d
03-06-2016	0.71 ±0.08 b	0.22 ±0.08 c	0.00 ±0.08 d
Mean between Jan and Mar-16	**0.80 ±0.06 a**	**0.34 ±0.06 b**	**0.18 ±0.06 c**

Means and standard errors (±S.E.) of twig daily growth rate of *P. tamarugo* observed under three water levels. Different letters indicate significant difference for water condition, according to the DGC test (α=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

Figure 5 shows the leaf water potential vs twig growth rate of tamarugo under three water level and six sampling dates. Each point represents a mean of the interpolated growth rate vs leaf water potential in six sampling dates. The twig growth rate decreases as the leaf water potential decreases, reaching zero at a leaf water potential of -3,16MPa (Figure 5), which found between February 08 and 14 and coinciding with the sampling date four (table 5), where growth rate was cero. Tamarugo maintained its growth at a low water potential.

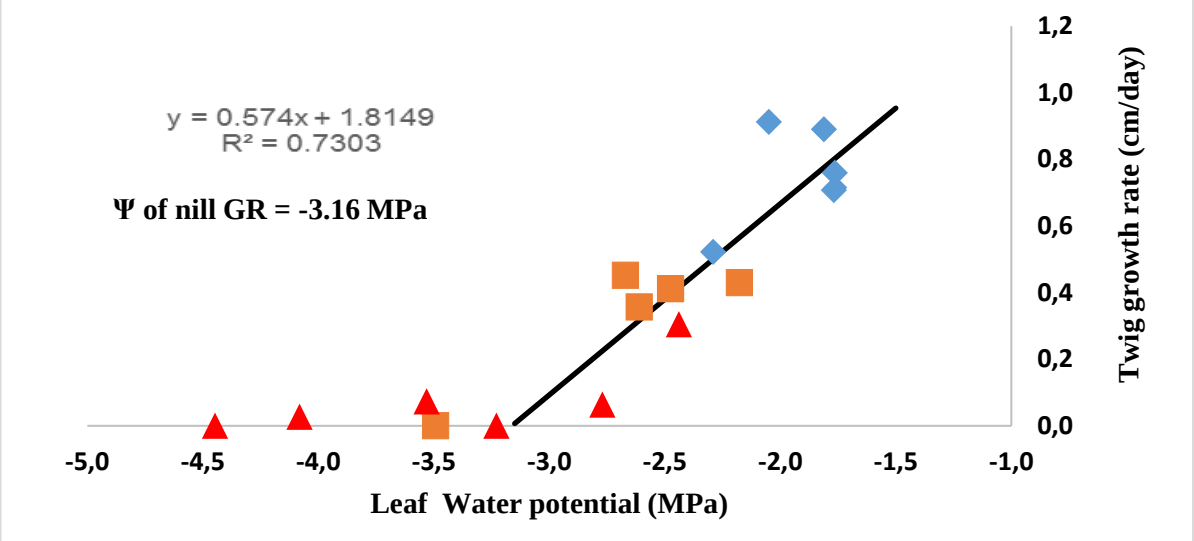

Figure 5. Mean twig growth rate for six sampling dates of the evaluation period as a function of mean leaf water potential. Each point represents the mean of growth rate of ten plants. The growth rate was calculated through the interpolation method. Diamonds represent the mean of well-watered plants; squares represent the mean of the low-watered plants and the triangles represent the mean of the non-watered plants. The water potential values of this graph represent the mean of predawn and mid-day leaf water potential for the same period of evaluation (shown in table 2 for Ψ value and sampling date).

6.2.2. Specific leaf area (SLA).

There was significant difference between WW and LW-NW. The mean of the well-watered between January and March was higher than the other water levels and statistically significant. There was no significant difference between low-watered and non-watered.

Table 6. Specific leaf area (SLA) (cm^2g^{-1}) of *P. tamarugo* observed under three water levels.

