

Germination and seedling growth of holm oak (*Quercus ilex* L.): effects of provenance, temperature, and radicle pruning

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This study tested acorn characteristics and the germination behavior of different provenances of Holm oak (*Quercus ilex* L.) and the effects of radicle clipping of germinated acorns on 1-0 year-old seedling morphology and their field performances. The results revealed that all acorn characteristics among different provenances were significant. *Quercus ilex* acorns germinated well at alternating temperatures of 20/10 °C compared to a constant 20 °C temperature. Radicle clipping of *Q. ilex* significantly increased the shoot height and root collar diameter. The average number of main roots resulting from radicle clipping was 2.8 times more than unclipped treatment. The results can contribute to nursery practices and artificial regeneration of this species.

Keywords: Holm Oak, Seed Germination, Radicle Pruning, *Quercus ilex*, Sowing, Artificial Regeneration

Introduction

The genus *Quercus* includes over 500 species worldwide (Oldfield & Eastwood 2007), of which 18 are present in Turkey (Hedge & Yaltirik 1982). Approximately 5.15 million ha of Turkish forests were covered by oak forests and more than half of this area (3.05 million ha) is degraded (Anonymous 2012). *Quercus ilex* (Holm oak), is an evergreen oak species that can be found both in shrub and tree forms (Yaltirik 1984), in pure or mixed stands, and is a typical Mediterranean sclerophyll species (Palacios et al. 2009). The holm oak is distributed primarily in three distinct areas: (i) north Africa from Tunisia to Morocco; (ii) on several large islands, e.g., Crete, Sicily, and Corsica; and (iii) in southern (continental) Europe, along a continuum from Turkey to Portugal (Michaud et al. 1995). In Turkey, *Q. ilex* occurs in western Black Sea coasts and in southwestern Anatolia mixed with other pseudo-maquis and maquis species, respectively. Maquis is the dominant vegetation type growing in Mediterranean climate conditions (Boydak et al. 2006, Kaya & Aladag 2009).

Heavy degradation of vegetation in large areas of the Mediterranean basin call for re-

habilitation with native coniferous and broadleaves species (Boydak et al. 2006). Mediterranean landscapes have been modified since long time by human activities. Fire, grazing pressure, shifting agriculture, and overcutting for fuel and other purposes are important degradation and/or selection factors in all the Mediterranean countries (Aschmann 1973, Naveh & Dun 1973, Fox 1982, Naveh & Lieberman 1984, Boydak & Dogru 1997, Zagas et al. 1998, Vallejo et al. 2000, Boydak et al. 2006). Moreover, it is expected that climate change could make Mediterranean ecosystems even more vulnerable (Gorissen et al. 2004, Wessel et al. 2004, Gratani et al. 2012). A decrease in Mediterranean shrublands cover might accelerate soil degradation and erosion (Haase et al. 2000, Gratani et al. 2012). The Holm oak plays a vital role in soil and water conservation and its acorns are important nourishment for many wild and domestic animals (Shakesby et al. 2002, Plieninger et al. 2003, Linan et al. 2011). On the other hand, *Quercus ilex* is an element of maquis vegetation that can play an important role in the rehabilitation and restoration of the western and eastern Mediterranean countries. Holm oak

gains more interest for Mediterranean forestry, along with other evergreen species such as *Quercus suber*, *Ceratonia siliqua*, and *Olea europaea*, especially in restoration and reforestation activities (Galván et al. 2012). Similarly, Holm oak plays an important role in reforestation programs in Spain (Pausas et al. 2004, Galván et al. 2012), and in restoration and rehabilitation programs of degraded maquis and forest areas in Turkey.

Successful restoration and rehabilitation in Mediterranean areas strongly depend on the quality of seeds and seedlings used. So far, the influence of oak species seedling quality on artificial regeneration performance has received little attention. Moreover, fibrous rooted seedlings are highly desirable for restoration and rehabilitation activities, especially in Mediterranean countries. Such seedlings may be produced by undercutting or transplanting, though this is generally expensive and time consuming (Calikoglu et al. 2007). However, it has been reported that pruning of the emerged radicle promotes the branching of the taproot in several oak species (Barden & Bowersox 1989, McCreary 1996, Ertas 2002, Tilki & Alptekin 2006, Devine et al. 2009). Bonner (1982) also stated that sowing pre-germinated acorns with damaged radicles resulted in multiple branched taproots in two southern red oak (*Quercus falcata* var. *pagodaefolia* and *Quercus shumardii*). Seedlings may benefit of branched multiple taproots in that root surface area becomes larger than that of seedlings with a single taproot (Devine et al. 2009).

