



RESEARCH ARTICLE

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Phytohormone effect on seedling quality in Hungarian oak

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Abstract

Aim of study: to find out the effects of four phytohormones, including Indole Butyric Acid (IBA), Indole Acetic Acid (IAA), and Gibberellic acids (GA₃, GA₄) on seedling growth of Hungarian oak which has a natural distribution in the northwestern of Turkey.

Area of Study: Mature acorns of Hungarian oak were collected from a natural stand in Zonguldak-Ereğli at northwestern region of Turkey.

Material and Methods: Collected acorns of the species were pretreated with 300 ppm of each phytohormone for 24 hours and sown in Yenihan State Forest Nursery of Bartın, Turkey. Two years old bare-root seedlings were used for the measurements of seedling height, root collar diameter, stem, root and seedling fresh weights, and stem, root and seedling dry weights of two bare-root seedlings for each phytohormone treatment.

Main results: Best performance of all studied seedling traits with the exception of root collar diameter was revealed in seedlings with IBA. In contrast, treatment of seeds with GA₃ reduced all seedling traits apart from seedling height and root collar diameter. According to Dickson quality index, IBA was displayed to be the most beneficial phytohormone application for generating Hungarian oak seedlings with better mechanical resistance during transplanting.

Research Highlights: IBA should be adopted in planting the seedlings of Hungarian oak for plantation activities.

Additional keywords: Field performance; Morphology; Phytohormone, Quality; *Quercus frainetto*.

Abbreviations used: Dickson quality index (DQI), gibberellic acid (GA), indole acetic acid (IAA), indole butyric acid (IBA), root collar diameter (RCD), root dry weight (R_{DW}), root fresh weigh (R_{FW}), seedling dry weight (SE_{DW}), seedling fresh weight (SE_{FW}), seedling height (SH), Statistical Package for the Social Sciences (SPSS), stem dry weight (ST_{DW}), stem fresh weight (ST_{FW}).

Authors' contributions: Original idea: HBO and NB. Acorn collection: HBO and CY. Study design: HBO, NB and CY. Data collection: HBO and CY. Data analysis: NB and CY. Manuscript preparation and revisions: HBO, NB and CY.

Citation: Yücedağ, C., Bilir, N., Barış Özel, H. (2019). Phytohormone Effect on Seedling Quality in Hungarian Oak. Forest Systems, Volume 28, Issue 2, e005. <https://doi.org/10.5424/fs/2019282-14604>

Received: 27 Jan 2019. **Accepted:** 04 Jul 2019.

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Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Turkey has 22.3 million hectares' forest area and is one of the regions of high diversity for *Quercus* species with 18 species of oak (Yaltrık, 1984) which have a natural distribution of about 5.9 million ha (OGM, 2015). One out of the 18 oak species in Turkey, is Hungarian oak (*Quercus frainetto* Ten.). Hungarian oak (also known as Italian oak) is a large deciduous tree, reaching heights of more than 30 m tall (Yaltrık, 1998; Mauri *et al.*, 2016). It is native to Balkan Peninsula, and also present in South Italy and North-West Turkey (Mauri *et al.*, 2016). This species grows up to 1000 m above sea level in Thrace, Northwest Anatolia and Marmara Regions of Turkey (Yaltrık, 1998). Its wood is widely used for firewood and timber production. It

is an element of the sub-Mediterranean flora, and is usually associated in mixed groups (as well as hybrids) with other oak species across its distribution range. Hungarian oak, a meso-xerophilous species, grow in heavy acidic soils and tolerates some water-logging (Mauri *et al.*, 2016). It is an intermediate ability of resistance to drought (Manes *et al.*, 2006).

231,692,750 conifers, 90,558,250 broadleaved and 2,241,750 *Quercus* seedlings per year in Turkey are produced (OGM, 2017). The production of high-quality seedling in the nursery is considered a prerequisite for plant survival and successful establishment in the field (Mohamed, 2013) particularly in extreme field conditions (Şevik *et al.*, 2003; Demircioğlu *et al.*, 2004; Avanoğlu *et al.*, 2005). Qualified seedling is defined as superior seedling in terms of their morphological,

physiological and genetic characteristics in comparison with even-aged seedlings from the same seed origin that are grown at similar conditions with regard to irrigation, weed control, hoeing and manuring (Gezer & Yücedağ, 2006). Nursery managers use various cultural practices (e.g. seedbed density and root pruning for barer-root seedlings; cell density and volume for container seedlings; fertilization, irrigation, and top-pruning for all stock types etc.) to produce seedlings with desired morphological and physiological characteristics (Ayan, 2007; Grossnickle & South, 2017). Knowledge in seed technology and nursery practice has important roles in field performance of the species because qualified seedlings were produced with the proper nursery techniques. One of the important nursery practices used to produce qualified seedlings is application of plant hormones.