	Water conditions		
Sampling date	**WW**	**LW**	**NW**
02-04-2016	099.2 ±5.44 c	106.1 ±5.44 b	108.6 ±5.44 b
02-16-2016	124.5 ±5.44 b	113.9 ±5.54 b	115.8 ±5.44 b
02-25-2016	126.7 ±5.44 b	119.9 ±5.44 b	125.3 ±5.44 b
03-01-2026	142.7 ±5.44 a	113.6 ±5.44 b	121.9 ±5.44 b
Mean between Jan and Mar-16	**123.3 ±3.14 a**	**113.3 ±3.14 b**	**117.9 ±3.14 b**

Means and standard errors (±S.E.) of SLA of tamarugo observed under three water level. Different letters indicate significate difference for water condition, according to the DGC test (α=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

Figure 6 shows the relation of leaf dry weight vs leaf area of *P. tamarugo* under the three water levels. The leaf area was proportional with dry weight in all water levels.

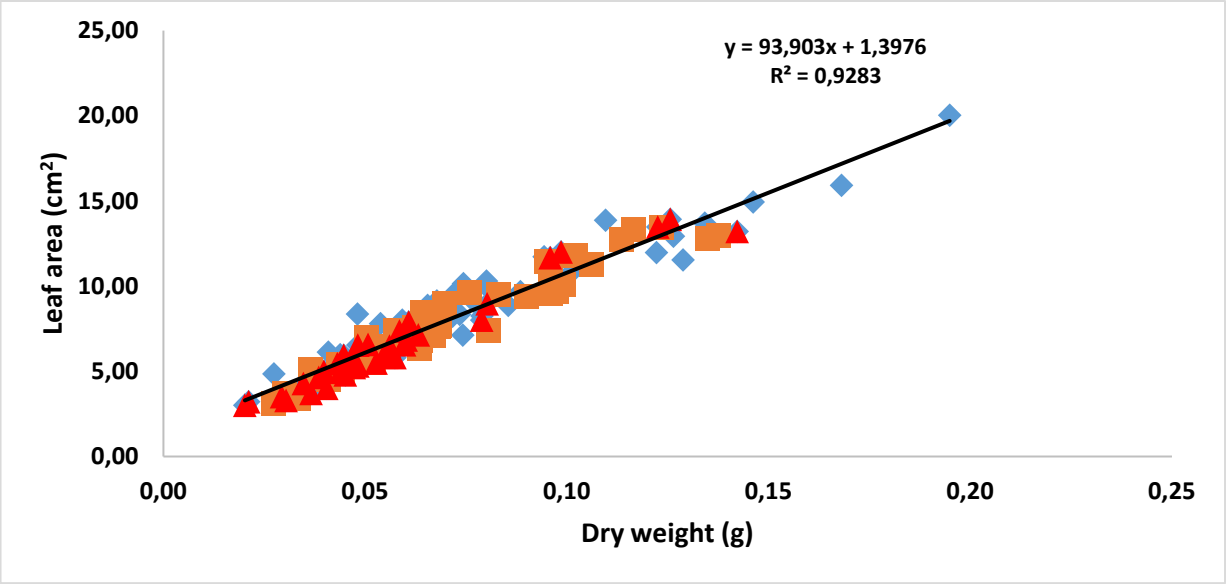

Figure 6. Relation of leaf dry weight vs leaf area of *P. tamarugo* under three water levels. Diamonds represent the well-watered; squares represent the low-watered and the triangles represent the non-watered.

6.2.3. Branching architecture (Apical Dominance Index)

Table 7 shows the branching architecture, expressed in term of apical dominance index of tamarugo per water levels. There was no significant difference between well-watered and low-watered in the three first measurement time. The well-watered and low-watered show an identical behavior for all the sampling date. Difference between the first and last sampling date was observed in the well-watered and low-watered.

Table 7. Branching architecture (ADI) (cm^{-1}) of *P. tamarugo* observed under three water levels.

	Water conditions		
Sampling date	**WW**	**LW**	**NW**
01-29-2016	0.04 ±0.01 b	0.03 ±0.01 b	0.06 ±0.01 a
02-02-2016	0.04 ±0.01 b	0.03 ±0.01 b	0.07 ±0.01 a
02-05-2016	0.04 ±0.01 b	0.03 ±0.01 b	0.06 ±0.01 a
02-12-2016	0.06 ±0.01 a	0.04 ±0.01 a	0.08 ±0.01 a
02-16-2016	0.07 ±0.01 a	0.05 ±0.01 a	0.08 ±0.01 a
02-19-2016	0.07 ±0.01 a	0.05 ±0.01 a	0.08 ±0.01 a
02-23-2016	0.07 ±0.01 a	0.05 ±0.01 a	0.08 ±0.01 a
03-01-2016	0.07 ±0.01 a	0.05 ±0.01 a	0.08 ±0.01 a
03-04-2016	0.07 ±0.01 a	0.05 ±0.01 a	0.08 ±0.01 a
03-09-2016	0.07 ±0.01 a	0.05 ±0.01 a	0.08 ±0.01 a
Mean between Jan and Mar-16	**0.06 ±0.01 b**	**0.04 ±0.01 b**	**0.08 ±0.01 a**