A limited number of investigation have been carried out regarding Holm oak in maquis vegetation (Özalp 1993, Kaya & Aladag 2009), and oak seed and seedling morphology in Turkey (Ertas 1996, Genc et al. 2000, Ertas 2002, Tilki & Alptekin 2006). Information on morphological variation in seed characteristics among the natural populations of a species is useful for tree improvement programs in that survival, growth and biomass allocation performances of seedlings largely depend on seedling quality (Singh et al. 2010). The objectives of this study were to determine: (a) acorn characteristics and germination behavior of different provenances of *Quercus ilex*; and (b) the effects of radicle clipping of germinated acorns on 1-0 year Holm oak seedling morphology, in order to assess their future field performances in rehabilitation and planting programs.

Material and methods

Acorn collection and determination of morphometric characteristics

Acorns were collected from mature trees in Canakkale (40° 10' N, 25° 50' E, elevation

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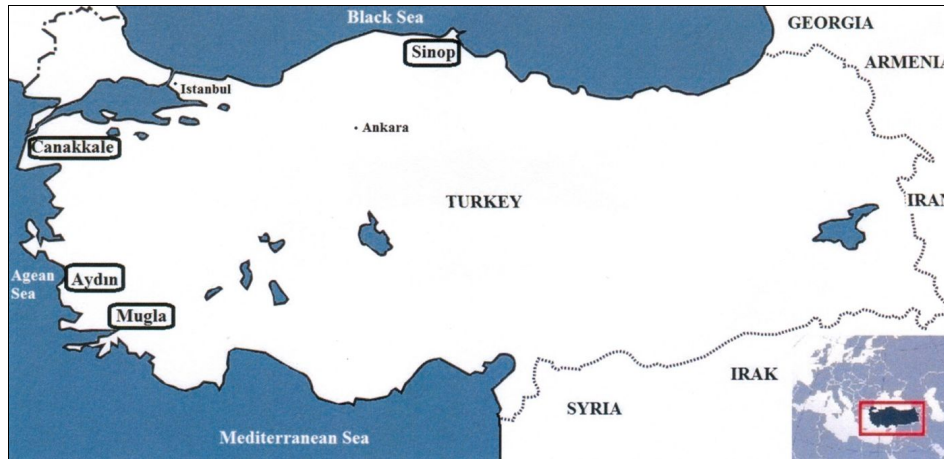


Fig. 1 - Map showing the locations of acorn collection sites.

175 m a.s.l.), Sinop (40° 01' N, 35° 00' E, 50 m a.s.l.), Aydin (37° 39' N, 27° 05' E, 10 m a.s.l.), and Mugla (36° 43' N, 27° 32' E , 850 m a.s.l.- Fig. 1). The climate at all sites is a typical maritime climate. The annual mean precipitation and annual mean temperature are respectively: 521 mm and 14 °C in Sinop; 513 mm and 15 °C in Canakkale; 525 mm and 18 °C in Aydin; and 979 mm and 15 °C in Mugla (Turkish State Meteorological Service 2013). Bedrocks in all sites are mainly limestone.

Collected acorns were placed in partially sealed plastic bags and transported to the laboratory. Acorns were floated in water to eliminate insect damaged or dead acorns. Sinking acorns were then selected if no damage was visible. Weight, length, and width were singly measured on 100 randomly drawn seeds from each provenance in five replications each of 20 seeds. The coefficient of variation (CV) was determined based on the overall mean and total variance for each measured trait.

Germination tests

Germination tests were carried out on 18 x 11 x 4.5 cm trays in a germination chamber. Trays were filled with sieved sand, autoclaved at 121 °C for 20 minutes, and then acorns placed on moist sand. Tests were performed with five replicates of 20 acorns for each provenance. Trays were examined daily for 30 days following the first signs of germination. Seeds were considered germinated when radicles were more than 5 mm long and showing geotropism.

