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Effects of Stratification and Growth Regulators on Seed Germination and Seedling Growth of *Quercus ilex* L.

M. Ghasemi and M. Khosh-Khui
Department of Horticultural Science, College of Agriculture,
Shiraz University, Shiraz, Iran

Abstract: In order to study the nature of seed dormancy of *Quercus ilex* L., in two separate experiments pericarp-removed seeds were stratified for 0, 1, 2, 3 and 4 months or treated with concentrations of benzyl adenine (BA) (50, 100, 200 and 400 mg L^{-1}), gibberellic acid (GA_3) (100, 200, 400 and 800 mg L^{-1}) or combinations of them (50 mg L^{-1} BA+100 mg L^{-1} GA_3 or 200 mg L^{-1} BA+400 mg L^{-1} GA_3 or 400 mg L^{-1} BA+800 mg L^{-1} GA_3). Stratification (1 to 2 months in 5°C) increased seed emergence up to 66.66%. Application of 200 mg L^{-1} BA+400 mg L^{-1} GA_3 resulted in highest emergence rates. In average, the highest leaf number, fresh and dry weights (5.23, 0.746 g and 0.296 g, respectively) were obtained when 200 mg L^{-1} BA alone was used. Using of 800 mg L^{-1} GA_3 resulted in longest shoot (6.82 cm) after two months.

Key words: Emergence, growth regulators, *Quercus ilex*, seed dormancy

INTRODUCTION

Holm oak (*Quercus ilex* Ballota syn. *Q. rotundifolia* Lam.) is an evergreen tree used in both silviculture and landscape plantings. The genus *Quercus* includes about 500 species and is an important group of temperate-zone forest trees. Oaks are subdivided into two subgenera, i.e., Lepidobalanus (white oaks) and Erythrobalanus (red and black oaks) (Bonner and Vozzo, 1987).

Acorns of the subgenus Lepidobalanus do not normally exhibit dormancy but there is epicotyl dormancy (dormancy of the shoot bud after the root has started to grow) in some white oak species. Red and black oaks (subgenus Erythrobalanus) exhibit variable dormancies. Not only do species differ in dormancy, but within a species variability can be seen according to geographical or altitude positions. The natural mechanism for breaking embryo dormancy is simply the cold, wet environment in which acorns over winter in litter on the forest floor. In forestry, these conditions provide by storing fully imbibed acorns at temperatures just above freezing for long periods of time (Bonner and Vozzo, 1987).

The chilling process appeared to enhance the production of some type of growth-promoting substances such as gibberellic acid (GA_3) (Powell, 1987). Exogenous GA_3 and stratification stimulated the seed germination and seedling growth of some *Quercus* species (Vogt, 1970; Farmer, 1974; Bonner, 1976; Youssef and Abou-Dahab, 1991). Also the pericarp is significantly involved in seed dormancy that may delays seed germination via chemical and physical methods (Bonner, 1968; Hopper, 1982; Peterson, 1983; Hopper *et al*., 1985).

Epicotyl dormancy has been reported in white oak (*Quercus alba*) and chestnut oak (*Q. prinus*) seeds (Farmer, 1977). Epicotyl dormancy occurs in seeds germinated at typical autumn temperature (10-15°C) and is subsequently broken by chilling (Farmer, 1977; Kondo *et al*., 2002). GA_3 and

Corresponding Author: M. Khosh-Khui, Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran

benzyl adenine (BA) have been effective in removing epicotyl dormancy in *Paeonia ostii* var. *lishizhenii* (Wang and Staden, 2002), *Coffea arabica* San Ramon (Sahoo *et al*., 1999) and *Paeonia lactiflora* (Buchheim *et al*., 1994).

In *Quercus robur* L. epicotyl dormancy resulted in faster shoot growth in the spring. Seedlings from acorns with autumn radicle emergence had faster growth rates than those without and were correlated with longer periods of cold treatment (Wigston, 1987). Furthermore, GA_3 and stratification improved the seed germination and seedling growth of *Quercus robur*.

A challenge in cultivation of *Q. ilex* L. is that seeds easily germinate but most of them will die after protruding from radicles. To our knowledge, so far there is no report on seed dormancy and germination of this species. The purpose of the present investigation was to study seed germination and seedling emergence and also to determine the effects of GA_3 and BA on seed germination and seedling growth of *Q. ilex* L.

MATERIALS AND METHODS

The seeds were collected from a population of *Q. ilex* L. plants grown in Isfahan, Iran on 11 December 2005.

Experiment No. 1

An experiment in a complete randomized design was carried out and seeds that were already maintained in 3°C for 1 month were used. The pericarp was removed and safe seeds were selected. Seeds were sown in 3-4 cm depth of soil in clay pots with 15 cm diameter and covered by peat moss. After 15 days, when seeds were germinated, 5 stratification treatments (0, 1, 2, 3 and 4 months in 5°C), in 5 replications were applied. Then they were placed in a greenhouse at 20±5°C. After 1 month the numbers of emerged seeds from the soil were recorded.