Plant hormones (also known as phytohormones) are naturally occurring organic substances that affect growth and development of plants in very low concentrations (Srivastava, 2002; Hajam *et al.*, 2017). Exogenous applications of growth regulators have marked influence on seedling growth (Kumaran *et al.*, 1994). Indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) and gibberellic acid (GA₃) are examples used as plant hormones (Maku *et al.*, 2014a). On the other hand, for prolife producing in nursery environment, use of ways that decrease the growth period of plant (like effective hormones in getting root) will be profit in the case of economic (Rahdari *et al.*, 2014).

This study was carried out to find out the effect of different phytohormones on the seedling growth of Hungarian oak for subsequent recommendation in forestry and to enhance large scale propagation of the species for plantation establishment.

Material and Methods

Mature acorns of Hungarian oak used for this study were collected from a natural stand in Kocaman forest district within the boundaries of Ereğli Forest Management Directorate affiliated Zonguldak Forest Regional Directorate at northwestern region of Turkey (41° 06' N, 31° 30' E, 650 m asl.) in September of 2013. Trees were about 50-60 years old and 30-40 cm in diameter at breast height. A total of 2000 acorns were obtained from 20 individuals keeping a minimum distance of 30 m between trees and taken to the laboratory as soon as collected. Collected acorns were mixed and 120 acorns were randomly selected for each treatment. Selected acorns were soaked in 300 ppm concentration of Indole Butyric Acid (IBA), Indole

Acetic Acid (IAA), and Gibberellic acids (GA₃, GA₄) for 24 hours before sowing. And also, no any treatment was used called as Control. 300 ppm concentration as solution was tested by taking into consideration researches exploring the effects of phytohormone on seedling growth of different species.

The study was conducted in the Yenihan State Forest Nursery of Bartın-Turkey (41°30' N, 32°29' E, 450 m asl). Annual average precipitation is about 387 mm with an average temperature of 11.9°C based on 90 years' climatic data (MGM, 2019). The growing season is about 220 days long. The nursery experiment was arranged using a completely randomized design with three replications of 40 acorns per replication. Pretreated acorns were sown in September of 2013 with 4 cm of sowing depth. Each seed-bed was oriented in east-west direction in the nursery and had five rows and a standard width of 1.2 m. Irrigation was performed twice a day by sprinkling, and weed control was manually done once a month for each seed-bed in the nursery during the growing season of 2014 and 2015. In November of 2015, a total of 150 two years old bare-root seedlings (30 seedlings for each treatment) were uprooted without harming the roots. The hand-lifted seedlings were examined in terms of morphological traits, such as seedling height (SH - cm), root collar diameter (RCD - mm), stem fresh weight (ST_{FW} - g), root fresh weight (R_{FW} - g), seedling fresh weight (SE_{FW} - g), stem dry weight (ST_{DW} - g), root dry weight (R_{DW} - g) and seedling dry weight (SE_{DW} - g). Calculations of fresh and dry weights were based on the weights determined before and after oven drying samples at 102±3 °C for 24 h.

Water contents of seedlings (%) for each treatment were determined (Saura-Mas & Lloret, 2007) as:

$$\text{Water Content of Seedling (\%)} = 100 \times \left[\frac{\text{SE}_{\text{FW}} - \text{SE}_{\text{DW}}}{\text{SE}_{\text{DW}}} \right]$$

As no single traits can determine seedling quality, the Dickson quality index (DQI) has been considered a promising tool or good indicator of seedlings quality (Mohamed, 2013). DQI was calculated according to Dickson *et al.* (1960) using the following formula:

$$\text{Dickson Quality Index} = \left[\frac{\text{SE}_{\text{DW}}}{\left(\frac{\text{SH}}{\text{RCD}} \right) + \left(\frac{\text{ST}_{\text{DW}}}{\text{R}_{\text{DW}}} \right)} \right]$$

Statistical analysis was carried out by using SPSS (SPSS Inc., 2002). An ANOVA was performed to test the effect of different treatments on seedling growth in Hungarian oak, and Duncan test was done as multiple comparison ($p < 0.05$). In addition, correlations between pairs of the measured seedling traits were evaluated by using Pearson's correlation coefficients.