Means and standard errors (±S.E.) branching architecture of tamarugo observed under three water levels. Different letters indicate significant difference for water condition according to the DGC test (**α**=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

6.3. Enrichment of ^{18}O ($\delta^{18}O$) and Isotopic Discrimination of ^{13}C ($\Delta\ ^{13}C$).

Significant difference was observed for $\delta\ ^{18}O$. There was no significant difference between low and non-watered. A clear enrichment of ^{18}O increasing was observed in the water stress levels. The well-watered had the lowest value of $\delta\ ^{18}O$ (Table 9).

Significant difference was observed for $\Delta\ ^{13}C$. There was no significant difference between low and non-watered. The discrimination of ^{13}C was affected by water stress. There was a decline in the discrimination of ^{13}C in the low and non-watered together, what can explain a partial stomatal closure in that group of plants for the period. The results of the analysis of the discrimination of ^{13}C indicate that the photosynthesis is affected by the water stress in tamarugo (Table 8).

Table 8. Isotopic Discrimination of ^{13}C ($\Delta\ ^{13}C$) of *P. tamarugo* observed under three water levels.

	Water conditions		
Sampling date	**WW**	**LW**	**NW**
02-16-2016	18.9 ±0.26 a	17.2 ±0.26 b	17.6 ±0.26 b
02-25-2016	19.2 ±0.26 a	18.0 ±0.26 b	18.2 ±0.26 b
03-01-2016	19.4 ±0.26 a	17.8 ±0.26 b	17.7± 0.26 b
Mean between Jan and Mar-16	**19.2 ±0.15 a**	**17.7 ±0.15 b**	**17.8± 0.26 b**

Means and standard errors (±S.E.) of $\Delta^{13}C$ (‰) of *P. tamarugo* observed under three water levels. Different letters indicate significant difference for water condition, according to the DGC test (**α**=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

Table 9. Enrichment of ^{18}O ($\delta^{18}O$) of P. tamarugo observed under three water levels.

	Water conditions		
Sampling date	**WW**	**LW**	**NW**
02-16-2016	29.1 ±0.29 a	29.7 ±0.29 a	29.8 ±0.29 b
02-25-2016	28.9 ±0.29 a	29.3 ±0.29 b	29.8 ±0.29 b
03-01-2016	28.5 ±0.29 a	29.1 ±0.29 b	29.8± 0.29 b
Mean between Jan and Mar-16	**28.8 ±0.17 b**	**29.4 ±0.17 a**	**29.8± 0.17 a**

Means and standard errors (±S.E.) of $\delta^{18}O$ (‰) of *P. tamarugo* observed under three water levels. Different letters indicate significant difference for water condition, according to the DGC test (**α**=0, 05). WW: mean of well-watered; LW is the mean of low watered and NW is the mean of no watered.

7. DISCUSSION

7.1. *P. Tamarugo* Water Status and Stomatal Functioning in Different Water Condition Levels.

The results of this study demonstrate that the water stress affects the water status of *P. tamarugo*. In the medium and intense stress, the predawn and midday water potential, and the stomatal conductance were lower compared to the well-watered. The minimum mean of midday water potential values observed were -3.17 and -3.82MPa for medium and intense stress, respectively. The maximum of pre-dawn water potentials recorded were -2.00 (three day after being watered) and -4.15MPa (17 day after a watering) for medium and intense stress, respectively. *P. tamarugo* in the medium stress decreased its water potential in -3.85MPa and in the intense stress the decrease in water potential was -4.99MPa. The difference between predawn and midday leaf water potentials was relatively conserved independently of the water condition level. This means that in the experimental conditions, tamarugo is able to maintain the soil-leaf water potential gradient necessary to ensure the flow of water through the continuous soil-plant-atmosphere. The average of pre-dawn and midday water potential was affected as the drought progressed.

