Phenotypic plasticity for physiological and life-history traits of *Quercus ilex* L.

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Abstract

Seedlings of *Quercus ilex* germinated from seeds of parent plants from four different localities within the distribution area in Italy were analysed. The plasticity index for physiological leaf traits is higher than for morphological ones. The most favourable environmental conditions of Castelporziano increase the plasticity index, while the climate of Nago, Bellegra and Frassanito allows the specialization of leaf traits, determining a higher efficiency of the resource use in response to specific stress factors. Because plasticity influences environmental tolerance, different plastic responses may contribute to differences in the range of environments that species inhabit, and in the specific capability to maintain functioning in contrasting conditions.

Keywords: climate change, *Quercus ilex*, plasticity index, seed, seedling

Introduction

Drought, high air temperatures and high irradiance, for short or long periods influence Mediterranean species productivity (Gratani & Varone, 2004, Ósorio *et al*., 2006). Nevertheless, plant response may vary among species, and among populations of the same species, depending on their adaptive capability. Considerations of adaptability involve an interest in plant species' response to the forecasted increase of air temperature and drought in the Mediterranean Basin.

Among the Mediterranean evergreen species, *Quercus ilex* L. is widely distributed in the Mediterranean Basin, extending longitudinally from Portugal to Syria, and latitudinally from Morocco to France (Valladares *et al*., 2000a). It seems to be limited in its southern range by increased summer drought and in altitude by factors associated with low air temperature. Michaud *et al*. (1992) observed a homogeneous genetic structure of *Q. ilex* in the Mediterranean region, with only slight geographic variations due to isolation (i.e. North Africa and Sicily). *Q. ilex* is used for restoration of heavily deforested areas and abandoned croplands of the Mediterranean Basin; nevertheless its seedlings have a low survival in plantations compared to other Mediterranean woody species, depending on their vulnerability to water stress in early life, particularly during the first summer drought following planting (Villar-Salvador *et al*., 2004).

The main objective of this research was to analyse the plasticity index of *Quercus ilex* seedlings developed from different populations of acorns in Italy. Plasticity is considered a measure of the phenotypic difference of a species in various environments (Valladares *et al*., 2000a), and it may also play a critical role in the response of natural populations to selective pressure in variable environments in respect of global change. In the last 15 years there has been an increasing interest in the capacity of a given genotype to express different phenotypes in different environmental conditions, nevertheless, only recently has plasticity been recognized as a significant way to evaluate diversity.

Materials and methods

Study localities

Four different localities along a gradient from the north to the south of Italy were considered: Nago (site N, 45°55’ N, 10°53’ E, 260 m a.s.l.) at the northernmost distribution limit, characterized by a transitional climate with intermediate characteristics between the pre-alpine and the Mediterranean climate (low winter air temperatures and frequent frost periods); Bellegra (site B, 41°53’ N, 13°01’ E, 815 m a.s.l.) under a temperate type of climate (low air temperature in winter); Castelporziano (site C, 41°45’ N, 12°26’ E, at sea level) under a Mediterranean type of climate (moderate drought stress); Frassanito (site F, 40°13’ N, 18°26’ E, at sea level) under a Mediterranean type of climate (strong drought stress).

Acorn collection and measurements

Quercus ilex acorns were collected at the end of November 2006 from 10 representative shrubs per locality. The acorns were transported to the Botanic Garden of Rome. The Botanic Garden area’s climate was of the Mediterranean type.

The acorn fresh mass (FM) and the maximum acorn diameter (D, mm) were measured, this last by a digital micro calliper (Haglöf, S). The acorn volume (V, cm^3) was calculated (Aizen & Patterson 1990).

Acorn sowing and germination

At the beginning of December 2006, 20 acorns per locality were sown in plastic pots and filled with a sandy-peat mixture, according to Aranda *et al*. (2004). Acorns were sown at approximately 2 cm depth in the topsoil, and covered with the respective litter collected in the original localities. The pots were kept outdoors under natural conditions and watered regularly to the field capacity.

Seedling measurements

Seedling height (H) was monitored 18 months after germination on 10 seedlings for each locality. The relative growth rate in height (RGR_H) was calculated by the equation: $RGR_H = (\ln H_2 - \ln H_1) / t_2 - t_1$, where t was the time in days and H_1 e H_2 were the seedlings’ height at t_1 and t_2.

Leaf morphology and anatomy

Morphological and anatomical traits were analysed on 20 fully expanded leaves (per locality). Measurements included leaf area (LA, by the Image Analysis System Delta-T Devices, LTD, England), leaf dry mass (DM, oven-dried at 90°C to constant mass), leaf mass area (LMA, leaf dry mass per unit leaf area). Leaf tissue density (LTD) calculated by the ratio of DM, and leaf volume (V, by the product of LA and leaf thickness). Leaf thickness was measured by leaf sections hand-cut from fresh fully expanded leaves per locality and analysed by light microscopy.

