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Relationship between acorn size and seedling morphological and physiological traits of *Quercus ilex* L. from different climates

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Abstract

Effects of *Quercus ilex* acorn size on seedling morphological and physiological traits were analysed. The study was carried out with five populations from different geographical areas and covering an aridity gradient. Seedling morphological traits (height and total leaf area) were analyzed during the first growing year. Physiological traits (net photosynthesis, stomatal conductance, leaf transpiration, and intrinsic water-use efficiency) were analysed at different air temperatures during spring, summer, and winter months, and in response to water stress. There were significant correlations among the considered acorn (volume, fresh mass) and seedling traits. Differences in growth and physiological traits among the considered populations were found. The larger differences in the growth parameters were observed during the first growing season, and they could be justified by the significant differences among acorn size. On the contrary, the physiological response to air temperature and aridity was more related to the geographical origin of the considered populations than to acorn size. *Q. ilex* acorn and seedling traits more tolerant to drought might have a high potential for vegetation recovery in afforestation projects and restoration programmes particularly under water-limited environments or in degraded areas.

Additional key words: acorn size; gas exchange; predawn leaf water potential; relative growth rate in height; relative water content at predawn; total leaf area.

Introduction

Mediterranean shrublands are largely distributed in areas around the Mediterranean region, which is one of the most heavily utilised by man (Boix-Fayos *et al.* 2009). Nevertheless, large areas in the Mediterranean region have suffered degradation and habitat loss by human activities (Evrendilek *et al.* 2006). Moreover, most major responses of Mediterranean shrublands to global change seem to be variations in their structure and productivity in the long term (Haase *et al.* 2000, Saxe *et al.* 2001). There is an increased interest in the restoration of savannah-like oak forests on abandoned agricultural areas in the Mediterranean region (DOCE 1992, BOE 1994, BOJA 1995, Oliet *et al.* 2007), where the establishment of forest plantations in areas subjected to seasonal drought is strongly limited by water availability (Padilla and Pugnaire 2007, Pérez-Devesa *et al.* 2008).

Seedling stage is a critical phase in the regeneration of woody species (Leiva and Fernández-Alés 1998, Zheng *et al.* 2009), and plantation success can be greatly dependent upon seedling morphological and physiological traits (Trubat *et al.* 2010). The seedling establishment capability confers a high potential for the vegetation recovery (Bognounou *et al.* 2010). Also seed size plays an important role in the establishment of woody species (Meyer and Carlson 2001, Cordazzo 2002, Gomez 2004a). Seed size variation often occurs within a species, with important consequences for the reproductive success of plant species (Gomez 2004a, Baraloto *et al.* 2005). Seed germination capability is significantly affected by seed size, with large seeds having a greater germination success than small seeds (Pizo *et al.* 2006). In order to create systems able to self-regenerate in afforestation projects, it is important to take into account seed and seedling traits more related to drought tolerance (Leiva

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Abbreviations: D – maximum acorn diameter; FM – acorn fresh mass; g_s – stomatal conductance; IWUE – intrinsic water use efficiency; LA – leaf area; P_N – net photosynthetic rate; PAR –photosynthetically active radiation; RGR_H – relative growth rate in height; RWC_{pd} – relative water content at predawn; SH – seedling height; SH_6 – seedling height 6 months after germination; SH_{18} – seedling height 18 months after germination; TLA – total leaf area per seedling; TLA_6 – total leaf area per seedling 6 months after germination; TLA_{18} – total leaf area per seedling 18 months after germination; V – acorn volume; Ψ_{pd} – predawn leaf water potential.

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and Fernández-Alés 1998, Cordazzo *et al.* 2000). However, there is no consensus on the set of plant traits that determine establishment success in water-limited environments (Cortina *et al.* 2006).

Q. ilex L. is an evergreen species widely distributed in the Mediterranean Basin (Barbero *et al.* 1992). It seems to be limited in its southern range by increased summer drought (Terradas and Save 1992), and in altitude by factors associated with low air temperatures (Nardini *et al.* 2000). *Q. ilex* occurs in mesic through xeric habitats (Archibold 1995), and it is an important species for the restoration of heavily deforested areas and abandoned croplands of the Mediterranean Basin (Rodà *et al.* 1999, Rey-Benayas and Camacho-Cruz 2004, Oliet *et al.* 2007). *Q. ilex* seedlings have a low survival and growth rates in plantations compared to other Mediterranean woody species due to its vulnerability to water stress during the early life stages, particularly during the first summer drought following planting (Villar-Salvador *et al.* 2004). Moreover, studies about seed germination capacity according to seed provenance and along latitudinal gradients are scarce (Gratani *et al.* 2003, Yakimowski and Eckert 2007, Bognounou *et al.* 2010). Adaptation of species to geographic environmental variations often depends on genetic variations among seed sources (Gratani *et al.* 2003, Ramírez-Valiente *et al.* 2009).

The main objective of this research was to analyse the relationship among acorn size, and seedling morphological and physiological traits of *Q. ilex* shrubs developed from acorns collected in different populations growing in areas characterised by different climates. We wanted to analyse their response to air temperature and water availability. We hypothesized that the seedlings for the more xeric locality had a higher acorn size and tolerance to water stress than those from the other localities. Understanding plant species functioning in water-limited environments is crucial in order to hypothesize reliable potential responses and make informed land management decisions (Maseyk *et al.* 2008).

Materials and methods

Study localities: Five different localities along a gradient from the north to the south of Italy, characterised by different climates were considered: Nago (N) at the northernmost distribution limit, Bellegra (B) and Castelporziano (C) at the centre of the distribution area, Monte Albo (A) at the south-west of Italy, and Frassanito (F) at the south-east of Italy.

At N and B, *Q. ilex* developed on a slope at the edge of a steep cliff, on soil pockets (30–40 cm deep), and it co-occurred with *Ruscus aculeatus* L., *Hedera helix* and *Viola odorata* L. at N, and with *Ostrya carpinifolia* Scop., *Fraxinus ornus* L., *Pistacia terebinthus* L., *Phillyrea latifolia* L. at B.