Germination tests were carried out at constant (20 ± 0.5 °C) and alternating (20/10 ± 0.5 °C) temperatures. Germination data were expressed as germination percentage (GP), peak value (PV), and germination value (GV). GP is the percentage of seeds germinated at the end of the test. PV is the highest number obtained dividing GP by the number of days elapsed. GV was calculated as follows (Djavanshir & Pourbeik 1976 - eqn. 1):

$$GV = \frac{\sum dGS}{N} \cdot \frac{N_{gs}}{100}$$

where GV is the germination value, dGS is daily germination speed, N is the frequency or the number of dGS calculated during the test, N_{gs} is the number of germinated seeds, and 10 is a constant.

Seedling growth and radicle pruning

Pre-germinated acorns from Sinop and Mugla provenances were analysed for seedling morphology, and sowed in a forest nursery in Istanbul-Bahçeköy. The nursery is located 41° 10' N, 28° 59' E, and elevation 116 m a.s.l. The climate at the nursery is a humid, mesothermal, and maritime climate with a moderate water deficit over summer months according to the Thornthwaite's classification method. Annual mean precipitation is around 1091 mm, and mean annual temperature is 12.8 °C. Most of the precipitation falls between October and March (Balci et al. 1986, Özhan et al. 2010, Akburak et al. 2013).

Acorns were divided into two different groups: radicles left intact and radicles

pruned back to 0.5 cm. Acorns were individually sown on seedbeds with five replicates of 12 acorns for each treatment. Survival percentage, shoot height, root collar diameter, number of main roots, total diameter of main roots, and total diameter of main roots / root collar diameter were determined in 1-0 year Holm oak seedlings.

Data analysis

Parameters related to germination, such as germination percentage, peak value, and germination value, were considered as dependent variables in the analysis of variance carried out. Germination percentages were transformed using arcsine square root transformation. Population, temperature, and replications were used as independent variables (predictors). ANOVA for the germination traits were based on the following model (Sokal & Rohlf 1995 - eqn. 2):

$$Y_{ijkl} = \mu + P_i + T_j + R_k + (PT)_{ij} + e_{ijkl}$$

where Y_{ijkl} is the observed value of k-th replication of the j-th temperature of the i-th population (provenance), μ is the overall mean, P_i the population effect, T_j the effect of temperature, R_k the effect of replication, and e_{ijk} is the error.

In the radicle pruning treatments, seedling traits such as survival, shoot height, root collar diameter, number of main roots, total diameter of main roots, and total diameter of main roots (mm) /root collar diameter (mm) (dependent variables) were analyzed using treatments as independent variables. Survi-

Tab. 1 - Morphometric characteristics of *Quercus ilex* L. acorns. Means within each column followed by different letters are significantly different (p<0.01). (SE): standard error.

Provenance	Acorn width (mm)			Acorn length (mm)			Acorn weight (gr)		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Canakkale	12.8 ^a	0.15	9.0-16.3	28.84 ^a	0.38	17.6-34.7	2.95 ^a	0.38	0.55-4.96
Sinop	13.03 ^b	0.12	10.8-16.2	24.41 ^b	0.22	19.0-30.9	2.64 ^b	0.07	1.50-4.84
Aydin	13.15 ^b	0.09	10.4-15.5	28.81 ^a	0.34	13.1-35.6	3.06 ^a	0.07	1.53-4.98
Mugla	13.66 ^b	0.16	9.7-17.4	26.06 ^c	0.37	14.2-33.9	2.90 ^a	0.09	0.98-5.13
Over four provenances	13.16	0.07	9.0-17.4	27.03	0.19	5.6-13.1	2.89	0.04	0.55-5.13

val percentages were transformed using arcsine square root transformation. ANOVA for the radicle pruning treatments were based on the following model (Sokal & Rohlf 1995 - eqn. 3):

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the observation of j -th seedling of the i -th treatment, μ is the overall mean, T_i is the effect of treatment, and e_{ij} is the error.

Duncan's multiple range test was applied to determine statistically significant differences among subsets ($\alpha=0.05$).