Tamarugo maintained high its Gs for a low Ψl (Figure 4). The figure 4 shows that tamarugo stomata had an anisohydric behavior with a diminution of the 50% of the stomata conductance at -2.6MPa. Similar species such as *Acer saccharum*, *Helianthus annus* and *Eucalyptus gomphocephala* have a greater Ψl range than isohydric species (Barnes, 1986, Loewenstein and Pallardy, 1998; Tardieu and Simonneau, 1998; Franks *et al.*, 2007; West *et al*., 2008, MCDowell *et al.*, 2008). A low stomatal conductance was observed for the medium and intense stress conditions at the two measurement periods of the day (Table 4), what could explain the partial stomatal closing of tamarugo described by Garrido *et al.* (2016) in condition of reduced water availability, where the minimum water potential measured in leaf at midday was -2.8MPa, quite superior than that measured in this study. Furthermore, instant measurements of Gs of this study indicated that there were differences between trees crosswise the three-water stress and foliar isotopic composition indicated the same.

The declining tendency in stomatal conductance of the stressed plants observed in Table 4 suggests a stomatal control of transpiration (Tardieu and Simonneau, 1997; Valladares *et al*., 2004) in these plants, to reduce water expenses.
In addition to this, Tardieu and Simonneau (1997) mention how the water potential of well-hydrated plants fluctuates during the day due to the evaporative demand and stomatal opening, with water potential having its maximum at predawn. The predawn water potential as an in-situ and daily value may not have a direct relationship with daily stomatal resistance.

A decline in the discrimination of ^{13}C in the low and non-watered was observed, what could be explained by the decrease of the stomatal conductance in the stress conditions (Table 8). The trees growing without stress had a higher mean value of $\Delta^{13}C$ (Table 8) and a lower mean $\delta^{18}O$ (Table 9) compared to the trees growing in the medium and intense water stress. This higher value of Δ in the well-watered may be linked to a high rate of photosynthesis and stomatal conductance as suggested by (Farquhar *et al.* 1982). Furthermore, these results

indicate that under reduced water availability, *P. tamarugo* has lower assimilation (lower $\Delta^{13}C$ isotopic discrimination) and a lower integrated gs, indicated by higher values of ^{18}O isotopic composition at the leaf level (Hasselquist *et al.*, 2010). These results of ^{13}C and ^{18}O, go in the direction of results found by Garrido *et al*. (2016) in natural tree of tamarugo in the Pampa of Tamarugal. The high photosynthetic capacity observed through $\Delta^{13}C$ in the well-watered could be correlated to the high growth rate of the same water level (Poorter *et al*., 1990; Lambers and Poorter, 1992).

Increased enrichment of ^{18}O was observed as the water stress was more intense. The values found in this study (between 28.5 and 29.8) are consistent with those reported by Reed and Billings (2007) in a study of trees of the genus *Quercus* rubra after 30 years of monitoring, where they found that the values of $\delta^{18}O$ during periods of rainy years ranged between (25.5 and 29.5 ‰) and for period of dry years (between 26.9 and 31 ‰). The high value of leaf $\delta^{18}O$ measured in the medium and intense stress could be associated to the low Gs values measured on the same date (Table 4 and Table 9) and the low $\delta^{18}O$ observed in the well-watered could be associated with high stomatal conductance recorded between January and March (Barbour, 2007).

7.2. *P. Tamarugo* Growth at Different Water Levels.

The results of this study demonstrate that the water stress affects the twig growth of *P. tamarugo*, as it affects all species. In the medium water stress, the twig growth rate was lower compared to well-watered. In the intense water stress, tamarugo twig growth decreased along with tamarugo twig water potential. But Tamarugo has the capacity to growth at low leaf water potential, therefore its growth rate reached zero at a leaf water potential -3.16 MPa (Figure 5), what could explain the potential of tamarugo to grow under extreme conditions (characteristic of desert plants).

The results show that it is possible to conclude that the tamarugo trees of this study are being affected in twig elongation, which could generate further limitations. The results of this study show that growth is mostly sensitive to changes in cell turgor and often declines before reductions in leaf photosynthesis in response to water scarcity, coinciding to what was reported by Hsiao *et al.* (1976).

