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Research article

Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina)

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ABSTRACT

Summary: Plants exposed to drought stress, as usually occurs in Patagonian shrublands, have developed different strategies to avoid or tolerate the lack of water during their development. Production of phenolic compounds (or polyphenols) is one of the strategies used by some native species of adverse environments to avoid the oxidative damage caused by drought. In the present study the relationship between phenolic compounds content, water availability and oxidative damage were evaluated in two native shrubs: *Larrea divaricata* (evergreen) and *Lycium chilense* (deciduous) of Patagonian shrublands by their means and/or by multivariate analysis. Samples of both species were collected during the 4 seasons for the term of 1 year. Soil water content, relative water content, total phenols, flavonoids, flavonols, tartaric acid esters, flavan-3-ols, proanthocyanidins, antioxidant capacity and lipid peroxidation were measured. According to statistical univariate analysis, *L. divaricata* showed high production of polyphenols along the year, with a phenolic compound synthesis enhanced during autumn (season of greatest drought), while *L. chilense* has lower production of these compounds without variation between seasons. The variation in total phenols along the seasons is proportional to the antioxidant capacity and inversely proportional to lipid peroxidation. Multivariate analysis showed that, regardless their mechanism to face drought (avoidance or tolerance), both shrubs are well adapted to semi-arid regions and the phenolic compounds production is a strategy used by these species living in extreme environments. The identification of polyphenol compounds showed that *L. divaricata* produces different types of flavonoids, particularly bond with sugars, while *L. chilense* produces high amount of non-flavonoids compounds.

Synthesis: These results suggest that flavonoid production and accumulation could be a useful indicator of drought tolerance in native species.

1. Introduction

Water deficit is the dominant environmental factor that shapes the pattern of vegetation in semi-arid ecosystems. Because of this, vegetation is heterogeneously distributed forming isodiametric patches with shrubs acting as colonizers or initiators and grasses surrounding the patches, alternating with areas of bare soil (Noy-Meir, 1973). The coexisting species of these ecosystems have different strategies to survive to the different environmental factors. Under field conditions, the response to one factor, such as water stress, is complex and difficult to study in isolation because an overlapping with others environmental factors (intensity of winds, solar radiation, temperature fluctuation, etc.) occur; however, this kind of research can provide an approach about the behavior and the adaptation of the species in study.

Native plants of semi-arid ecosystems have developed different mechanisms to face drought, which involve different strategies and adaptive changes depending on the genotype (Chaves et al., 2003). Plants were classified in two main ecophysiological groups

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according to the strategy used: drought avoidant plants and drought tolerant plants (Levitt, 1980). Drought avoidant species are characterized by high relative growth rate, deciduous phenology, high-faster metabolism, high potential for resource capture, and low investment in secondary metabolites. Thus, in water-limited environments, avoidant plants with high stomatal conductance and good photosynthetic capacity demonstrate opportunistic and rapid growth during short periods of water availability (Hetherington and Woodward, 2003). Drought tolerant plants have the ability to maintain their normal physiological parameters even during long periods of drought by employing different strategies like osmotic adjustment, changes in cellular/tissue elasticity (elastic properties of cell walls), different antioxidant mechanisms and high production of secondary metabolites (Reddy et al., 2004; Westoby et al., 2002). Regardless the strategies used to face drought, plants exposed to long periods of abiotic stress enhance the secondary metabolites production as part of the non-enzymatic mechanisms. These compounds generally have a strong antioxidant capacity to protect cellular structures from the oxidative damage caused by the increase of reactive oxygen species (ROS) production in cells (Sies, 1993; Naczk and Shahidi, 2004; Reginato et al., 2014).

In recent years, the research about non-enzymatic components with antioxidant capacity has focused on phenolic compounds (Surweswaran et al., 2007; Krishnaiah et al., 2011). Phenolic compounds, also known as polyphenols, include a large variety of molecules and could be classified in 3 different groups: 1) Non-flavonoids, molecules that have at least one phenolic ring with different reactive groups (hydroxyl, nitrosyl, SH, etc), including simple phenolic acids, phenyl alcohols, stilbenes, chalcones and lignans; 2) flavonoids, molecules with a phenyl chromane structure of C15 formed by 2 aromatic rings bind with a carbon chain (C6–C3–C6), sub-classified into anthocyanidins, flavonols, flavones, isoflavones, flavanones, flavan-n-ols (n refers to the carbon number of the phenyl chromane structure where the hydroxyl group is bonded. Ex: flavan-3-ols, flavan-4-ols, flavan-3,4-ols), etc, depending on the amount, position and type of reactive group (Crozier et al., 2009; Motilva et al., 2013); and 3) tannins, subdivided in a) condensed tannins: flavonoids polymers type A (C7–C2 and an ether bind) and type B (C4–C8 or C4–C6) and b) hydrolyzable tannins: phenolic acids polymers bind to a 5 or 6 carbons ring (Khanbabaee and Van Tee, 2001). Phenolic compounds have antioxidant functions in response to severe abiotic stress, complementing the roles played by the enzymatic antioxidant system, with great potential to reduce ROS and to avoid cell damage (Hatier and Gould, 2008; Agati and Tattini, 2010). Fast growing plants generally have a low investment in defense and protection. Therefore, this species tend to have low phenolic compounds concentrations. Conversely, slow growing species have high phenolic compounds concentrations. This could be attributed to a long-term probability that these long-lived plant species evolved to confront both biotic and abiotic (primarily water shortage) stress factors (Karabourniotis et al., 2014).