Gas exchange measurements

Net photosynthesis (P_N), stomatal conductance to water vapour diffusion (g_s), transpiration rate (E) and leaf temperature (T_l) were monitored in January, April and July 2009, with an infrared CO_2 gas analyser (ADC-LCA4, UK), equipped with a leaf chamber PLC4 (ADC, Hoddesdon, UK). Measurements were made on cloud-free days, in the morning (08:30 to 10:30 a.m.), on four leaves per seedling (per each sampling occasion). The collected gas exchange data (P_N, g_s) were grouped in sequences of air temperatures (Gratani *et al*. (2003).

Experiment of imposed water stress

The experiment of imposed water stress was carried out in July 2009. Water was withheld from 5 seedlings per locality to generate stress. The control seedlings were watered regularly to field capacity. During the experiment, diurnal air temperature was 24 to 29°C, and air humidity 70 per cent in the early morning and 40 per cent at midday.

P_N, E and g_s were monitored during the imposed water stress experiment on fully expanded leaves.

Predawn leaf water potential (Ψ_{pd}) was measured the first and the last day of the experiment on fully expanded leaves (control) and on fully expanded stressed leaves, with a pressure chamber (SKPM 1400 Skye Instruments, UK).
Relative water content at predawn (RWC_{pd}) was determined on the same leaves used for Ψ_{pd} measurements. RWC_{pd} was calculated as RWC = (FM – DM)/(TM – DM) x 100, where FM was the leaf fresh mass, TM the leaf mass after rehydration until saturation for 48 h at 5°C in the darkness.

Index of phenotypic plasticity
The index of phenotypic plasticity was calculated for each variable as the differences between the minimum and the maximum mean values divided by the maximum mean value, according to Valladares *et al*. (2000b).

Statistics
The differences of acorn and seedling traits were determined by the analysis of variance (ANOVA), and Tuckey test for multiple comparisons.

All statistical tests were performed using a statistical software package (Statistica, Statsoft, USA).

Results

Acorn traits
F and B acorns had the significant ($p \leq 0.05$) highest V and FM, while N acorns had the lowest. C acorns were in an intermediate position.

Seedling growth
Seedling growth occurred when mean minimum air temperature was 10.2±1.0°C at the middle of March for B, F, and C seedlings, and at the end of March for N seedlings. The observation of seedling growth finished at the middle of June when mean maximum air temperature was 29.9±1.9°C.

B and F seedlings had the highest RGR_{max} (0.235±0.028 mm mm^{-1} d^{-1} and 0.203±0.011 mm mm^{-1} d^{-1}, respectively), while N seedlings had the lowest (0.087±0.005 mm mm^{-1} d^{-1}). C seedlings had intermediate RGR_{max} value (0.140±0.005 mm $mm^{-1}d^{-1}$).

B, F and C seedlings showed the highest H (258±21 mm, mean value), and N seedlings the lowest (118 ± 37 mm)

Leaf morphology
LMA was the highest in B and F seedlings (14.1±0.8 mg cm^{-2}, mean value). LMA of C and N seedlings was 15 per cent lower than B and F ones. LTD was 729±25, 602±29, 538±35 and 534±39 mg cm^{-3}, in N, B, F and C, respectively.

Gas exchange
At the lowest air temperatures (7 to 9 °C) N and B seedlings had the significant ($p \leq 0.05$) highest P_N (7.8±0.2 μmol CO_2 m^{-2} s^{-1}, mean value), while at the highest air temperatures (29 to 31 °C) F seedlings had the significant ($p \leq 0.05$) highest P_N (7.5±0.4 μmol CO_2 m^{-2} s^{-1}).

g_s had the same P_N trend, with the highest g_s rates for N and B seedlings (0.05±0.01 mol H_2O m^{-2} s^{-1}, mean value) at the lowest air temperatures, and for F seedlings (0.11±0.01 mol H_2O m^{-2} s^{-1}) at the highest air temperatures.

Experiment of imposed water stress
By day four of the experiment, P_N of N stressed seedlings had the highest decrease (37 per cent) and F the lowest (10 per cent). By day five, P_N of all the considered seedlings was near zero.

g_s of the considered seedlings had the same P_N trend.

On the first day, ψ_{pd} did not significantly differ among the considered stressed seedlings (0.9±0.1 MPa, mean value). By the fifth day, ψ_{pd} had decreased on an average 74 per cent in N and B seedlings, and 67 per cent in C and F seedlings.