C and A were characterized by the typical Mediterranean maquis including *Q. ilex*, *Pistacia lentiscus* L., *P. latifolia* L., *Arbutus unedo* L., *Cistus incanus* L., *Cistus monspeliensis* L., *Erica arborea* L., *Erica multiflora* L., *Daphne gnidium;* L., *Lonicera implexa* Ait., and *Rosmarinus officinalis* L.

F was characterized by the same species of C and A co-occurring with *Quercus coccifera* L. and *Rhamnus alaternus* L.

The climate of the considered localities was analysed using data from the nearest Meteorological Stations to the localities where acorns were collected, for the period 1985 to 2006 (Table 1).

N had a transitional climate with intermediate characteristics between the pre-alpine climate (frequent rainfall in summer, low air temperatures and frequent frost periods in winter of *ca*. 50 days) and the Mediterranean type climate. B was under a temperate climate. C, A, and F were characterised by a Mediterranean climate but with a different drought intensity and length period.

Soil characterization: The physical soil characterization of the considered localities was carried out in May 2006, on soil samples collected using an hand auger to 40-cm depth.

Soil samples (500 g each per locality) were air-dried, and then sieved with a sieve of 2 mm in diameter for particle size analysis. Analyses were performed according to the National Soil Resources of the Department of Agriculture, Food and Forestry Methods (1999).

The soil texture of N and B had the highest gravel content (73.9 ± 0.7%, mean value), while C and F the highest soil separates content (99.3 ± 0.3%, mean value). A had 50.8 ± 3.2% and 49.2 ± 3.1% of gravel and soil separates content, respectively. C had the highest sand content (96.5 ± 3.1%) and the soil of B the highest silt content (73.7±5.0%) (Table 1).

Acorn collection: *Q. ilex* acorns were collected at the end of November 2006 (200 per locality) from 10 representative *Q. ilex* shrubs (on an average 2.5 m height, with well developed crown and abundant acorns) per locality.

The acorns were reaped directly from the selected shrubs (acorns of each shrub per each population were maintained separated) at the time of natural dispersal, and they were immediately transported to the Botanical Garden of Rome (Italy). The Botanical Garden area's climate was of the Mediterranean type. During the study period (December 2006 to December 2009) the total annual rainfall was 843 mm, and the mean air temperature was 16.8°C (data from the Meteorological Station of Regional Agency for Development and Technological Innovation of Lazio Agriculture).

Acorn size measurements: The collected acorns were surveyed to discard aborted, dry and infected ones. They were floated in water (Bonfil 1998) to separate viable acorns from those parasitized ones (Seiwa 2000, Puerta-Piñero *et al.* 2006).

The acorn fresh mass (FM) was measured. The maximum acorn diameter (D) was measured individually by a digital micro calliper (Haglöf, S). The acorn volume (V) was calculated according to Aizen and Patterson (1990).

Acorn sowing and germination: At the beginning of December 2006, 20 acorns per locality were sown in a plastic pot (60 × 20 cm; 5 pots per locality for a total of 100 acorns per locality). They were filled with a sandy-peat mixture (2.7 kg of dry peat/10 l of sand), according to Aranda *et al.* (2004). Acorns were sown at approximately 2-cm depth in the topsoil, and covered with the respective litters, collected in the original localities, according to Gomez (2004a). The pots were kept outdoors under natural conditions, and watered regularly to the field capacity.

The germination process was carried out without pretreatments. The germination time was monitored daily, and the beginning of germination was considered when the percentage of germination for each pot was 20%, according to Castro-Díez and Montserrat-Martí (1998). After germination 20 seedlings per each locality were selected for measurements.

Seedling RGR and height measurements: Seedling height (SH, mm) was monitored weekly, 7 to 120 days after germination (*i.e.* at the end of the spring vegetative period), on 10 seedlings for each locality, in the period March to July 2007. Moreover, seedling height was monitored at 6 and 18 months after germination (SH_6 and SH_{18}, respectively), at the end of September 2007 and 2008, respectively.

The relative growth rate in height (RGR_H, mm mm^{-1} d^{-1}) was calculated by the equation: $RGR_H = (\ln SH_2 - \ln SH_1)/(t_2 - t_1)$, where t was time in days and SH_1 e SH_2 were the seedlings height at t_1 and t_2. (Hunt 1982).

Leaf area and total leaf area per seedling: Fully expanded leaves of the cultivated seedlings were collected at the end of August 2007 and 2008. Leaf area (LA) was measured using the Image Analysis System (*Delta-T Devices*, UK). Measurements were carried out on 10 seedlings per each locality (3 leaves per each seedling).

The total leaf area per seedling (TLA) was calculated by multiplying the total number of leaves and the mean LA of each seedling, 6 (TLA_6) and 18 (TLA_{18}) months after germination. Measurements of TLA were carried out on 10 seedlings (seedlings used for LA measurements were excluded in order to did not underestimate TLA).

Gas exchange measurements: Photosynthetic photon flux density (PPFD), net photosynthetic rate (P_N), and stomatal conductance to water vapour diffusion (g_s) were monitored in January, April, and July 2009 (five days of measurements per month) with an infrared CO_2 gas analyzer (*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 h), on four leaves per seedling (5 seedlings per locality) in each sampling occasion.

The collected gas-exchange data (P_N, g_s) were grouped in sequences of air temperatures, according to Zangler and Bazzaz (1983), and Gratani *et al.* (2003). The air temperature ranges were 7 to 9°C, 19 to 21°C, and 29 to 31°C.

Experiment of imposed water stress: The experiment of imposed water stress was carried out in July 2009 on 10 seedlings per each locality. All the seedlings were irrigated until July 13th, when the experiment started.

The stress treatment was induced by withholding the irrigation on 5 seedlings per locality (stressed seedlings) until net photosynthesis approached zero, according to Pesoli *et al.* (2003), and Gomes *et al.* (2008). At the same time, 5 seedlings per locality (control seedlings) were watered to field capacity every day during the experiment.

During the experiment, diurnal air temperature ranged from 24 to 29°C, air humidity was 70% in the early morning and 40% at midday, and PAR ranged from 1,300 to 1,500 µmol(photon) m^{-2} s^{-1}. During the water stress experiment rain did not occur.

