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THE EFFECT OF ALTITUDE ON THE GROWTH AND DEVELOPMENT OF TROJAN FIR (*Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen) SAPLINGS

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HIGHLIGHTS

The growth and development of sapling are closely related to altitude.

Morphology, anatomy, and wood density were determined for each Trojan fir saplings between two altitudinal steps (795 m and 1350 m).

Saplings grown at high-altitude showed better growth and development in their stems than saplings grown at low-altitude.

Keywords:
Altitude
Sapling
Trojan fir
Stem Morphology

ABSTRACT

The altitude is an important factor to affect the growth and development of saplings of the tree. However, the effect of altitude on the growth and properties of wood during their young stage it has been little studied. This study, therefore, aimed to evaluate the influence of two different altitude steps: 795 m (a.s.l. low-altitude) and 1350 m (a.s.l. high altitude) on the morphological, anatomical and wood density properties of saplings of *Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen (Trojan fir). Trojan fir is an endemic species in Turkey and its morphology and anatomy have less studied in the literature. The functional traits and wood density properties differed significantly between the two altitudes. The saplings grown at low-altitude showed greater taper degree, pith radius, pith proportion, and bark proportion than high-altitude. However, stem height, stem diameter, node number, and xylem proportion were found to be higher in saplings grown at high-altitude than low-altitude. Wood cell anatomy also varied significantly between two altitudes such that ring width, ray numbers, tracheid length, and tracheid width were higher at low-altitude, whereas ray height, ray width, tracheid lumen width, and tracheid wall thickness were greater at high-altitude. This study, therefore, suggested that the growth and development of fir saplings were better when they were grown at high-altitude than low-altitude.

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INTRODUCTION

The growth and establishment of trees can be influenced potentially by various climatic and environmental factors which are light regime, air temperature, water availability, wind, soil characteristics, altitude, aspect, and slope (Fritts, 1976; Kramer and Kozlowski, 1960; Oliver and Larson, 1996; Hicks, 1998; Desta et al., 2004; Körner, 2007; Topaloğlu et al., 2016). Altitude is one of the important physiographic factors that affect plant growth and development since functional traits could show great variance depending on the altitude level. Highest altitudes generally show different environments such as low air temperature and atmospheric pressure, precipitation prediction complexity, extreme climates (climatically unusual), strong winds and high rates of global warming (Grabherr et al., 1994; Beniston, 2003; Körner, 2003; Currie et al., 2004; Pepin and Lundquist, 2008; Rangwala and Miller, 2012; Dalerum et al., 2019). The altitude step also closely related to the light intensity which is the most significant ecological resource that providing photosynthesis and so directly affecting the survival and growth of the tree (Chen et al. 2004). Higher altitudes have generally higher light intensity than lower altitudes and higher light intensity provide greater photosynthetic rates, transpiration, stomatal conductance, leaf area, dry weight, apical dominance, the thickness of leaves, the biomass of tree (e.g. the biomass of roots and stems), root length and stem diameter (Machler and Nosberger, 1977; Zhang et al., 2003; An and Shangguan, 2009; Wang et al., 2009; Mielke and Schaffer, 2010; Zervoudakis et al., 2012; Yang et al., 2014, 2017). However, the plant diversity (richness of plant species), plant productivity, plant height, and a leaf or needle length dropped with increasing altitude (Nagy et al., 2003; Luo et al., 2004; Coomes and Allen, 2007; Körner, 2007; Abdusalam and Li, 2018). The limited environmental requirements and resources depending on the altitude can play a key role in the morphological and anatomical features of trees (Puijalon and Bornette, 2006; Matesans et al., 2010; Nicotra et al., 2010; Nascimbene and Marini, 2015). Along altitudinal gradients, the morphological and anatomical traits could show differences depending on the type of tree species (Gercek et al., 1998; Briceño et al. 2000; Tiwari et al., 2013; Topaloğlu et al., 2016). Trees however could develop different morphological, anatomical, and physiological strategies to survive and grow in different altitude steps. Morphologically, trees could adapt different altitudinal gradients by changing their height and diameter of the stem, number, and size of leaves and needles, internode length, and bark thickness (Poorter 2001; Dorken and Barrett, 2004; Gómez-Aparicio et al., 2005; Huber et al., 2009). Trees growing at low altitudes generally had greater stem heights since trees more likely grow vertically to capture more light, while trees growing at higher altitudes produce thicker stems in which the temperature is colder at higher altitudes so stems grow more likely in radially (Briceño et al., 2000; Cavieres, 2000). In different altitudes, wood anatomy could also show great variance due to changes in environmental conditions. Anatomically, wood is formed by different cells which are tracheids, fibers, vessels, parenchyma cells, and rays. However, tees could be better adapted to different altitudes by changing the size, number, thickness, and distribution of cells and width of annual growth rings (Coomes and Allen, 2007). The size, number, and distribution of each cell type, therefore, show how plants grow and develop at different altitudes. In each year of the plant growth, trees produce new cells that are oriented in concentric circles in a cross section of the stem which is called an annual growth ring. Previous studies showed that trees growing at low altitudes had larger growth rings width, the higher diameter of vessels, and higher fiber length than trees growing at higher altitudes (Coomes and Allen, 2007; Topaloğlu et al., 2016).