Furthermore, the results of this study demonstrate that the water stress affects the specific leaf area of *P. tamarugo*, by decreasing the leaf area. The well-watered leaf area values were higher compared to low and non-watered (Figure 6). According to these results, *P. tamarugo* could regulate its water demand via partial stomatal closure and through leaf area reduction under water deficit situation. On the other hand, the leaf area was proportional with the leaf dry weight in all water levels. Furthermore, the reduction of the development of leaf area is considered as an early response to water deficit, through this strategy plants tend to reduce their transpiration rates, and thus facilitates the conservation of water (Westgate and Boyer, 1985). Additionally, the results of this study demonstrate that the water stress affects the apical dominance index in the branching architecture of *P. tamarugo*. There were high values of the apical dominance index in the branching architecture of *P. tamarugo* in the non-watered (Table 7). There was no significant difference between the first and the last sampling date, what can be attributed to the low growth in height of the branches and no ramification of the Tamarugo under water stress. Whereas, significant differences between the first sampling date and the last sampling date was observed in the well-watered and the low-watered plants. According to the behavior observed for this index, it possible to conclude that, being in the optimal conditions, *P. tamarugo* maintains a high number of ramifications per cm of branch (Table 7).

The apical dominance index could be a good monitoring indicator of growth in trees under no optimal water status, for conservation purposes.

Hsiao and Acevedo, (1974) pointed out that at the cell scale, there is a sequence of the process starting with cell growth, inhibition of cell division, inhibition of wall and protein synthesis, accumulation of solutes, closing of stomata, and inhibition of photosynthesis that are affected by the water stress. In addition, Passioura (1996) pointed out that at the whole plant scale, there is a sequence of events during a gradual water deficit that usually starts with a decrease in twig growth, followed by a decrease in stomatal conductance by reducing the rate of assimilation of CO_2, photosynthesis is affected, the accumulation of solutes in the cells, the growth of roots decreases and finally a senescence of leaves is observed which would lead to a decline of the whole plant.

8. CONCLUSIONS

As tamarugo has an anisohydric behavior, facing water stress, tamarugo lets the leaf water potential decrease as the soil water potential decreases, with a partial stomatal closure strategy reflected in the tendency to the decrease of the stomatal conductance. However, tamarugo can maintain a high stomatal conductance at low leaf water potential. In addition, tamarugo reduces its leaf area as a strategy to diminish the water demand. Furthermore, as growth is particularly sensitive to changes in cell turgor associated with water condition, tamarugo twig growth decreases along with the leaf water potential. However, tamarugo has the capacity to growth at low leaf water potential probably due to its anisohydric behavior, its growth rate was practically nil at a low leaf water potentials.

The hypothesis "*Prosopis tamarugo* Phil., being a desert plant, is able to grow at medium to low leaf water status" was maintained.

9. REFERENCES

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10. APPENDIX

Appendix I ANOVA for water relation (Predawn and Midday leaf water potential) of *P. tamarugo* Phil under three water condition levels.

MD WP factor	numDF	F-value	p-value
(Intercept)	1	3629,63	<0,0001
WC.	2	101,22	<0,0001
SD	5	33,51	<0,0001
WC.xSD	10	10,59	<0,0001

PD WP factor	numDF	F-value	p-value
(Intercept)	1	4402,5	<0,0001
WC	2	120,48	<0,0001
SD	5	44,44	<0,0001
WC.xSD	10	15,95	<0,0001

Appendix II ANOVA for Stomata functioning (AM and PM stomata conductance) of *P. tamarugo* Phil under three water levels.

Gs (A.M) factor	numDF	F-value	p-value
(Intercept)	1	993,70	<0,0001
WC.	2	51,75	<0,0001
SD	5	24,32	<0,0001
WCxSD	10	8,38	<0,0001

Gs (P.M) factor	numDF	F-value	p-value
(Intercept)	1	915,60	<0,0001
WC.	2	107,64	<0,0001
SD	5	19,30	<0,0001
WCxSD	10	14,60	<0,0001

WC. Means water condition that include WW=well-watered, LW=low watered and NW=non-watered, SD means sampling date between January and March 2016. AM is the abbreviated ante meridiem and PM is the abbreviated of post meridiem, that's mean before noon and afternoon respectively.