P_N and g_s were monitored during the imposed water stress experiment on 2 fully expanded leaves per seedling. Moreover, the intrinsic water-use efficiency (IWUE) was calculated as the ratio between P_N and g_s (Gulías *et al.* 2009).

Predawn leaf water potential (Ψ_{pd}) was measured the first and the last day of the experiment on the control and on stressed seedlings with a pressure chamber (*SKPM 1400 Skye Instruments*, UK), using 2 fully expanded leaves per each stressed and control seedlings. Relative water content at predawn (RWC_{pd}) was determined on the same leaves used for Ψ_{pd} measurements. RWC_{pd} was calculated as $RWC = (FM - DM)/(TM - DM) \times 100$, where FM was the leaf fresh mass, DM the leaf mass after drying at 90°C to constant mass, and TM the leaf mass after rehydration until saturation at 5°C in the darkness (Lo Gullo and Salleo 1988).

Statistics: The differences of acorn size and physiological traits among seedlings from different localities were determined by the analysis of variance (*ANOVA*), and *Tukey* test for multiple comparisons.

The correlation among the considered acorn size and physiological traits of the seedlings from different localities were evaluated by Pearson's correlation coefficient.

The Principal Component Analysis (PCA) was performed using the acorn and seedling data set on the basis of a matrix of the normalised data. The matrix was subjected to a rotated principal component analysis with the objective of summarising the main factors determining the variations of the analysed traits in the considered seedlings, according to García-Plazaola *et al.* (2000). All statistical tests were performed using the statistical software package (*Statistica, Statsoft*, USA).

Results

Acorn traits and seedling growth: There were no significant intra-population differences among acorn size. F and B acorns had the significant ($p \leq 0.05$) highest D, V and FM, while N acorns the lowest ones. A and C acorns were in an intermediate position (Fig. 1). The germination time varied among the considered acorns: B had the shortest acorn germination time (93 ± 3 days after sowing), followed by F (95 ± 2 days after sowing), C (101 ± 3 days after sowing), A (103 ± 2 days after sowing), and N (109 ± 2 days after sowing).

Seedling growth started when the mean minimum air temperature was 10.2 ± 1.0°C in mid March for B, F, C, and A seedlings, and at the end of March for N seedlings. The seedlings growth ended in mid June when the 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 the lowest RGR_{max} (0.087 ± 0.005 mm mm^{-1} d^{-1}) during the growth period in the first year (Fig. 2). A and C seedlings had intermediate RGR_{max} values (0.138 ± 0.007 mm mm^{-1} d^{-1}, mean value).

SH of the considered seedlings at 6 and 18 months after germination is shown in Fig. 3. B and F seedlings showed the significantly ($p \leq 0.05$) highest SH_6 (115 ± 25 mm and 81 ± 24 mm, respectively) 6 months after germination, while N seedlings the lowest one (35 ± 10 mm). A and C seedlings had intermediate SH_6 values (70 ± 1 mm, mean value). B, F and C seedlings showed the significantly ($p \leq 0.05$) highest SH_{18} (258 ± 21 mm, mean value), and N seedlings the lowest one (118 ± 37 mm).

Total leaf area per seedling: B and F seedlings had the significantly ($p \leq 0.05$) highest TLA_6 (66.1 ± 7.5 cm^2, mean value), and N seedlings the lowest TLA_6 (17.9 ± 7.0 cm^2), while A and C seedlings had intermediate values (42.1 ± 3.0 cm^2, mean value) (Fig. 3). F, B and C seedlings had significantly ($p \leq 0.05$) higher TLA_{18} (235.4 ± 19.9 cm^2, mean value) than N seedlings (30.7 ± 0.5 cm^2) (Fig. 3).

Gas exchange: All the considered seedlings had the highest P_N in the range 19 to 21 °C [10.3 ± 0.3 μmol(CO_2) m^{-2} s^{-1}, mean value] (Fig. 4). At the lowest air temperatures (7 to 9°C) N and B seedlings had the significantly ($p \leq 0.05$) 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 significantly ($p \leq 0.05$) highest P_N [7.5 ± 0.4 μmol(CO_2) m^{-2} s^{-1}].

g_s had the same trend as P_N with the highest g_s rates observed for N and B seedlings [0.05 ± 0.01 mol(H_2O)

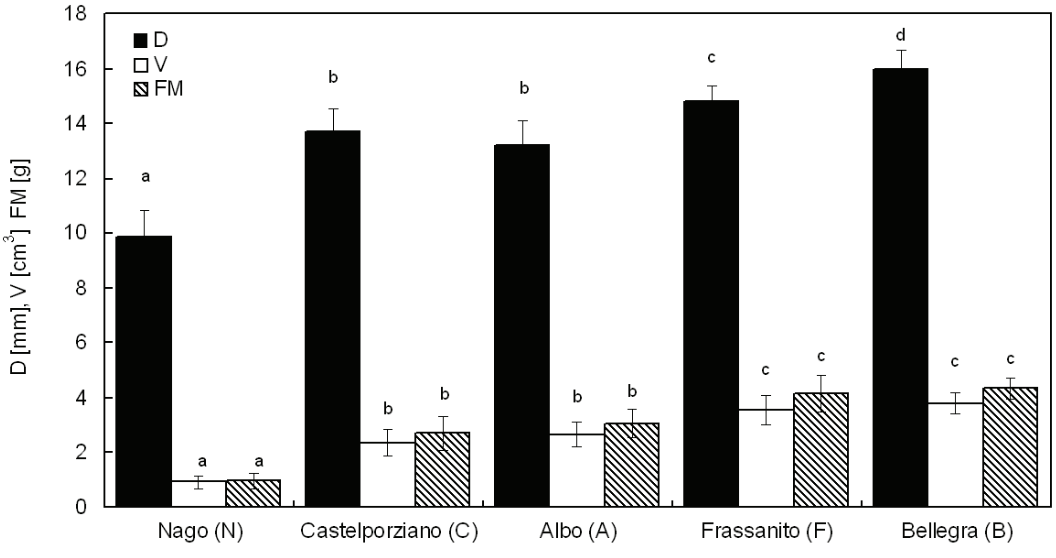

Fig. 1. Maximum acorn diameter (D), acorn volume (V), and acorn fresh mass (FM) of the seedlings from the considered populations. Mean values (± SD, $n = 100$) are shown. For each parameter mean values with the same *letters* indicate not significant differences among the seedlings (*Tukey* test, $p \geq 0.05$).