Fir species have also great ecological importance due to their survival adaptation at different altitude steps since they are relatively shade-tolerant species which can be considered as the plant tends to survive and permit good development and succession in their trees under minimum light levels or minimum light quantity (Ward and Stephens, 1993; Valladares and Niinemets, 2008). The firs (*Abies*) generally grow in mountain sides with higher altitudes (above 400m, up to 2400m) (Atalay, 1987c; Bozkuş, 1987; Kaya et al., 2008; Akkemik and Oral, 2011; Atalay and Efe, 2015) and temperate altitudes (Frampton and Benson, 2012). In Turkey, there are four species of fir taxa are naturally distributed from the eastern part of the Kızılırmak River, Kazdağı, Mount Uludağ, Mount Taurus along the Mediterranean coast, western Black Sea and to Kocaeli basin (Kaya et al., 2008; Atalay and Efe, 2015). The naturally distributed fir taxa are *Abies nordmanniana* Stev. (Caucasian or Nordmann fir), *Abies bornmuelleriana* Mattf. (Uludağ fir), *Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen (Kazdağı or Trojan fir), and *Abies cilicica* subsp. *isaurica* Coode & Cullen (Taurus fir) (Kurt et al., 2016). The firs however have special importance in Turkey since two species of fir taxa are distributed locally and thus they are endemic which are *Abies nordmanniana* subsp. *equi-trojani* [Asch.

& Sint. ex Boiss] Coode & Cullen , and Abies *cilicica* subsp. *isaurica* Coode & Cullen.

Numerous studies have focused on understanding the effect of changes in environmental and climatic conditions with altitudinal gradients on the physiological, morphological, anatomical, and mechanical properties of different tree species (Coomes and Allen, 2007; Topaloğlu et al., 2016; Lopez-Mata, 2017). However, little attention has been paid to determine how morphological and anatomical properties of fir trees are influenced by the change of altitude particularly during their young stage of growth (sapling stage). This study, therefore, investigated the effect of two different altitudes on the morphological, anatomical, and wood density properties of the saplings of *Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen (Trojan fir) to understand how saplings are at similar age can show differences in their growth and development depending on the altitudinal gradient. The present study hypothesized that as altitude increases stem diameter, height, tracheid wall thickness, and ray width increase, while annual ring width and ray number decrease. Therefore, it can be suggested *Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen (Trojan fir) saplings grow and develop better at high-altitude. This study, therefore, could provide a better understanding of the ecological requirements of Trojan fir particularly at the sapling stage so the findings of this study may help to produce the successful performance and growth over the life cycle of a Trojan fir tree.