Experiment No. 2

In the second experiment pericarp removed seeds were soaked in GA_3 (100, 200, 400, 800 mg L^{-1}, BA (50, 100, 200 and 400 mg L^{-1}), (BA 50 mg L^{-1}+GA_3 100 mg L^{-1} or BA 200 mg L^{-1}+GA_3 400 mg L^{-1} or BA 400 mg L^{-1}+ GA3 800 mg L^{-1}) and water (control) and sown as mentioned in previous experiment. After 20 days the numbers of emerged seeds were recorded in a 4-day period and the peak value (P.V.) was measured (Hopper *et al*., 1985). P.V. = germination %/germination time (days). Seedlings were harvested after 2 months and shoot length, leaf number, fresh and dry weights of plants were measured.

Statistical Analyses

Data were subjected to analysis of variance procedures (ANOVA) (SAS Institute, Cary, USA). Treatments means were separated by standard error (SE), $p = 0.05$.

RESULTS

Results of first experiment showed that stratification had positive effect on seed emergence in all treatments compared to untreated control treatment. Highest germination (66.66%) was obtained after 3 months of cold treatment that was not significantly different ($p = 0.05$) with treatments of 1 and 2 months (Table 1).

In second experiment the highest peak value (4.06) was observed in 200 mg L^{-1} BA+400 mg L^{-1} GA_3 and 800 mg L^{-1} GA_3. Lowest ratio was in 400 mg L^{-1} BA treatment. All treatments were significantly different with control (Table 2).

Table 1: Effect of stratification period on seedling emergence of Quercus *ilex*. (Mean±SE)

Stratification (month)	Seedling emergence (%)
0 (control)	17.32±1.641
1	65.34±4.428
2	62.68±4.515
3	66.66±4.716
4	52.00±4.428
LSD (0.05%)	11.52

Table 2: Effect of plant growth regulators on seed peak value of *Quercus ilex*. (Mean±SE)

Treatments (mg L^{-1})	Seed peak value
0 (Control)	1.72±0.136
100 GA_3	2.89±0.111
200 GA_3	3.44±0.142
400 GA_3	3.72±0.141
800 GA_3	4.06±0.068
50 BA	3.72±0.188
100 BA	3.72±0.142
200 BA	3.72±0.208
400 BA	2.44±0.136
50 BA+100 GA_3	3.39±0.184
200 BA+400 GA_3	2.89±0.068
400 BA+800 GA_3	4.06±0.111
LSD (0.05%)	0.33

GA_3 = Gibberellic acid, BA = Benzyl adenine

Table 3: Effects of GA_3 and BA concentrations on leaf number, shoot length (cm), plant fresh and dry weights (g) of *Quercus ilex* L. (Mean±SE)

	Characteristics			
Treatments (mg L^{-1})	Leaf No.	Shoot length	Plant fresh weight	Plant dry weight
0 (Control)	2.84±0.446	3.76±0.502	0.574±0.062	0.192±0.021
100 GA_3	2.57±0.332	3.35±0.242	0.549±0.176	0.172±0.054
200 GA_3	2.50±0.198	3.47±0.381	0.674±0.066	0.217±0.021
400 GA_3	3.14±0.280	4.38±0.349	0.658±0.060	0.212±0.019
800 GA_3	4.87±0.538	6.82±0.504	0.520±0.034	0.193±0.013
50 BA	3.37±0.713	3.69±0.525	0.594±0.091	0.212±0.032
100 BA	4.26±0.370	4.10±0.207	0.719±0.049	0.276±0.013
200 BA	5.23±0.367	5.31±0.316	0.746±0.035	0.296±0.013
400 BA	1.90±0.249	2.83±0.429	0.498±0.067	0.178±0.024
50 BA+100 GA_3	3.07±0.438	2.85±0.414	0.636±0.062	0.245±0.024
200 BA+400 GA_3	2.62±0.226	2.93±0.085	0.434±0.027	0.163±0.010
400 BA+800 GA_3	3.43±0.351	4.34±0.377	0.503±0.028	0.186±0.008
LSD (0.05%)	1.142	1.105	0.158	0.055

GA_3 = Gibberellic acid, BA = Benzyl adenine

The highest leaf number (5.23) was obtained in 200 mg L^{-1} BA that did not show a significant difference with 800 mg L^{-1} GA_3. (Table 3). Furthermore, the longest shoot (6.82 cm) was observed in 800 mg L^{-1} GA_3 (Table 3). The highest fresh weight (0.746 g) was obtained by using 200 mg L^{-1} BA and the highest dry weight (0.296 g) in 200 mg L^{-1} BA without any significant difference with 100 mg L^{-1} BA (Table 3).