The variance model of one-way ANOVA given below was used

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

Where; Y_{ij} is the observation from the j^{th} seedling of the i^{th} treatment, μ is overall mean, T_i is the random effect of the i^{th} treatment, and e_{ij} is random error.

Results

Mean values, standard deviations and range related to the traits were determined (Table 1). Accordingly, it was found that there were significant differences at 0.01 level among the studied treatments in terms of the traits with the exception of root collar diameter. The overall mean values of seedling height, root collar diameter, stem fresh weight, root fresh weight, seedling fresh weight, stem dry weight, root dry weight and seedling dry weight were 30.8 cm, 9 mm, 6.3 g,

13.9 g, 20.2 g, 8 g, 3.8 g and 11.7 g, respectively. IBA increased all seedling traits with the exception of root collar diameter while GA_3 decreased them apart from seedling height and root collar diameter. Only minor differences in mean values were recorded between treatment IAA and GA_4 . The highest mean values for seedling height and root collar diameter were found 44 cm in treatment IBA and 10.9 mm in treatment IAA (Table 1).

Pearson's correlation coefficients among pairs of traits (Table 2) revealed positive correlations ($p < 0.05$) between pairs of seedling traits of the species apart from root collar diameter. A strong positive correlation was found between root fresh weight with both seedling fresh weight ($r = 0.948$) and seedling dry weight ($r = 0.921$).

Phytohormone affected both DQI and seedling water content. DQI had the highest value (2.3) on IBA and the lowest value (1.8) on GA_3 . The highest value of seedling water content was 44.4 % on IAA, and

Table 1. Phytohormone effects on seedling growth in Hungarian oak (SH: seedling height, RCD: root collar diameter, ST_{FW} : stem fresh weight, R_{FW} : root fresh weight, SE_{FW} : seedling fresh weight, ST_{DW} : stem dry weight, R_{DW} : root dry weight and SE_{DW} : seedling dry weight).

	SH (cm)	RCD (mm)	ST_{FW} (g)	R_{FW} (g)	SE_{FW} (g)	ST_{DW} (g)	R_{DW} (g)	SE_{DW} (g)
IBA								
Mean	44.0c*	8.9a	9.7b	16.0c	25.7b	9.2c	5.7b	15.0c
Range	14.0–69.0	6.1–11.8	4.0–21.6	7.1–29.0	13.1–43.3	4.1–16.1	2.4–12.6	7.6–25.4
Standard deviation	13.1	1.3	4.2	5.6	8.8	3.3	2.5	5.0
IAA								
Mean	30.7b	10.9a	5.7a	13.2ab	18.9a	7.6ab	3.4a	10.5ab
Range	16.3–51.6	5.7–90.2	2.8–11.6	7.3–23.5	10.1–32.5	3.7–14.5	1.6–6.7	5.0–17.6
Standard deviation	9.2	15.0	2.1	4.2	6.0	2.5	1.3	3.4
GA_3								
Mean	25.6ab	8.6a	4.8a	11.9a	16.8a	6.9a	2.9a	9.8a
Range	10.0–52.5	4.6–11.0	1.5–10.8	4.8–19.4	6.3–30.2	3.0–11.0	1.0–5.9	4.0–16.6
Standard deviation	9.7	1.5	2.3	3.9	5.9	2.2	1.2	3.0
GA_4								
Mean	29.7b	8.3a	5.9a	14.7bc	20.6a	8.8bc	3.6a	12.4b
Range	11.5–47.3	5.9–12.4	1.6–19.2	2.0–34.4	6.3–50.6	3.6–20.5	1.0–11.0	4.5–30.7
Standard deviation	8.7	1.5	3.9	6.4	9.9	3.6	2.3	5.8
Control								
Mean	24.0a	8.3a	5.4a	13.8abc	19.1a	7.9abc	3.2a	11.0ab
Range	13.5–43.0	6.9–10.5	2.7–8.7	6.5–18.3	10.1–25.9	3.8–11.1	1.5–5.6	5.5–15.3
Standard deviation	7.3	0.9	1.8	3.2	4.3	1.9	1.2	2.8
Overall								
Mean	30.8	9.0	6.3	13.9	20.2	8.0	3.8	11.7
Range	10.0–69.0	4.6–90.2	1.5–21.6	2.0–34.4	6.3–50.6	3.0–20.5	1.0–12.6	4.0–30.7
Standard deviation	11.9	6.8	3.5	4.9	7.8	2.9	2.0	4.5