Results

Results from ANOVA applied on acorn characteristics of *Quercus ilex* showed that differences among provenances were significant for all the traits considered (Tab. 1). The maximum range of variation in acorn width, length, and weight was observed for Mugla, Aydin, and Canakkale provenances (9.7-17.4 mm, 13.1-35.6 mm, and 0.55-4.96 gr, respectively). Largest width, length, and weight were observed for acorns from Mugla, Canakkale, and Aydin provenances, respectively. According to the results of the Duncan's test on acorn width and weight (Tab. 1), Canakkale population is distinct from Sinop, Aydin, and Mugla provenances. As for acorn length, no significant differences were found in between Canakkale and Aydin, though they showed significant differences with both Sinop and Mugla. Maximum coefficient of variation (CV) was observed in acorn weight for all provenances (Fig. 2). For acorn weight, the maximum CV was recorded in the Canakkale provenance (34.8%) with a minimum of 23.2% in the Aydin provenance. For acorn width and acorn length, the maximum CV was observed in Mugla provenance (11.8% and 14.3%, respectively).

Results from ANOVA showed that GP , GV , and PV were significantly different among provenances ($p=0.0001$). The effect of temperatures was significant only for GV and PV ($p=0.0108$ and $p=0.00018$, respectively). Interaction between provenances and temperatures did not reveal any significant effect (Tab. 2).

Germination percentage, germination value and peak value were the highest in the Sinop provenance at both constant (20 ± 0.5 °C) and alternating ($20/10 \pm 0.5$ °C) temperature regimes. Germination performances of Canakkale, Mugla, and Aydin provenances were higher at the alternating than at the constant temperature regime. Contrastingly, Sinop provenance acorns revealed better performances in terms of germination parameters at constant rather than alternating temperature regime (Tab. 3, Fig. 3, Fig. 4).

Germination percentage, germination value, and peak value at 20 °C and 20/10 °C

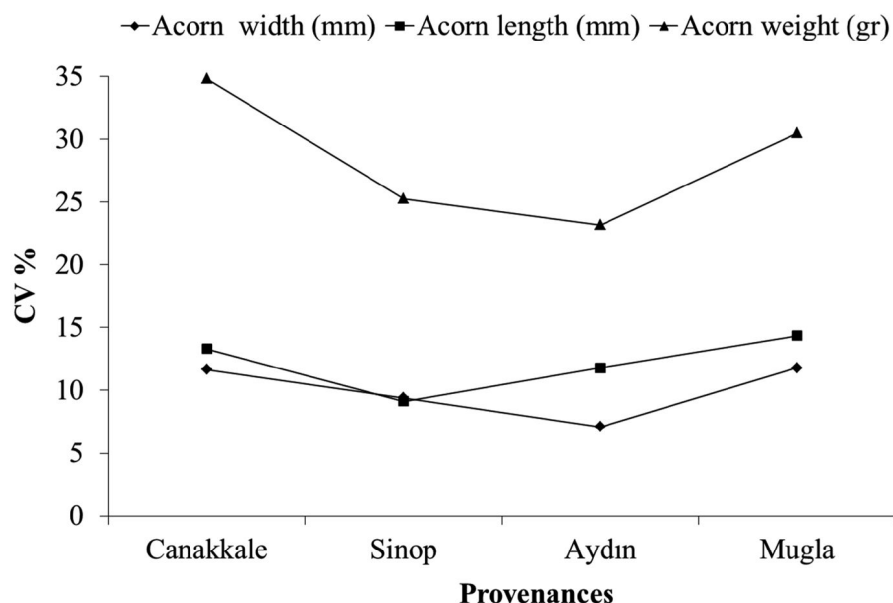


Fig. 2 - Coefficient of variation (CV) among different provenances for different acorn characters in *Quercus ilex*.

Tab. 2 - Results of the ANOVA carried out on germination percentage (GP), germination value (GV) and peak value (PV) of the acorns of *Quercus ilex*.