Patagonian shrub lands are located in the North-East of Patagonian Monte, Argentina, specifically between 42 and 44° 20′S and 64–68° W, with a surface of 4200 km^2 approximately (Soriano, 1950). This region is characterized by a semi-arid climate with large daily and seasonal temperature variations and with annual rainfalls below 200 mm, generally concentrated in winter and/or spring (Cabrera, 1976). The landforms and soils of Patagonian shrub lands enable heterogeneity in vegetation distribution (from dense scrub to steppes) with large variety of species, dominated by grasses and shrubs alternating with bare soil (Bertiller et al., 2004). The most common shrub species are *Larrea divaricata*, *Chuquiraga hystrix*, *Lycium chilense*, *Junellia alatocarpa*, *Condalia microphylla*, *Prosopidastrum globosum*, *Schinusjohn stonii* and *Monttea aphylla* (Cabrera, 1976; León et al., 1998); all of these species have a variety of adaptive strategies related with water and nutrient conservation (Bertiller et al., 2005, 2006). Two of the dominant shrubs in this region are *L. divaricata* and *L. chilense*, both being considered as colonizers or initiators of the patches. The contrasting functional traits and phenologic behavior of both species were previously described (Bertiller et al., 2004; Soriano et al., 1995). *L. divaricata* is an evergreen shrub, with a perennial behavior and low leaf shedding during the whole year (Soriano and Sala, 1983; Campanella and Bertiller, 2008). *L. chilense* is a deciduous shrub with phenological activity occurring during winter–spring and sometimes until early summer when water availability in soil is greater (Soriano et al., 1995; Campanella and Bertiller, 2008).

The aim of this work was to perform a field study along the different seasons in two native species of Patagonian shrub lands with contrasting mechanisms of drought resistance, *L. divaricata* and *L. chilense*, to evaluate the oxidative damage in tissues, the levels of different groups and types of polyphenols and their antioxidant capacity. In theory, plants from semi-arid regions are classified according to their ecological niches, but in practice it becomes very difficult to catalog a plant species in one of the main ecophysiological groups because of the overlapping of different environmental factors and plant responses. It has been proposed that, independently of the ecophysiological strategies used, coexisting species in semi-arid regions have a gradient of mesophytism to xerophytism that allows them to coexist and survive in the same habitat (Cenzano et al., 2013).

Species from semi-arid ecosystems like Patagonian shrub lands, with different behavior and phenological activity to face drought, are supposed to have powerful antioxidant enzymatic and non-enzymatic mechanisms. Considering the severe stress that these species have to face during long periods, we hypothesize that polyphenols may be an important component of their antioxidant system. However, the role of polyphenols as a mechanism of drought response and their suitability as indicators of drought tolerance in native species of the Patagonian shrub lands has not been explored yet.

2. Materials and methods

2.1. Study site and plant material

This study was carried out in the wildlife refuge “La Esperanza” of Natural Patagonia Foundation, located in the northeast of Chubut province (67 km^2 surface, 42°7′ 43.92″S and 64°57′ 40.99″ W). Leaves and roots of *L. divaricata* and *L. chilense*, (five plants per specie), were randomly collected in autumn (May), winter (August), spring (November) and summer (February). Plant material was frozen immediately after collection and kept at −80 °C until biochemical analyses were performed. Samples were lyophilized previously to the extraction procedure.

2.2. Weather data

To determine seasonal changes in water availability, rainfall and temperature averages were recorded using an automatic data recorder (21× Micrologger, Campbell Scientific) located in the study site. Data were compared between the four seasons to establish the dry and wet seasons.

2.3. Soil water content (SWC)

Soil water content was measured by the gravimetric method (Peters, 1965). During each season sampling, five soil samples were taken at three different depths (10, 20 and 30 cm) from the