MATERIAL AND METHODS

Study site and sampling

In this study, three-years-old *Abies nordmanniana* subsp. *equi-trojani* [Asch. & Sint. ex Boiss] Coode & Cullen (Trojan fir) seedlings were obtained from the Kastamonu-Gölköy forest nursery in Turkey. The seedlings had the same 'Gölköy' local provenance, same nursery-grown conditions, and planted out as a containerized planting stock. The seedlings were obtained in similar age (three-years-old) to keep all the factors in the same set, so the effect of altitude could be understood in detail. The study was conducted from May 2017 to September 2019. Total fifty young tree seedlings were planted at two altitudes: twenty-five seedlings were grown at 795 m altitude (a.s.l.) and the other twenty-five were grown at 1350 m altitude (a.s.l.). On the same day, the plants were hand-planted and the plants are planted about 1-m away from each other to limit the competition. All plants were carefully grown on east-facing slopes to provide the same growth conditions for two altitudes. The first site was set up at 795 m altitude (a.s.l) and was located in the Subaşı, Kastamonu, Turkey (N 41°26´, E 33°42´). The second site was set up at 1350 m altitude (a.s.l) and was located İğdir, Kastamonu, Turkey (N 41°19´, E 33°11´). The distance between the two study sites were approximately 80 km. The soil type in the study areas was classified as lithic Leptosol soil which is hallowed over hard rock and comprise of very gravelly or highly calcareous material (European Soil Bureau Network, 2005). The study sites are located in the Western Black Sea region of Turkey and characterized as a European-Siberian floristic region. In this region, generally, the climate is cold and humid (Atalay and Efe, 2015). The average precipitation and temperature at two altitudes between the 2017-2019 time-period are given in Table 1. A weather station at low-altitude on the study area has recorded a mean air temperature of 10 °C and a mean precipitation of between 390.7 mm·year^{-1} to 497.4 mm·year^{-1}. A weather station at high-altitude on the study area has recorded a mean air temperature of 11 °C and a mean precipitation of between 465.4 mm·year^{-1} to 552.1 mm·year^{-1}.

TABLE 1 The weather records at two altitudes between 2017-2019 years. Altitude L means saplings grown at relatively low-altitude (795 m a.s.l.) and Altitude H means saplings grown at relatively high-altitude (1350 m a.s.l.).

The weather records	Altitude L			Altitude H		
	2017	2018	2019	2017	2018	2019
Precipitation (mm·year^{-1})	390.7	514.5	497.4	465.4	618.9	552.1
Average temperature (°C)	8.5	11.1	9.6	11	12.2	11.2

Measurements of morphological properties

All plants were collected during September 2019 from fifty individual saplings at two altitudes. Each plant was 5-year-old when they were harvested. After collection, the saplings were stored in paper bags at room temperature until the measurements of anatomical and morphological properties. The following morphological data were collected from fifty sample individuals: above-ground stem height (cm), stem diameter, the degree of taper, pith radius and proportion, bark proportion, xylem proportion, and node number per length. For each sapling, stem height was measured from above-ground. The stem heights were measured considering both straight distance from base to tip and any curve along the stem from base to tip. Stem diameter was measured over the bark on both sides of the stem: in the plane and perpendicular to the plane of the stems, then the average diameter was obtained. The degree of taper was measured as the ratio between the stem diameter at the base and the stem diameter at the tip. Nodes were counted along with the stem height and recorded.

Measurements of anatomical properties

The wood anatomical analysis was performed on the stem wood. The specimens were taken from the same site (east) to keep all parameters similar to determine only the altitude effect on wood anatomical properties. Wood specimens (1 cm in height) were softened in the boiled water then immersed in equal parts of water, glycerol, and ethanol to cut into thin sections Softened specimens were then sectioned in transverse, tangential and radial sections of the thickness of 20-25 μm using a sliding microtome (Yaltirik, 1971; Yaman, 2008). To measure tracheid anatomical properties, wood specimens were cut into strips (around 1x10 mm) and were then macerated using Franklin's (1945) method (1:1 (v:v) equal parts of hydrogen peroxide and concentrated glacial acetic acid). The sections were then stained with safranin for anatomical investigations of wood cells (Bond et al., 2008). The cell anatomical variables measured were annual ring width, tracheid length, and width, tracheid lumen width, tracheid wall thickness, ray number per mm^2, ray height, and width. The tracheid properties and annual ring width were measured in the transverse section, ray number per mm, and ray size (height and width) were determined in the tangential section. Tracheid wall thickness was measured using the ratio of double wall thickness and the lumen diameter in the radial direction (IAWA, 2004). For each cell anatomical characterization, twenty-five measurements were carried out (IAWA, 2004; Yaman, 2007). The sections were observed under a light microscope (Leica DM750). Leica Application Suite (LAS EZ) microscope software was used to capture images and measure the cell sizes and numbers. The cell sizes and numbers were measured using a LAS EZ Image Analysis Software.