DISCUSSION

According to data obtained in this study, stratification improved *Q. ilex* L. seedling emergence. These results revealed that apparently there was an epicotyl dormancy present in *Q. ilex* seeds. These results are in agreement with the findings of Farmer (1977) and Wigston (1987). Farmer (1977) studied epicotyl dormancy in white oak (*Quercus alba*) and chestnut oak (*Q. prinus*) seeds and concluded that epicotyl dormancy occurred in seeds germinated at typical autumn temperature (10 to 15°C) and was subsequently broken by chilling. Wigston (1987) in *Quercus robur* L. showed that cold treatment resulted in a faster shoot growth.

The phenology of radicle and cotyledon emergence has been reported for several species with epicotyl dormancy including *Hydrophyllum macrophyllum* (Baskin and Baskin, 1983), *Hydrophyllum appendiculatum* (Baskin and Baskin, 1985), *Asarum canadense* (Baskin and Baskin, 1986), *Erythronium japonicum* (Kondo *et al*., 2002) and *Hexastylis heterophylla* (Adams *et al*., 2003). The temperature requirements (season) for root or cotyledon emergence also are basically the same, although there are some differences among the species. Radicle emergence occurs in autumn at low temperatures followed by a period of high temperatures in summer and cotyledons emerge in spring after a period of low temperatures during winter.

Seed dormancy involves the interaction between plant growth regulators. These regulators may be either inhibitors or promoters and act together to influence the net result of germination or dormancy. In *Quercus*, Indole-3-acetic acid (IAA) and abscisic acid (ABA) levels increase with acorn development but decrease with acorn maturity. IAA content is always greater in the cotyledon than in the pericarp, while ABA is equally distributed between the two. Conversely, cytokinin activity decreases during maturation (Blanche *et al*., 1980; Hopper and Vozzo, 1982). During stratification of water oak (*Quercus nigra*), IAA decreased from day 10 to day 50, The inhibitor-promoter balance in the embryo axis changed between day 30 and day 50, as ABA ecreased and GA_3 increased (Hopper and Vozzo, 1982). In this study it was shown that BA and GA_3 improved seed emergence percentage and rate and shoot growth of *Q. ilex* which are in accordance to the results reported by Youssef and Abou-Dahab (1991) in *Quercus robur*, Buchheim *et al*. (1994) in herbaceous peony and Sahoo *et al*. (1999) in coffee. Youssef and Abou-Dahab (1991) working on *Q. robur* showed that the best seed germination percentage (100% compared with 34.7 in control) occurred in the 200 ppm GA_3+low temperature for 4 weeks treatment. GA_3 at 200 ppm+low temperature for 2 weeks and GA_3 at 100 ppm+low temperature for 4 weeks gave the tallest seedlings, the most leaves/seedling and the highest leaf index. Low temperature for 2 and 4 weeks gave the largest leaf surface, longest roots and most dry weight per seedling. In coffee (*Coffea arabica* 'San Ramon'), treatment with 20 ppm kinetin resulted in the highest germination percentage 80 days after sowing (86.2% compared with 73.0% in control seeds) and the shortest time for initiation of germination (26.10 days compared with 34.60 days in control seeds). Also treatment with 40 ppm kinetin resulted in the greatest seedling vigor (Sahoo *et al*., 1999).

Buchheim *et al*. (1994) showed that exogenous GA_3, ABA and BA on epicotyl dormancy of cultured *Paeonia lactiflora* embryos. Epicotyl dormancy was broken in cultured embryos after topical application of agarose gels containing GA_3, with optimum growth at 1.5 mM GA_3. Epicotyl dormancy was also broken in embryos by culture on media containing 1 or 10 mM BA. A highly significant increase in leaf number occurred when embryos cultured on medium containing BA and treated topically with GA_3.

The inhibitory effects of retardants may be overcome by GA_3 (Giba and Konjevic, 1993). cytokinins (Hossa *et al*., 1990) and gibberellins (Liu and Loy, 1976). Gibberellins can affect growth and development by increase in hydrolytic activity (Mozer, 1980) and cell plasticity (Taylor and Cosgrove, 1989).

In present study increase in BA concentration up to 200 mg L^{-1} affected all characteristics measured but 400 mg L^{-1} had negative effects on percentage of seedling emergence, leaf number and shoot length which indicates that this species is not tolerant to higher concentrations of BA.

In general, first report of presence of epicotyl dormancy in *Q. ilex* as it is evident from data obtained in this investigation, is resemble to epicotyl dormancies which have been reported in several species in the families Aristolochiaceae, Caprifoliaceae, Hydrophyllaceae, Liliaceae, Paeoniaceae, Ranunculaceae (Baskin and Baskin, 1998) and Fagaceae (Farmer, 1977).

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