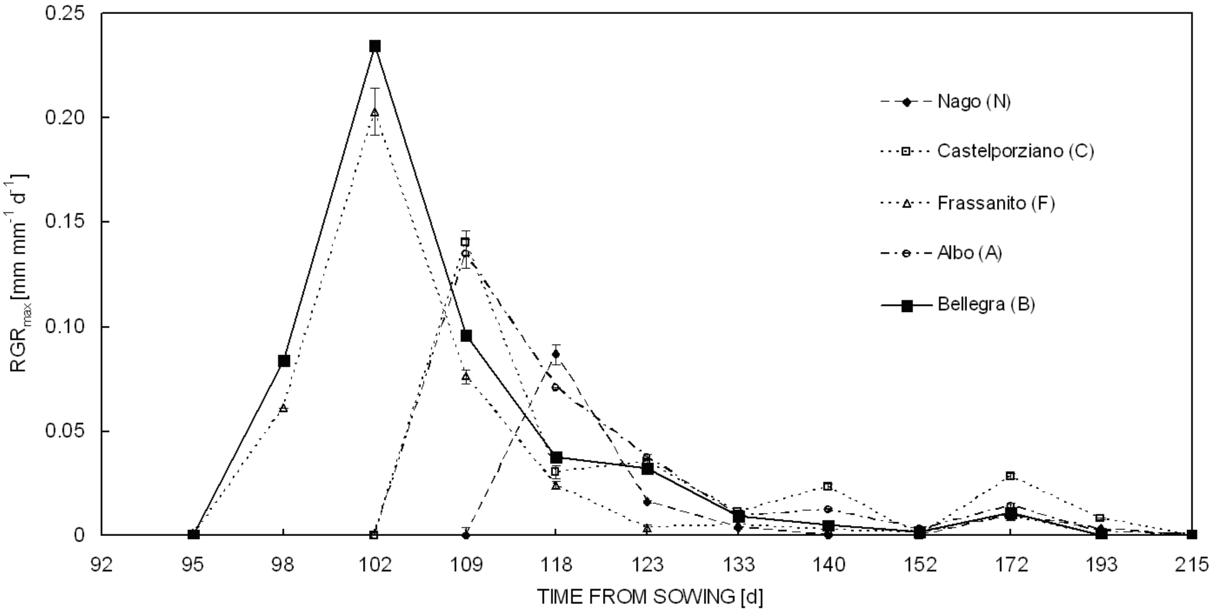

Fig. 2. Maximum relative growth rate (RGR_{max}) of the seedlings from the considered populations during the study period. Mean values (± SD, $n = 10$) are shown.

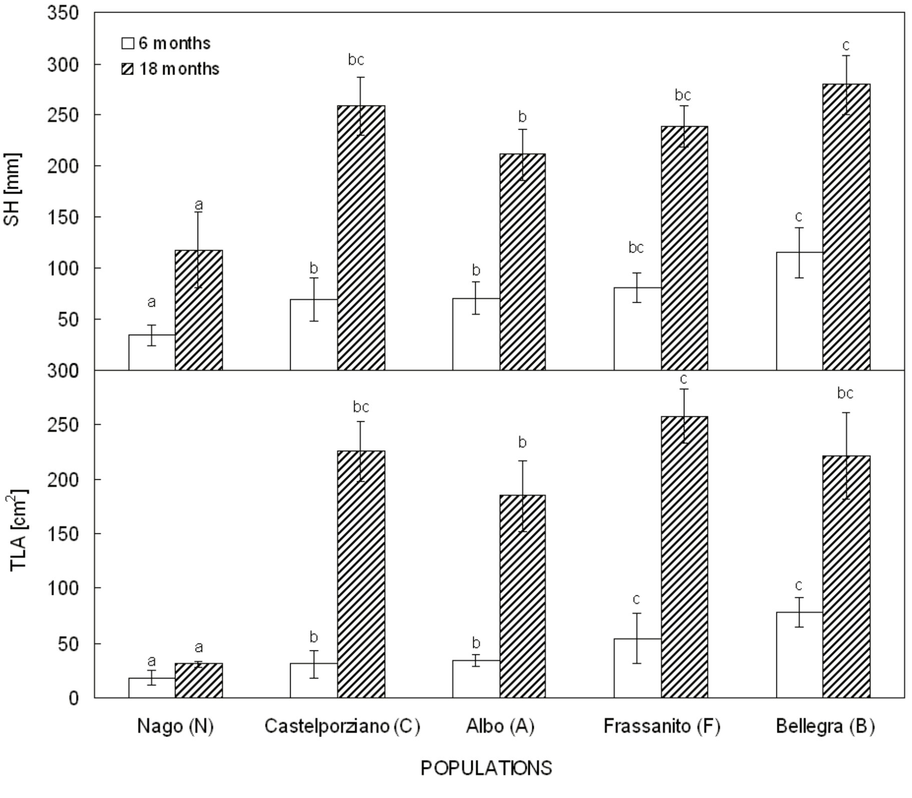

Fig. 3. Seedling height (SH) and total leaf area per seedling (TLA) of the seedlings from the considered populations 6 and 18 months after germination. Mean values (± SD, $n = 10$) are shown. For each seedling age mean values with the same *letters* are not significantly different (*Tukey* test, $p \geq 0.05$).

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 (Fig. 4).

Experiment of imposed water stress: On the 1st experimental day ψ_{pd} did not significantly differed among the stressed seedlings (–0.9 ± 0.1 MPa, mean value). On the 5th experimental day, ψ_{pd} decreased by 71% in N, B and A seedlings, and by 67% in C and F seedlings (Table 2).

On the 5th experimental day, RWC_{pd} was 86 ± 2% in F, C, A and B seedlings (mean value) and 81 ± 1% in N seedlings (Table 2).