Variable	Parameter	Source of variation				
		Provenance (A)	Temperature (B)	Interaction (A x B)	Replications	Error
	df	3	1	3	4	28
GP	MS	3610.635	180.625	119.375	102.292	121.518
	F	29.71	1.49	0.98	0.84	-
	P-value	0.000	0.2329	0.4331	0.4825	-
GV	MS	2419.881	761.508	80.797	214.348	102.204
	F	23.68	7.45	0.79	2.10	-
	P-value	0.000	0.0108	0.5412	0.1232	-
PV	MS	51.287	22.695	1.048	3.933	1.910
	F	26.85	11.88	0.55	2.06	-
	P-value	0.000	0.000	0.7011	0.1283	-

Tab. 3 - Germination of *Quercus ilex* L. acorns from four provenances at 20 °C and 20/10 °C. Means in the same column followed by different lowercase letter are significantly different ($p<0.05$); means in the same column followed by different uppercase letter are significantly different ($p<0.05$).

Temp. Regime	Provenance	Germination Parameters		
		GP	GV	PV
constant (20 °C)	Canakkale	30 ^a	5.0 ^a	2.1 ^c
	Sinop	81 ^b	49.0 ^b	8.7 ^a
	Aydin	70 ^{bc}	35.6 ^c	6.2 ^b
	Mugla	60 ^c	27.2 ^{cd}	5.4 ^b
	Mean	60 ^A	29.2 ^A	5.6 ^A
alternate (20/10 °C)	Canakkale	41 ^a	6.6 ^a	2.3 ^{dc}
	Sinop	77 ^b	29.4 ^{cd}	5.6 ^b
	Aydin	73 ^{bc}	22.6 ^{cd}	4.7 ^{cb}
	Mugla	68 ^{bc}	17.5 ^d	3.9 ^{cd}
	Mean	65 ^A	19.0 ^B	4.1 ^B

Tab. 4 - Survival and seedling morphology from different radicle pruning treatments (average for two provenances). Means in the same row followed by different letters were significantly different ($p < 0.05$).

Parameters	Sowing Treatments	
	Radicle not clipped	Radicle clipped
Survival (%)	66 ^a	62 ^a
Shoot height (mm)	147 ^a	169 ^b
Root Collar Diameter (mm)	3.5 ^a	3.8 ^b
Number of the main roots	1 ^a	2.8 ^b
Total diameter of the main roots (mm)	3.5 ^a	7.1 ^b
Total diameter of the main roots (mm) / Root Collar Diameter (mm)	1 ^a	1.9 ^b

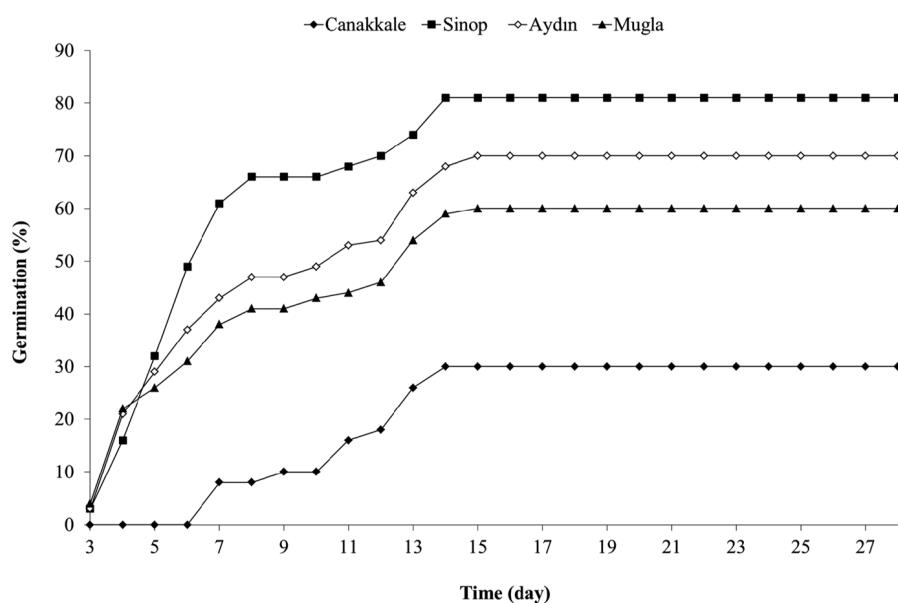


Fig. 3 - Germination of *Quercus ilex* acorns at the constant (20 ± 0.5 °C) temperature regimes.

