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Growth and physiological sapling responses of eleven *Quercus ilex* ecotypes under identical environmental conditions

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ABSTRACT

Studies with holm oak indicate that genetic variation may result in substantial differences in drought tolerance among its ecotypes. However, few trials have studied this variation under common environmental conditions. This study aimed to assess physiological and morphological responses of holm oak saplings for 11 ecotypes that represent a longitudinal transect across south-central Spain (Andalusia). Drought resistance was assessed by measuring growth, xylem water potential, chlorophyll fluorescence, and photosynthesis. Possible relationships among morphological and physiological responses across ecotypes were determined using Pearson product-moment correlations and multiple linear regressions. The response variables were used in multivariate analyses including discriminant function analysis, principal component and cluster analyses. Last, we used sparse Partial Least Squares regression (sPLS) to analyse the relationships between the morpho-physiological responses and biophysical parameters of the parent locations. Our results indicate that *Q. ilex* ecotypes growing in a common garden setting exhibited substantial variation in morphological and physiological traits. At the end of the growth trial (65 months post-planting), basal diameter, leaf area, and midday water potential were higher in *Q. ilex* ecotypes from western sites compared to eastern sites across Andalusia. PCA and clustering revealed clear morphological and physiological differentiation in response to gradients of geographical and ecological variation in ecotype origin. Variables that were related to the water regime of the ecotypes, such as seasonal precipitation and evapotranspiration, showed stronger correlations with ecotype responses. Consequently, eastern ecotypes were more likely to spread in response to projected increases in temperatures and declines in summer precipitation; however, western ecotypes would likely decrease in response to hotter and drier summers.

1. Introduction

The holly or holm oak (*Quercus ilex*) grows in parts of southern Europe, Asia Minor, and North Africa, surrounding the Mediterranean Sea. Forests of this evergreen species grow on a range of substrates and in semi-arid and sub-humid climates of the Mediterranean region, although their optimal development occurs under annual rainfall regimes of 350–700 mm and mean minimum temperatures ranging between −2 and 10 °C (Barbero et al., 1992). Total forest cover is estimated to be about 3.9 million hectares, and has great ecological importance in the southern Mediterranean Basin (García-Nogales et al., 2016). Holm oak populations are often fragmented, resulting in prolonged isolation and complex geographic patterns of genetic variation (Guzmán et al., 2015). Mediterranean savanna-like agrosylvopastoral systems (*dehesas* in Spain, *montados* in Portugal and the *terroir* of France) are managed and highly manipulated systems (Joffre et al., 1999), which may help explain the observed genetic variability from location to location.

Quercus ilex L. subsp. *ballota* (Desf.) Samp. is the subspecies of holm oak that is native to Spain. It is one of the most widely planted trees in reforestation and afforestation sites, especially within the southern part of the country. These areas cover more than 82,755 ha in Andalusia, particularly in abandoned agricultural lands. Holm oak has been selected for afforestation because of its high drought tolerance (Barbeta and Peñuelas, 2016) and plasticity of response to varying edaphic conditions (Laureano et al., 2016). In southern Spain, holm oak forests occur over a wide range of rainfall regimes, i.e., from drought-affected marginal lands with annual rainfall as low as 260 mm to wet climates with annual rainfall over 1000 mm. These populations may be particularly suitable for forest conservation and afforestation projects that place a premium on stress tolerance over growth rate. Although several

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studies have investigated the relevant seedling traits in nurseries prior to out-planting (Villar-Salvador et al., 2004), physiological and morphological analysis of these populations and their responses to water deficits and identification of the traits conferring drought resistance are required (Costa-Saura et al., 2016), once the plants (saplings) have become established in the field after several years.

Adaptation of species to geographic variation in the environment often depends upon genetic variation among seed sources. Genetic inventories using biochemical and DNA markers have demonstrated that genetic variability is geographically structured. Variation in holm oak is greatest in France (Lumaret et al., 2002; Vitelli et al., 2017) and Spain (Guzmán et al., 2015; García-Nogales et al., 2016), most likely due to the genetic structure of populations and their historical management. Genetic studies, together with ecological information, have been used to define seed orchards of holm oak in several countries. In general, seed sources from drier, inner portions of this species' range show greater pre-adaptation to drought than populations originating close to the coast (Matías et al., 2010). Studies with holm oak indicate that genetic variation may promote significant differences in drought tolerance among ecotypes (Peguero-Pina et al., 2014). However, the production of *Q. ilex* nursery stock suffers from a low degree of genetic variability, which may limit the success of more drought tolerant *Q. ilex* ecotypes in restoration and reforestation programs.

Quercus ilex occupies habitats that receive low amounts of precipitation, but elevated levels of annual radiation (Quero et al., 2006); under planting stress, seedlings may undergo morphological and physiological changes, such as leaf morphology and transpiration reduction, by decreasing the area of exposed leaf surface. Variation in stem and leaf morphology, seedling physiology, phenological stages, and growth have been reported among holm oak populations that were raised from different seed sources (Gratani et al., 2003; Gimeno et al., 2009; Peguero-Pina et al., 2014), but relatively little information exists concerning genetic variation in the major components of drought resistance in this species.