By the fifth day of the experiment, RWC_{pd} was 86±2 per cent in F, C, and B seedlings (mean value) and 81±1 per cent in N seedlings.

Plasticity index

The plasticity index of the seedlings was the lowest for the considered morphological traits (0.24, mean of the considered traits) than for the physiological ones (0.68, mean of the considered traits). The mean plasticity index (morphological and physiological traits) was the highest in C seedlings (Table 1).

Discussion

The results on the whole indicate that *Quercus ilex* seedlings grown in a common garden and developed from seeds of different provenance in Italy are characterized by several features, which seem to be the expression of the climate of the original localities, according to Baquedano *et al*. (2008) and Zheng *et al*. (2009).

The Nago acorns' small size could be related to the scarce plant assimilates for the developing acorns at the original locality (Gratani *et al*., 2000). At the other extreme, the large size of the Frassanito and Bellegra acorns might be justified by the drier climatic conditions of their original localities. Castelporziano intermediate acorn size is the result of the favourable climatic conditions of the original localities, according to the results of Valencia-Diaz & Montaña (2005). Seedling height and RGR_{max} are significantly related to FM; in particular, Frassanito seedlings have the highest RGR_{max} because there are sufficient acorn reserves to allow growth for a much longer period (98±3 days from acorn germination to the end of the spring vegetative period), according to Pons & Pausas, 2007).

At physiological level, N, C and B seedlings have a different response to air temperature and water stress. Frassanito seedlings show the highest stomata sensitivity to changes in leaf water potential, i.e. they adjust to drought by the best stomatal control of transpiration. The decrease of water loss per transpiration by stomatal closure paralleled by the water potential, allows Frassanito seedlings to maintain a sufficiently high RWC at predawn determining sufficient P_N rates. Moreover, drought stress has selected for more scleromorphic leaves (i.e. the highest LMA).

Seedlings from parent plants acclimatized to Nago and Bellegra are able to sustain sufficient P_N rates at the lowest air temperatures; moreover the small LA and the highest LTD appear to be the best adaptation in response to winter cold stress at both these localities.

Phenotypic plasticity for physiological and life-history traits may allow plants to grow and reproduce in spatially or temporally variable environments (Gratani *et al*. 2003). Traits involved in the same function are also more highly correlated at phenotypic and genetic levels than they are with traits which have different functions or developmental origins; selection for photosynthetic traits may often operate indirectly via correlations with other traits (Arntz & Delph, 2001). Because adaptive plasticity influences environmental tolerance, different plastic response may contribute to differences in the range of environments that species inhabit in the field (Ackerly *et al*., 2000).

The results underline that the plasticity index of the considered seedlings is significantly lower for morphological traits than for physiological ones. The mean plasticity index (morphological + physiological plasticity) is the highest in C seedlings, reflecting the most favourable environmental conditions, while the climates of Nago, Bellegra and Frassanito allow the specialization of leaf traits determining a higher efficiency of the resource use in response to specific stress factors**.**

Climate change may act as a potent agent of natural selection among *Quercus ilex* populations (Alistair & Peñuelas, 2007). Box & Choi (2000) indicate an expansion of *Q. ilex* northward and inland under warming. Thus, plasticity index may be used to draw the species response to increasing stress factors. *Q. ilex* acorn size and seedling traits from different localities in Italy might be used in afforestation and restoration programmes; in particular, Frassanito ecotype could have great potential in resource-limited areas of the Mediterranean; its high RGR and low vulnerability to water stress could be advantageous in the establishment phase during the first summer drought following planting in afforestation projects.

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Table 1. Plasticity index for *Quercus ilex* morphological (LA = leaf area, DM = leaf dry mass, LMA = leaf mass area and LTD = leaf tissue density) and physiological traits (P_N = net photosynthesis, g_s = stomatal conductance, E = leaf transpiration, WUE = water use efficiency, $\square_{pd}$ = leaf water potential at predawn)

Morphological traits	Seedlings			
	N	C	B	F
LA	0.16	0.44	0.25	0.28
DM	0.18	0.47	0.32	0.35
LMA	0.17	0.24	0.12	0.11
LTD	0.17	0.26	0.10	0.11
Mean value	0.17	0.35	0.20	0.21
Mean value of considered traits	0.23			

Physiological traits	Seedlings			
	N	C	B	F
P_N	0.39	0.40	0.41	0.45
g_s	0.75	0.83	0.73	0.88
E	0.76	0.78	0.77	0.75
WUE	0.82	0.75	0.82	0.57
$\square_{pd}$	0.72	0.73	0.70	0.65
Mean value	0.69	0.70	0.69	0.66
Mean value of considered traits	0.68			

N = Nago, C = Castelporziano, B = Bellegra, F = Frassanito