Measurement of wood density

Collected wood specimens were cut into small pieces (2 cm in height). Density samples were taken from the same stem region that anatomy samples were taken from. The water displacement method was used to determine the green volume of small pieces of wood. Each wood piece firstly was kept in water using an airtight container until all hydrated. Then, each specimen was immersed in water using a needle in a beaker standing on an electronic weighing balance that gave a mass of water displacement. After that, the specimens were oven-dried at 103°C to constant mass. The wood density was calculated by dividing the oven-dried mass to volume (Barnett and Jeronimidis, 2003).

Statistical analysis

To determine the effect of altitude step on the morphological (stem height, stem diameter, node number, taper degree, pith radius, pith area, bark area and xylem area), anatomical (ring width, ray number, ray height and width, tracheid length and width, tracheid lumen width, tracheid wall thickness) and wood density traits, statistical analysis was performed using the SPSS 19.0. One-way analysis variance (ANOVA) was used to test the statistical significance in different properties between two altitude steps.

RESULTS AND DISCUSSION

Morphological properties

The morphological parameters showed differences between the two altitudes (low vs. high). Height is an important morphological parameter that shows the quality of the growth of the saplings. The results showed that fir saplings were grown better at high-altitude. One-way ANOVA results found significant differences in the height of saplings between two altitudes ($F_{1, 48} = 7.55$ $p < 0.05$). The height of saplings was almost 17% greater in saplings grown at high-altitude than saplings grown at low-altitude (Figure 1a). The diameters in saplings grown at high-altitude were found to be significantly greater than the diameters in saplings grown at low-altitude ($F_{1, 48} = 14.71$ $p < 0.05$). Saplings at high-altitude showed more than 1.1 times greater diameter in their stems than the sapling at low-altitude (Figure 1b).

The saplings taper (i.e. variation of diameter from stem base to tip) was also measured for each individual (Figure 1d). The degree of taper is an important parameter for the growth of the plant since it is directly related to both height and diameter. Particularly, trees in their young growth period (sapling stage) are so vulnerable to environmental conditions therefore the asymmetric structure of plants could be balanced equally from above the ground (at the bottom) to tip to maintain stem structure better (West et al., 1999). When the tree grows in height, the diameter starts to decrease from base to tip to make stem stronger. Therefore, tapering is a strategy or adaptation of the plant to balance its growth (increase in height and diameter) to a different environment. In this study, saplings grown at low-altitude showed a significantly higher degree of taper than the saplings grown at high-altitude ($F_{1, 48}$ = 13.68 $p < 0.05$). The taper degree was on average 1.05 at high-altitude and was on average 1.27 at low-altitude. It could be suggested that the stem form is poor in the fir saplings which grown at low-altitude, however, fir saplings which grown at high-altitude could have better form in their stem structure due to its lower degree of taper. As a result, a low degree of taper is a good function because increasing the taper degree makes stem structure weaker (Figure 1).