P_N of the considered stressed seedlings decreased with the increase of the imposed water stress (Fig. 5). P_N of N

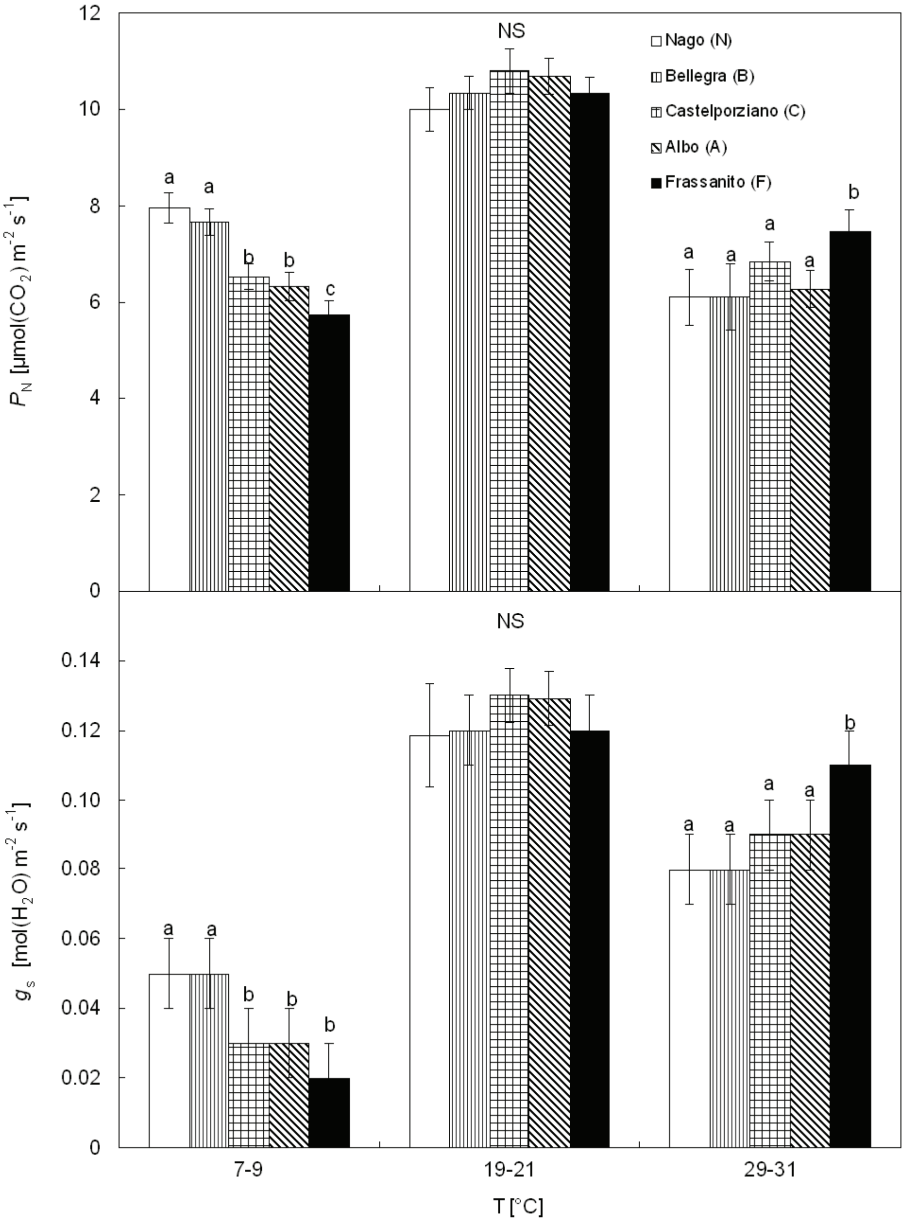

Fig. 4. Net photosynthetic rate (P_N) and stomatal conductance (g_s) of the seedlings from the considered populations measured at low (7–9°C, January), optimal (19–21°C, April) and high (29–31°C, July) air temperature. Mean values (± SD, $n = 100$) are shown. Within the same air temperature range, mean values with the same *letters* indicate not significant differences among the seedlings (*Tukey* test, $p \geq 0.05$).

and C seedlings decreased by 22% on the 3rd experimental day, while A and B seedlings decreased by 13%, and F seedlings by 10%. On the 4th experimental day, P_N of N stressed seedlings had the greatest reduction (by 37%) while F stressed seedlings had the lowest reduction (by 10%). On the 5th experimental day, P_N of all the considered seedlings was near zero.

g_s of the considered seedlings had the same trend as P_N (Fig. 5) with a significant ($p \leq 0.05$) decrease on the 3rd experimental day for all the stressed seedlings.

IWUE of all the considered stressed seedlings significantly ($p \leq 0.05$) increased on 4th experimental day respect to the 1st day; in particular, N seedlings had the highest IWUE increase (by 28%) and C, A and F seedlings the lowest IWUE increase (by 25%) (Fig. 5).

Main trends from statistical analysis: The *Pearson*'s correlation analysis showed significant correlations among the considered acorn and seedling traits (Table 3). PCA analysis extracted two factors accounting for 70% of the total variance among the considered seedlings (50 and 20% for the 1st and the 2nd factor, respectively). The 1st factor was manly related to FM, TLA$_{18}$, and P_N during summer, while the 2nd was mainly related to g_s measured at the highest air temperatures range (29–31 °C).

Considering both factors jointly, the seedlings were grouped in four groups (Fig. 6); the 1st group included seedlings from N locality, the 2nd group seedlings from C and A localities, the 3rd group seedlings from B locality; finally the 4th group included seedlings from F locality.

Table 1. Geographic, climatic and soil characteristics of the study localities where the acorns have been collected. Climatic data from 1985 – 2006. R – total annual rainfall; R_s – total annual rainfall during summer months (June to August); T_m – mean annual air temperature. Mean values (± SD) of the soil characteristics are shown. In each column mean values with the same letters are not significantly different (*Tukey* test, $p \geq 0.05$).