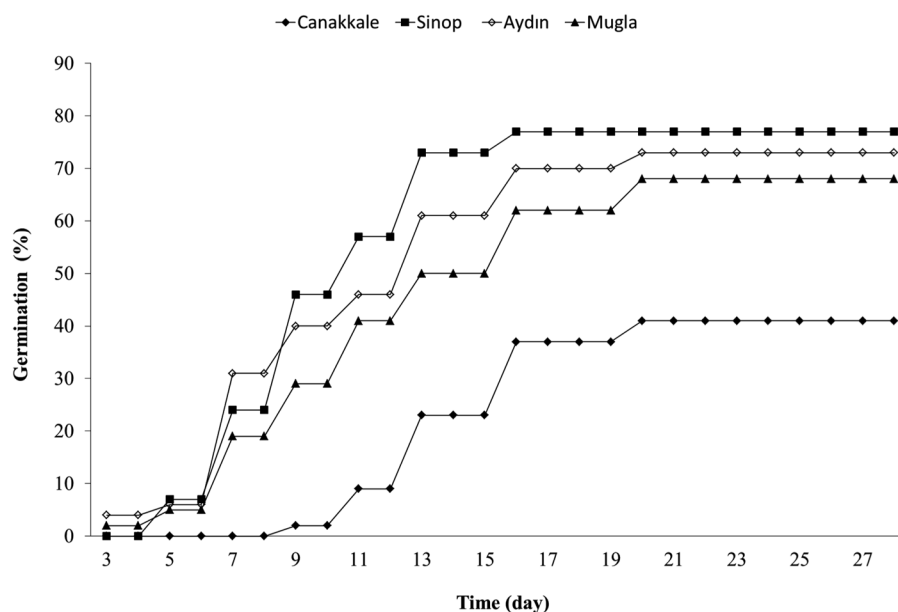


Fig. 4 - Germination of *Quercus ilex* acorns at alternating ($20/10 \pm 0.5$ °C) temperature regimes.

were the highest in the Sinop provenance with lowest seed weight, and the lowest in the Canakkale provenance with medium seed weight (Tab. 1 and Tab. 3).

The effects of radicle pruning of germinated acorns on 1-0 year Holm oak seedling morphology were tested. Significant differences were found in shoot height, root collar diameter, number of main roots, and total diameter of main roots/root collar diameter. On the other hand, no significant differences were observed in survival. Finally, all the seedlings from the unpruned radicle treatment had single roots, while multiple roots were observed for all seedlings from the pruned radicle treatment (Tab. 4). Overall, seedlings with radicle pruned at germination seemed to perform better than unpruned seedlings (Tab. 4).

Discussion

Most Mediterranean sclerophyllous species develop morphological and physiological adaptations to the long and dry summer period, such as small and thick leaves, dense pubescence, deep water extraction system, and high water use efficiencies (Ozturk et al. 2010). In fact, drought stress is responsible for growth and survival of evergreen woody species in the Mediterranean basin (Gratani & Varone 2004).

Local adaptations to specific ecological conditions may lead to differences among provenances in growth performances (Crescente et al. 2002, Ducouso et al. 1996). Population variability with respect to acorn morphology have been reported for *Quercus ilex* (Galván et al. 2012), *Q. glauca* (Singh et al. 2010), and *Q. suber* (Ramírez-Valiente et al. 2009). In a study on thirteen populations of holm oak throughout Spain (Galván et al. 2012), differences in acorn morphology between eastern and western provenances were reported, the latter showing also larger overall variation. The authors reported acorn weight ranging from 2.4 to 6.1 g (CV = 22-37%), length ranging from 40.4 to 22.8 mm (CV = 3-17%), and diameter ranging from 17.1 to 12.4 mm (CV = 13-35% - Galván et al. 2012). In the present study, ranges for acorn weight, length and width were 2.6-3.1 gr, 24.4-28.8 mm, 12.8-13.7 mm, respectively. On the other hand, CV ranges for weight, length, and width were 23-35%, 9-14%, and 9-12%, respectively. Moreover, significant differences among provenances were found for all the seed traits considered in this study. Afzal-Rafii et al. (1992) reported mean width values ranging from 12 to 13 mm for Holm oak acorns, while mean weight was 2.4-3 g, ranges similar to those observed in the present study (12.8-13.7 mm and 2.6-3.1 g, respectively).