Nodes were also counted along with the stem height (Figure 1c). The number of nodes differed significantly between the two altitudes. The number of nodes was on average 24.2 at high-altitude and

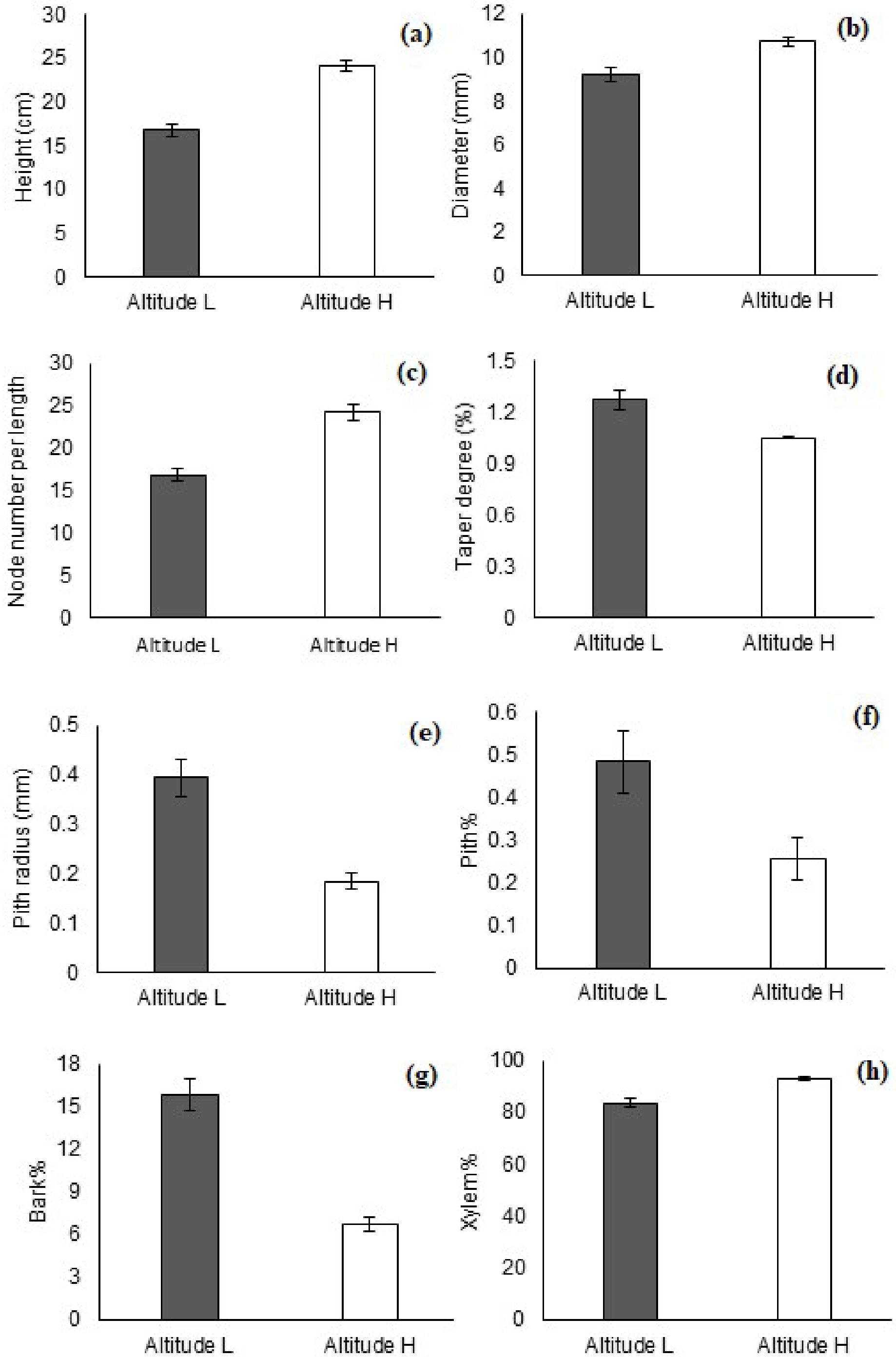

FIGURE 1 The morphological characteristics of saplings of Trojan fir grown at two different altitudes. Altitude L means saplings grown at relatively low-altitude (795 m a.s.l.) and Altitude H means saplings grown at relatively high-altitude (1350 m a.s.l.). A standard error is shown by the error bars.