Appendix III. ANOVA for $\Delta^{13}C$ of *P. tamarugo* Phil under three water levels.

	numDF	F-value	p-value
(Intercept)	1	45454,60	<0,0001
WC	2	30,54	<0,0001
SD	2	3,63	0,0367
WC:SD	4	0,89	0,4788

WC. Means water condition that include WW=well-watered, LW=low watered and NW=non-watered, SD means sampling date between January and March 2016.

Appendix IV. ANOVA for $\delta^{18}O$ of *P. tamarugo* Phil under three water levels.

	numDF	F-value	p-value
(Intercept)	1	90856,80	<0,0001
WC	2	8,42	0,0010
SD	2	1,32	0,2806
WC:SD	4	0,34	0,8514

WC. Means water condition that include WW=well-watered, LW=low watered and NW=non-watered, SD means sampling date between January and March 2016.

Appendix V. ANOVA for SLA of *P. tamarugo* Phil under three water levels

	numDF	denDF	F-value	p-value
(Intercept)	1	81	4103,89	<0,0001
WC	2	18	2,56	0,1048
SD	3	81	10,57	<0,0001
WC:SD	6	81	2,47	0,0305

WC. Means water condition that include WW=well-watered, LW=low watered and NW=non-watered, SD means sampling date between January and March 2016.

Appendix VI. ANOVA for growth rate of *P. tamarugo* Phil under three water levels.

	numDF	denDF	F-value	p-value
(Intercept)	1	216	132,23	<0,0001
WC	2	18	38,74	<0,0001
SD	8	216	7,34	<0,0001
WC:SD	16	216	10,14	<0,0001

WC. Means water condition that include WW=well-watered, LW=low watered and NW=non-watered, SD means sampling date between January and March 2016.

Appendix VII. ANOVA for apical dominance index (ADI) of *P. tamarugo* Phil under three water levels.

	numDF	denDF	F-value	p-value
(Intercept)	1	243	57,95	<0,0001
WC	2	18	1,36	0,2807
SD	9	243	6,68	<0,0001
WC:SD	18	243	0,32	0,9969

WC. Means water condition that include WW=well-watered, LW=low watered and NW=non-watered, SD means sampling date between January and March 2016.

Appendix VIII Mean of volumetric pot water content (VWC) per water level measured during the experimental period.

	Mean of VWC ($m^3 m^{-3}$) ±S.E. (0.006) (n=50)		
Sampling date	**WW**	**LW**	**NW**
2016-01-26	0.134	0.127	0.141
2016-01-27	0.123	0.121	0.136
2016-01-28	0.117	0.117	0.130
2016-01-29	0.111	0.113	0.121
2016-02-01	0.100	0.082	0.085
2016-02-03	0.148	0.087	0.092
2016-02-05	0.110	0.074	0.081
2016-02-09	0.105	0.104	0.066
2016-02-12	0.102	0.106	0.069
2016-02-15	0.109	0.086	0.069
2016-02-17	0.147	0.083	0.092
2016-02-19	0.097	0.069	0.078
2016-02-22	0.096	0.068	0.081
2016-02-24	0.144	0.059	0.063
2016-02-26	0.070	0.095	0.052
2016-02-29	0.094	0.098	0.063
2016-03-04	0.072	0.051	0.039

Appendix IX Physicochemical quality of the irrigation water used in the experiment.

			Cumplimiento de la norma	
Característica	Valor	Unidad	NCH 133	NCH 1333
pH	7,10		Sí	-
C.E.	0,24	mmhos/cm	Sí	-
Ca soluble	1,38	Meq/L	Sí	-
Mg soluble	1,07	Meq/L	Sí	-
Na soluble	0,42	Meq/L	Sí	-
K soluble	0,02	Meq/L	Sí	-
Cl soluble	0,25	Meq/L	Sí	-
SO_4 soluble	0,44	Meq/L	Sí	-
HCO_3 soluble	1,83	Meq/L	Sí	-
R.A.S ajust.	0,6		Sí	-
Coliformes Totales	Nd	NMP/100 mL	Sí	Sí
Coliformes Fecales	Nd	NMP/100 mL	Sí	Sí

Observaciones:
NCH 133: Norma Chilena de Clasificación del agua de riego según su salinidad.
NCH 1333: Norma Chilena de Clasificación del agua para riego de frutas y verduras que se desarrollan a ras de suelo y que se consumen crudas.