* Means within each column followed by the same letter are not significantly different ($p < 0.05$).

Table 2. Pearson coefficient of correlation between pairs of traits of the seedlings.

	RCD ¹	ST _{FW}	R _{FW}	SE _{FW}	R _{DW}	ST _{DW}	SE _{DW}
SH	0.144 ^{ns}	0.769*	0.538	0.685	0.753	0.507	0.651
RCD	-	0.135 ^{ns}	0.139 ^{ns}	0.148 ^{ns}	0.136 ^{ns}	0.120 ^{ns}	0.132 ^{ns}
ST _{FW}		-	0.701	0.891	0.965	0.659	0.838
R _{FW}			-	0.948	0.695	0.921	0.857
SE _{FW}				-	0.872	0.879	0.918
R _{DW}					-	0.690	0.873
ST _{DW}						-	0.899

¹: SH: seedling height, RCD: root collar diameter, ST_{FW}: stem fresh weight, R_{FW}: root fresh weight, SE_{FW}: seedling fresh weight, ST_{DW}: stem dry weight, R_{DW}: root dry weight and SE_{DW}: seedling dry weight; ^{ns}: Correlation is non-significant; *: Correlation is significant ($p < 0.05$).

the lowest value was 39.8 % on GA₄ (Figure 1). DQI showed strongly positive correlations with root and seedling fresh weights but no correlation with the other traits (Table 3).

Discussion

Strong positive correlations were found between root fresh weight with both seedling fresh weight and seedling dry weight. However, root collar diameter had no correlation with the studied traits in compliance with the result determined by Thangjam & Sahoo (2017) who investigate the effects of GA₃ on the seedling growth of *Parkia timoriana* (DC.) Merr. On the other side, Binotto *et al.* (2010) indicated that root collar diameter was strongly correlated with seedling height, stem and root weight, and seedling weight in both *Eucalyptus grandis* Hill ex Maiden and *Pinus elliottii* Engelm. var. *elliottii*. In fact, the architecture

of the root system directly influences seedling growth i.e. the greater the root area for nutrient absorption, the more positive the growth behavior (Yamada *et al.*, 2005; Binotto *et al.*, 2010).

Best performance of all studied seedling traits with the exception of root collar diameter was revealed in seedlings with 300 ppm IBA for 24 hours in this study. In similar way, Maku *et al.* (2014a and b) found that treatments of seeds with 5 and 30 ppm IBA for 24 hours enhanced seedling height and root collar diameter of *Tetrapleura tetraptera* (Schumach. & Thonn.) Taub. and *Mansonia altissima* A. Chev. seedlings, respectively. Contrary to these positive results from IBA application, Sale (2016) suggested that the performance of *Parkia biglobosa* (Jacq) Benth could be raised without treating the seeds with IBA.

In the present study, treatment of seeds with 300 ppm GA₃ for 24 hours reduced all seedling traits apart from seedling height and root collar diameter. Similar results have been found previously by Maku *et al.* (2014a) with