There is increasing interest in using species' traits to predict ecotype responses to environmental change (Niinemets, 2015, Cavender-Bares et al., 2016). This approach has been used in studies of *Quercus ilex* responses to drought (Fusaro et al., 2017), climate gradients (García-Nogales et al., 2016; Peguero-Pina et al., 2014) and other forms of disturbance (Chiatante et al., 2015). It has been proposed that attention be focused on plant organs such as leaves to facilitate functional comparisons of plants, since leaves are the most well-studied plant organs (Wright et al., 2004; Pérez-Harguindeguy et al., 2013). Leaf traits are strongly correlated among populations and species. This implies that multiple traits are associated with a singular trade-off in function (Wright et al., 2004).

Therefore, a wide range of ecophysiological responses is expected in *Q. ilex* ecotypes originating from habitats of differing moisture regimes, when grown under identical field conditions. In previous studies, differences in growth, leaf gas-exchange, and xylem water potential were observed, suggesting that the genetic diversity of *Q. ilex* ecotypes leads to differences in establishment success (Gratani et al., 2003; Pesoli et al., 2003). This differential response indicates that *Q. ilex* possesses highly effective physiological plasticity and can thus adapt to different environmental conditions (Gimeno et al., 2009; Peguero-Pina et al., 2014). Yet, the genetic sources of *Q. ilex* plasticity have been explored to a lesser degree than those of other forest species (Michaud et al., 1995; Valero-Galván et al., 2011, 2012) in long-term field trials. Hence, the results lead to reliance upon particular genotypes or ecotypes. Consequently, analysis of the physiological and morphological responses of *Q. ilex* ecotypes under identical environmental field conditions is crucial to the characterisation of the species and the selection of the more drought-tolerant genotypes (ecotypes) among the provenances. We hypothesised that the more drought-resistant *Q. ilex* ecotypes might be less susceptible to water stress, possibly due to lower rates of photosynthesis and growth; i.e. a more conservative resource-use strategy (sensu Valladares et al., 2000).

The aim of this study was to assess how morphological and physiological traits of 11 *Quercus ilex*ecotypes affect their performance under identical environmental conditions. Therefore, in this study we proposed and tested three non-exclusive hypotheses: (i) Are there differences in morphological and physiological traits among *Q. ilex* ecotypes? (ii) Are *Q. ilex* populations from drier habitats more drought-tolerant than those from moister ones? (iii) Can we identify, several years after establishment, which populations and plant traits may warrant further ecotype selection for the improvement of *Q. ilex* drought tolerance for forest conservation and afforestation programs in southern Spain? We expect that ecotypes responses vary according to the environmental conditions of populations' origin.

2. Materials and methods

2.1. Plant material and growth conditions

Since 2009, the University of Córdoba has hosted a collection of 11 different ecotypes of *Q. ilex*. Each one consists of a group of plants grown from acorns that were collected from controlled seed sources provided by the Andalusia Forest Department (southern Spain, Table 1, Fig. S1 Supplementary material). The ecotypes that were included in this study were selected according to their potential use in afforestation under drought conditions, covering a wide range of habitats in southern Spain (region of Andalusia). To establish this collection, one-year-old seedlings were grown with standard nursery practices (300-cm^3 containers and peat-vermiculite [3:1 v:v] as substrate). Average seedling height and basal diameter, measured just before planting, were 14.66 ± 0.81 cm and 3.45 ± 0.31 mm, respectively (means and standard errors, $N = 50$), with no significant differences according to the origin of the acorns. For the purposes of this study, we assumed that nursery cultivation and its environmental effects during subsequent growth were minimal.

Table 1
Environmental features of *Quercus ilex* ecotypes that were established in a common garden experiment in Hinojosa del Duque (Province of Cordoba, southern Spain). Average temperature of the coldest month (Tmin), average temperature of the warmest month (Tmax), and average annual rainfall (P) (see ecotype locations in Fig. S2, Supplementary Material).

Cod.	Ecotype id./Region. Province		Coordinates (ETRS89)	MASL (m)	T_{max} (°C)	T_{min} (°C)	P (mm)
	A	Almería/Sierra de Alhamilla. Almería	36°59′N, 2°05′W	1241	25.2	8.9	277.9
More eastern	S	Segura/Sierra de Segura. Jaén	38°17′N, 2°36′W	643	23.1	4.4	795.4
	Po	Pozo Alcón/Sierra del Pozo. Jaén	30°17′N, 2°36′W	643	24.1	4.4	795.4
	P	Valdepeñas/Sierra Sur. Jaén	37°30′N, 3°56′W	618	24.8	5.9	556.3
	G	Granada/Arenas del Rey. Granada	36°57′N, 3°54′W	489.3	24.7	11.5	489.3
↓	Poz	Pozoblanco/Valle de los Pedroches. Córdoba	38°22′N, 4°54′W	618	26.8	8.1	612.7
	Ca1	Benamahoma/Sierra de Grazalema. Cádiz	36°45′N, 5°27′W	649	24.9	9.8	1263.6
	Ca2	Puerto Serrano/Sierra de la Nava. Cádiz	36°54′N, 5°31′W	373	25.5	9.5	1000.5
	S1	Almadén/Sierra Norte. Sevilla	37°52′N, 6°05′W	482	26.4	9.5	722.1
	H2	Corteconcepción/Sierra de Aracena. Huelva	37°54′N, 6°30′W	369	26.3	5.5	845.6
More western	H1	Calañas/Andévalo Oriental. Huelva	37°52′N, 6°51′W	184	26.5	10.5	635.7