Locality	Geographic characteristics			Climatic characteristics			Soil characteristics				
	Latitude (N)	Longitude (E)	Altitude [m .a.s.l.]	R [mm]	R_s [mm]	T_m [°C]	Soil separate content [%]	Gravel [%]	Sand [%]	Silt [%]	Clay [%]
Nago (N)	45°55’	10°53’	260	899	260	13.2	25.6 ± 4.2^{a}	74.4 ± 4.1^{a}	65.2 ± 6.2^{a}	42.4 ± 4.1^{a}	7.5 ± 1.5^{a}
Bellegra (B)	41°53’	13°01’	815	1167	157	13.6	26.6 ± 5.4^{a}	73.4 ± 4.3^{a}	11.0 ± 2.3^{b}	73.7 ± 5.0^{b}	15.4 ± 3.4^{b}
Castelporziano (C)	41°45’	12°26’	0	707	64	15.8	99.1 ± 0.8^{b}	0.9 ± 0.4^{b}	96.5 ± 3.1^{c}	3.5 ± 1.3^{c}	-
Monte Albo (A)	40°34’	9°38’	790	700	76	15.6	49.2 ± 3.1^{c}	50.8 ± 3.2^{c}	37.3 ± 3.5^{d}	32.8 ± 4.6^{d}	2.0 ± 1.0^{c}
Frassanito (F)	40°13’	18°26’	0	679	39	16.3	99.6 ± 0.3^{b}	0.4 ± 0.2^{b}	50.1 ± 5.7^{e}	44.7 ± 4.5^{a}	18.0 ± 2.6^{b}

Table 2. Leaf water potential (Ψ_{pd}) and relative water content (RWC_{pd}) at predawn of the seedlings from the considered populations measured the 1st and the 5th day of the induced water-stress experiment for the control- and stressed seedlings. Mean values (± SD, $n = 10$) are shown. Mean values with the same *letters* are not significantly different (*Tukey* test, $p \geq 0.05$).

Populations	Control				Stress			
	ψ_{pd} [MPa] 1st day	ψ_{pd} [MPa] 5th day	RWC [%] 1st day	RWC [%] 5th day	ψ_{pd} [MPa] 1st day	ψ_{pd} [MPa] 5th day	RWC [%] 1st day	RWC [%] 5th day
Nago (N)	-0.9 ± 0.1^{a}	-1.0 ± 0.2^{a}	89 ± 1^{a}	88 ± 1^{a}	-1.0 ± 0.1^{a}	-3.6 ± 0.3^{a}	89 ± 1^{a}	81 ± 1^{a}
Bellegra (B)	-0.9 ± 0.2^{a}	-0.8 ± 0.2^{a}	90 ± 1^{a}	88 ± 1^{a}	-1.0 ± 0.2^{a}	-3.3 ± 0.2^{a}	89 ± 1^{a}	84 ± 1^{b}
Castelporziano (C)	-0.8 ± 0.1^{a}	-0.9 ± 0.2^{a}	91 ± 1^{a}	90 ± 1^{a}	-0.8 ± 0.1^{a}	-2.6 ± 0.3^{bc}	90 ± 2^{a}	87 ± 1^{c}
Albo (A)	-0.8 ± 0.1^{a}	-0.9 ± 0.1^{a}	90 ± 1^{a}	89 ± 1^{a}	-0.8 ± 0.1^{a}	-2.8 ± 0.3^{b}	90 ± 1^{a}	85 ± 1^{b}
Frassanito (N)	-0.7 ± 0.2^{a}	-0.8 ± 0.1^{a}	90 ± 4^{a}	90 ± 1^{a}	-0.8 ± 0.1^{a}	-2.3 ± 0.3^{c}	90 ± 1^{a}	87 ± 1^{c}

Discussion

Plant response to climate vary among different species, and species with extensive geographical ranges have the potential to exhibit large intraspecific variations in physiology, morphology, phenology, and growth rate; thus they may constitute good models for the study of local and regional adaptation (Soolanayakanahally *et al.* 2009).

The results of our research indicate that *Q. ilex* seedlings from five localities (Nago, Bellegra, Castelporziano, Monte Albo, and Frassanito) are characterised by different acorns size and morphological and physiological seedling traits probably driven by the climate of the localities from which they were originated, according to the results of Oleksyn *et al.* (1998) for *Picea abies* (L.) H. Karst., Palmroth *et al.* (1999) for *Pinus sylvestris* L., and Peuke *et al.* (2002) for *Fagus sylvatica* L., Zhao *et al.* (2007) for *Trollius ranunculoides* Hemsl., Baquedano *et al.* (2008) for *Q. coccifera* L. and *Pinus halepensis* Mill., Zheng *et al.* (2009) for *Trigtonobalanus doichangensis* (A. Camus) Forman.