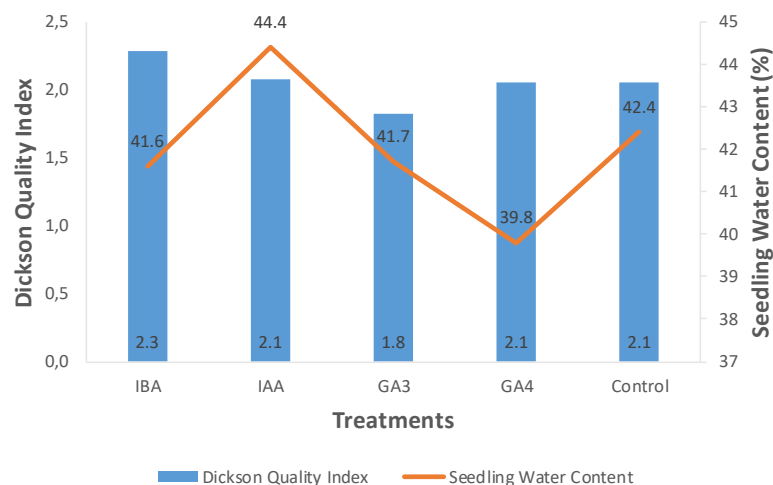
**Figure 1.** DQI and Seedling water content (%) for each treatment.

Table 3. Correlation between DQI with seedling traits.

Variable	Abbreviations	Coefficient of correlation (r)
Seedling height	SH	0.756 ^{ns}
Root collar diameter	RCD	0.163 ^{ns}
Stem fresh weight	ST _{FW}	0.812 ^{ns}
Root fresh weight	R _{FW}	0.954*
Seedling fresh weight	SE _{FW}	0.885*
Stem dry weight	ST _{DW}	0.873 ^{ns}
Root dry weight	R _{DW}	0.810 ^{ns}
Seedling dry weight	SE _{DW}	0.839 ^{ns}

^{ns}: Correlation is non-significant; *: Correlation is significant ($p < 0.05$).

30 ppm GA₃ for 24 hours for *Tetrapleura tetraptera* (Schumacher & Thonn.) Taub. and by Acar *et al.* (2017) with 500 ppm GA₃ for 24 hours for *Pistacia khinjuk* Stocks. - A genotype. In contrast, the opposite results such as for *Pistacia vera* L. with 200 ppm GA₃ for 12 hours (Ameen & Al-Imam, 2007), *Corylus colurna* L. with 80-120 ppm GA₃ for 24 hours (Arslan *et al.*, 2013), *Coriandrum sativum* L. with 100 ppm GA₃ for 24 hours (Kumar *et al.*, 2014), *Citrus limonia* Osbeck with 80 ppm GA₃ for 12 hours (Dilip *et al.*, 2017), *Parkia timoriana* (DC.) Merr. with 500 ppm GA₃ for 12 hours (Thangjam & Sahoo, 2017) and *Cassia fistula* L. with 760 ppm GA₃ for 24 hours (Rout *et al.*, 2017) have been reported. Sivacioğlu *et al.* (2007) were obtained best traits values via IBA+NAA mixed 17 hormones for root collar diameter and stem dry weight by production Scots pine seedlings. At the same time, Kumaran *et al.* (1994) stressed that the concentration and time of phytohormone application were inversely related with seedling growth of *Azadirachta indica* A. Juss. Positive results from seed treatment with IBA and IAA in comparison with GA₃ in the present study was in agreement with the findings reported from studies on woody species by Elo *et al.* (2009) that IAA, IBA and NAA were the predominant factors regulating cambium formation rather than GA₃.

Although nursery plants may have a good external appearance such as good seedling height or root collar diameter, it does not necessarily imply good survival in the field after transplanting. This information is obtained by the study of morphological attributes such DQI (Manas *et al.*, 2009). DQI integrating the aspects of total plant mass, the sturdiness quotient and the ratio of root collar diameter to seedling height explains over all plant potential for survival and growth in the field. Increasing values of DQI have showed the growing potential of the relevant species for field establishment success, i.e. its maximum values are desirable (Manas

et al., 2009; Chauhan & Sharma, 2017). DQI is in turn a useful predictor of plantation performance (Bayala *et al.*, 2009). Seedlings of Hungarian oak pretreated with IBA not only had the highest values for the studied traits apart from root collar diameter but also according to DQI, treatment IBA displayed to be the most beneficial phytohormone application for generating Hungarian oak seedlings.

As considered the results from the present study, it could be concluded that IBA should be adopted in planting the seedlings of Hungarian oak for plantation activities. However, various doses of phytohormones during different durations should be examined in order to achieve better growth results. The effects of phytohormones on seedling survival and establishment in field should be also investigated in a further research.

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