Gomez (2004) stated that large acorns of *Q. ilex* showed increased germination rate. In contrast, in the present study Canakkale

provenance had the largest acorns but showed the lowest germination percentage, while Sinop provenance had the lightest acorns and the highest germination percentage. In some tree species, large seeds showed increased germination and survival (Ke & Werger 1999, Cicek & Tilki 2007). On the contrary, the rule larger seeds = highest germination rate does not hold for some other species, as it was in our study (Khera et al. 2004, Tilki & Alptekin 2005, Tilki 2010). However, it has been reported that acorn size and morphology are also affected by soil properties and stand characteristics (Gea-Izquierdo et al. 2006).

In the present study, *Q. ilex* acorns showed better germination at alternating temperature regime (20/10 °C - 65%) than at constant regime (20 °C - 60%) temperature regime. However, similar differences among provenances in germination rate have been observed. Pasquini et al. (2011) reported for Holm oak germination percentages of two seed lots (68% and 63%) very similar to those observed in the present study.

Radicle pruning has revealed a positive effect on seedling performances in this investigation, determining a larger taproot production and increasing height and root collar diameter of seedlings. Moreover, significant differences were found in shoot height, root collar diameter, number of the main roots, and total diameter of the main roots/root collar diameter of the two tested provenances, though no significant differences in seedlings' survival were observed at the end of the growing season. However, in *Quercus vulcanica* radicle clipping had no effect on survival, shoot height, and diameter of seedlings obtained from spring-sown acorns (Tilki & Alptekin 2006). Radicle clipping had also no effect on shoot height and diameter of containerized *Q. vulcanica* 1-year-old seedlings from fall-sown acorns (Genc et al. 2000) and did not significantly alter height in *Fagus orientalis* (Calikoglu et al. 2007). In the present study, the average number of main roots was 2.8 times higher for seedlings undergoing radicle clipping in comparison to unclipped treatment, while it was close to 2.5 times higher for radicle-pruned seedlings of *Quercus vulcanica* after 8 months (Tilki & Alptekin 2006). Radicle clipping resulted in more seedling branch roots both in *Q. vulcanica* (Genc et al. 2000) and *Quercus douglasii* (McCreary 1996), and in a greater terminal elongation in greenhouse conditions in northern red oak (Barden & Bowersox 1989). Furthermore, radicle pruning prior to sowing did not adversely affect seedling production in two southern red oaks (*Quercus shumardii* and *Quercus falcata* var. *pagodaefolia* - Bonner 1982). Similar results were also obtained by Ertas (1996) in *Quercus hartwissiana*, where radicle pruning doubled the average number of

taproots and increased the root collar diameters of the seedlings, and by Ertas (2002) in *Quercus petraea*, where root surface area and number of taproots increased in 1-0 year old radicle-pruned seedlings.

The present study confirmed the importance of radicle clipping of *Q. ilex*. Radicle clipping generally inhibits the development of main carrot-type tap root, and causes the formation of several tap roots and a more fibrous root systems. It is thought that such a root system may confer an advantage on seedlings, providing greater root surface area for the absorption of moisture and nutrients (Tilki & Alptekin 2006). Radicle-pruning techniques may be adopted in nursery in order to produce vigorous material to be used in reforestation or rehabilitation activities in drought-prone or degraded areas frequently occurring in the Mediterranean basin. Moreover, it is well-known that height and root collar diameter of seedlings are among the major factors positively affecting planting success of broad leaved species. The slow growth and poor survival of planted oaks may be in part due to the root system (Barden & Bowersox 1989). Finally, Tsakalidimi et al. (2005) highlighted that grading criteria for oak seedlings' shoot height and root-collar diameter are important parameters to be considered for successful reforestation of sites with high environmental stress.

Conclusion

The aim of this study was to determine the acorn characteristics, germination behaviors, and effects of radicle clipping of different provenances of Holm oak (*Quercus ilex* L.) from northern and western regions of Turkey. *Quercus ilex* acorns better germinated at alternating temperatures rather than at the constant temperature regimes. Significant differences were detected among different provenances for all the measured seed traits. Pruning the radicle of germinated acorns before sowing has proven to positively affect 1-year-old seedlings of *Quercus ilex*, increasing their shoot height and root collar diameter.

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