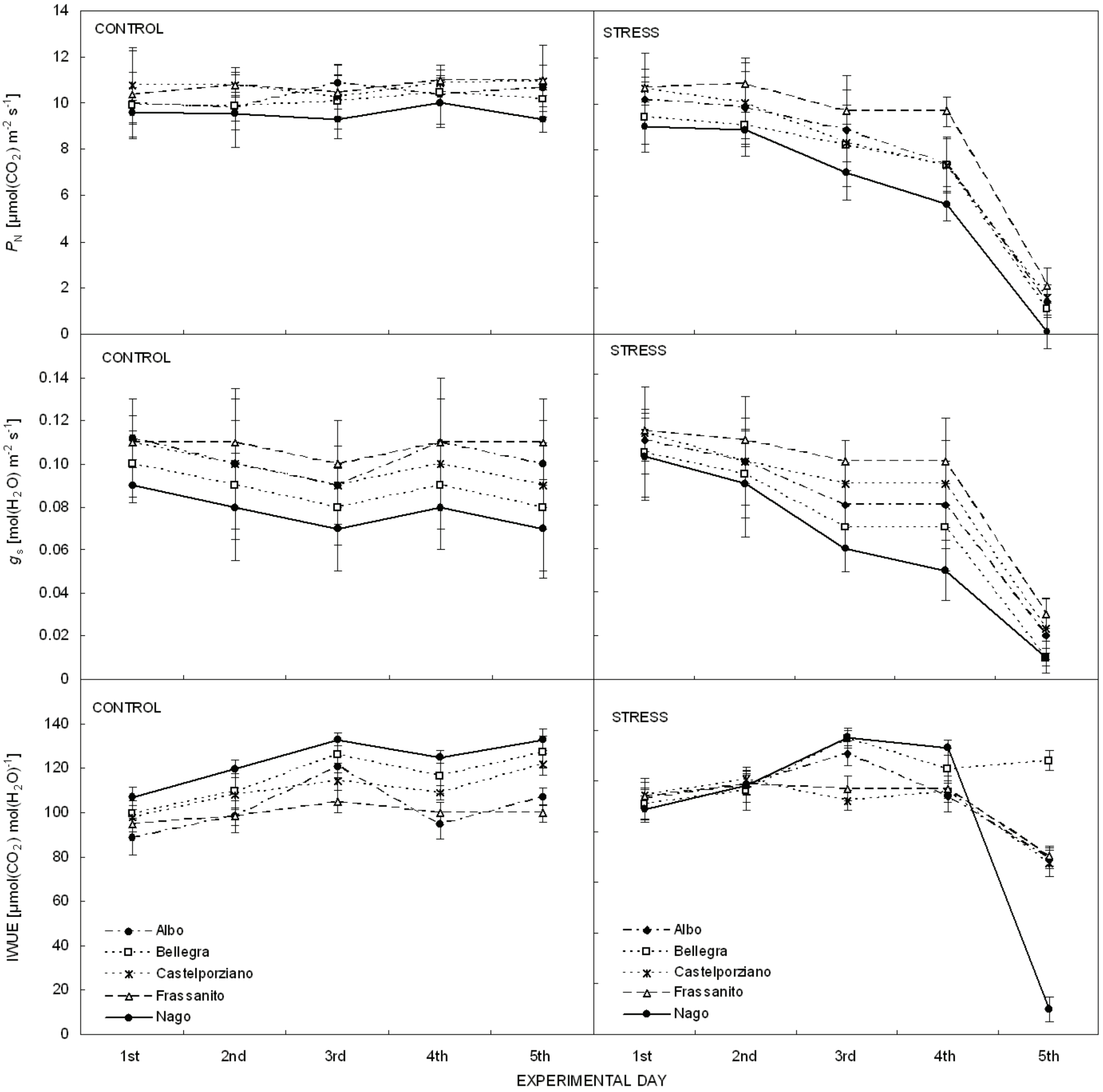

Fig. 5. Net photosynthetic rate (P_N), stomatal conductance (g_s) and intrinsic water-use efficiency (IWUE) of the seedlings from the considered populations during the induced water stress experiment for the control and stressed seedlings. Mean values (± SD, $n = 10$) are shown.

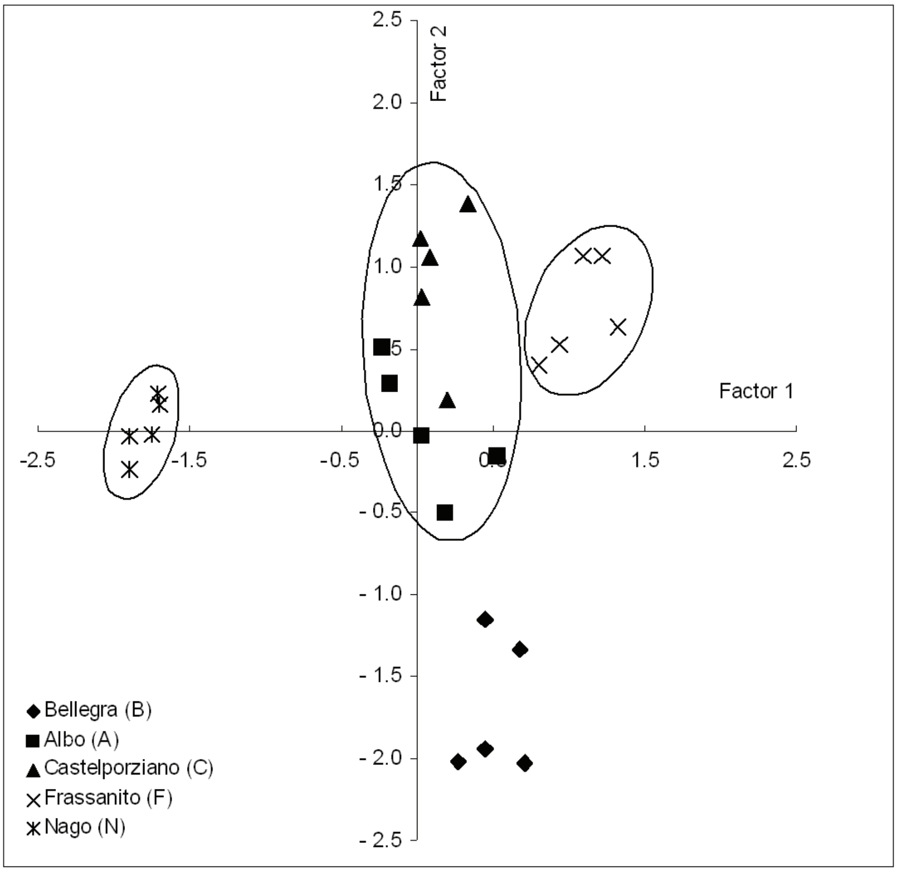

Fig. 6. Principal Component Analysis (PCA) performed using the acorn and seedling data set of the seedlings from the considered populations Factor 1 accounted for 50% of the total variance, and it was manly related to acorn fresh mass, total leaf area 18 months after germination and net photosynthesis measured at highest air temperature ranges. Factor 2 accounted for 20% of the total variance and it was manly related to stomatal conductance measured at the highest air temperatures (29–31°C).

Table 3. Results of the *Pearson*'s correlation analysis. V – acorn volume, FM – acorn fresh mass, RGR_{max} – maximum relative growth, TLA_6 – total leaf area 6 months after germination, TLA_{18} – total leaf area 18 months after germination, SH_6 – seedling height 6 months after germination, SH_{18} – seedling height 18 months after germination. Correlations are statistically significant ($p \leq 0.01$) except when indicated (N.S. – not significant). n = 50 for all the correlations, except for correlation between V and FM (n = 500).

	V	FM	RGR_{max}	TLA_6	TLA_{18}	SH_6	SH_{18}
V		0.90	0.96	0.93	0.88	0.93	0.87
FM			0.79	0.93	0.89	0.85	0.87
RGR_{max}				0.91	N.S.	0.92	N.S.
TLA_6						0.93	N.S.
TLA_{18}							0.93

The significant differences in the time of germination and size of the considered *Q. ilex* acorns seem to be in relationship with the local conditions under which the acorns mature, according to the results of Bognounou *et al.* (2010) for other species. In particular, the small Nago acorns size (0.91 ± 0.22 cm^3 and 1.0 ± 0.3 g, V and FM, respectively) can be related to the scarce plant assimilates for the developing acorns at the original locality, because of the lowest photosynthetic rates of the cold stressed *Q. ilex* parent plants (Gratani *et al.* 2000). In fact, the cold stress inhibits the activity of sucrose synthesizing enzymes like sucrose phosphate synthase which has a high sensitivity to low air temperatures. (Perz *et al.* 2001). Moreover, the extensive damage to the water-conducting system of roots and stems caused by freeze stress may induce significant leaf dehydration during winter (Nardini *et al.* 2000). The winter cold stress of Nago, with frost and subzero air temperatures until the beginning of March, justifies Nago acorn germination delay (on an average 13 days) compared to the other considered acorns, according to the results of Khurana and Singh (2000) for other species.

On the contrary, the large Frassanito and Bellegra acorns size (3.7 ± 0.2 cm^3 and 4.3 ± 0.1 g mean value of V and FM, respectively) may be justified by the drier climatic conditions of their original localities, according to Gomez (2004b), who underlines that large seed size accelerates germination time and increases germination rate, seedling survival, and seedling growth. In particular, in spite of the large total annual rainfall, water stress occurs in summer at Bellegra, due to the small soil pockets where shrubs naturally grow, and where the high soil gravel content (73.4 ± 4.3%) reduces water availability.

The Castelporziano and Monte Albo intermediate acorns size (2.5 ± 0.2 cm^3 and 2.9 ± 0.3 g mean value of V and FM, respectively) are justified by the favourable climatic and soil conditions of the original localities, according to the results of Valencia-Diaz and Montaña (2005).

The correlation analysis underlines that seedling height and RGR_{max} are significantly related to acorn FM: Frassanito seedlings have a higher RGR_{max} (0.203 ± 0.011 mm mm^{-1} d^{-1}) because of the sufficient acorn reserves sustain the seedling growth for a much longer period (98 ± 3 days from acorn germination to the end of the spring vegetative activity during the first year), allowing a less dependence of the growth on external factors, according to the results of Gomez (2004a), and Pons and Pausas (2007). This is also attested by the total leaf area per seedling, which is related to acorn size; in particular during the first six months after germination; the early-emerging seedlings of Frassanito and Bellegra are able to utilize a longer period of favourable conditions to grow reaching the highest TLA_6 (66 ± 18 cm^2, mean value) than the other seedlings; N has the lowest TLA_6 (18 ± 7 cm^2).

18 months after the emergences, the differences of TLA_{18} among the considered seedlings are lower (184 ± 90 cm^2, mean value). It will be important to monitor the considered seedlings in the next years to verify if the positive effects of acorn size on seedlings TLA persist because it might attest the genetic origin of this trait. The effect of seed mass can be of adaptive significance in establishing and maintaining populations in the original localities (Khurana and Singh 2000).

Mediterranean evergreen species are characterised by lower rates of photosynthetic carbon gain per unit of dry mass than deciduous ones (Reich *et al.* 1992); environmental stresses (*i.e.* high air temperatures and water stress associated to high irradiance) limit carbohydrate production (Ósorio *et al.* 2006, Behboudian *et al.* 2001, Yang *et al.* 2001).

At physiological level, N and B seedlings have higher P_N rates [7.8 ± 0.2 μmol(CO_2) m^{-2} s^{-1}, mean value] at the lowest air temperatures (7–9 °C) than the other seedlings, while F seedlings higher P_N rates (7.5 ± 0.4 μmol(CO_2) m^{-2} s^{-1}) at the highest air temperatures (29-31 °C) than the other seedlings.

There is the evidence for genetic variations in the efficiency of the water use among populations of the same species (Pou *et al.* 2008). Vassileva *et al.* (2010) underline that one of the approaches to improve plant performance of reforested areas under drought is the development and cultivation of plants with higher water-use efficiency. Our results underline that the considered seedlings have a different responses to the induced water stress: on the 4th experimental day N seedlings have the highest P_N decrease (by 37%) and F the lowest one (by 10%). Moreover, on the 4th experimental day all the considered seedlings show an IWUE increase respect to that monitored the 1st day, and it is due to g_s decrease less than 50%, indicating a predominant stomatal control over photosynthesis, according to the results of Gulias *et al.* (2009).

The PCA confirms the obtained results identifying on the basis of the considered acorn and seedling traits, four groups and underlining a complete separation between Nago and Frassanito seedlings along the 1st factor, which explains 50% of the total variance, and an intermediate position of Castelporziano, Monte Albo, and Bellegra seedlings. Nevertheless, Castelporziano and Monte Albo are closer to each other than to Bellegra seedlings, by their similar climatic and soil conditions.

Climate change may act as a potent agent of natural selection within populations, and its likely interaction with habitat fragmentation may alter population genetic structure (Alistair and Peñuelas 2007) in consideration of the expected increase of drought in the Mediterranean Basin. Moreover, the length and intensity of drought will affect to a great extent those species with a higher sensitivity to seasonal changes in water availability (Gulias *et al.* 2009).

Overall our results underline that *Q. ilex* is characterised by a large variability of acorn and seedling traits which could be considered for afforestation projects and restoration programmes. The knowledge of seed germination and seedling growth capability in response to the intensity and duration of water stress in the Mediterranean Basin is important because water availability is a limiting factor of prime importance in the oak regeneration (Ramírez-Valiente *et al.* 2009, Trubat *et al.* 2010). In particular, the large acorns from Frassanito may have a high potential in resource-limited or degraded areas by their better germination rate and growth seedlings capability in the establishment phase, and by the highest capability to maintain a more stable water-use efficiency which may be advantageous during the early life stages, particularly during the first summer drought following planting in afforestation projects. Physiological knowledge concerning populations of *Q. ilex* seedlings from different climate are important to forecast the potential productivity to increasing drought stress.

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