on average 20.2 at low-attitude (Figure 1). One-way ANOVA results also showed that the number of nodes was significantly greater in saplings grown at high- altitude than the saplings grown at low-altitude ($F_{1, 48}$ = 7.32 $p < 0.05$). The higher numbers of nodes could be related to the survival strategy of saplings' stems. Nodes are the growing points in which leaves/needles and lateral buds arise. During the growth and development of leaves and lateral buds, stems require more water content, but the stems need to use water potentials effectively. However, nodes provide less water potential since the surface area of the nodal region is quite narrow. This morphological structure could help to prevent large cavitation, therefore, acquired greater hydraulic conductivity in this region (Zimmermann1978a, b; Zimmermann and Sperry, 1983; Lo Gullo et al., 1995; Özden and Ennos, 2018). In this study, greater node numbers at high-altitude could be explained by the variations in environmental conditions since the temperature and rainfall show great variance at high-altitude thus saplings could improve the adaptational strategy to cope with all stresses. Therefore, making node numbers greater at high-altitudes may be a way to provide successful growth and development in stems.

The pith radius, the proportion of pith, the proportion of bark, and the proportion of xylem were determined for each sapling between two altitudes (Figure 1e, f, g, h). Saplings grown at low-altitude had significantly greater pith radius, the proportion of pith (pith%), and proportion of bark (bark%) than saplings grown at high-altitude. Saplings grown at low-altitude showed more than 2 times greater pith radius ($F_{1, 48}$ = 24.10 $p < 0.05$) and more than 2.3 times greater bark proportion ($F_{1, 48}$ = 25.31 $p < 0.05$) than the saplings grown at high-altitude (Figure 1e). Surprisingly, pith proportion did not differ significantly between two altitudes ($F_{1, 48}$ = 2.80 $p > 0.05$). Pith is placed in the center of vascular cambium of stems and composed of parenchyma cells which responsible for the transport of stored nutrients and minerals throughout the organs of saplings. The greater radius of pith at low-altitude could be due to the amount of precipitation which is higher at low-altitude. At low altitude, stems thus may have a greater pith radius to obtain more soil nutrients for growth. Previous studies have shown that precipitation and soil organic matter cycle are associated such as increased precipitation causes low pH and low concentrations of phosphorus (P) and total nitrogen (N) (Hall and Swaine, 1976; Swaine, 1996). Prior studies also showed that P and total N are important for the growth and development of trees such as increasing P and total N content provide better tree performance to environmental changes (Hall and Swaine, 1976; Jiang et al., 2015). In this study, fir saplings showed better growth and development at high-altitude which had a narrower radius of pith than low-altitude. It could be suggested the soil nutrients (i.e. P and total N contents) could be used effectively at high-altitude than at low-altitude. However, further research is needed to evaluate the relationships between soil nutrients and pith radius.

However, the proportion of stem area occupied by the xylem was found to be significantly higher in saplings grown at high-altitude than saplings grown at low-altitude ($F_{1, 48}$ = 24.97 $p < 0.05$) (Figure 1h). Xylem proportion at high-altitude was almost 11.1% greater than the low-altitude. Xylem is known the main skeleton of stem since it is the woody part which provides both mechanical and hydraulic support to stem. Xylem is also produced during the secondary growth which known as radial growth of stems (Murmanis, 1970; Larson, 1994). In the current study, the stem diameter was found to be greatest at high-altitude than low-altitude thus it could be suggested that saplings grown at high-altitude had greater radial growth than low-altitude due to the greater xylem area. At high-altitude, the wind-induced loads could be also more destructive for the plant growth therefore; saplings could adapt their habitat to modify their xylem area. Greater xylem area could provide greater mechanical resistance to environmental loadings so saplings could tend to continue growth and development throughout the stem (Özden and Ennos, 2018).

Wood density and anatomical properties

Saplings grown at high-altitude had almost 17% greater wood density than saplings grown at low-altitude ($F_{1, 48}$ = 115.79 $p < 0.05$). Wood density was found to be on average 0.53 g cm^{-3} at high-altitude and 0.45 g cm^{-3} at low-altitude (Fig. 2a). The average ring width also differed significantly between two altitudes. The decline of ring width with altitude was recorded in this study. One-way ANOVA found that saplings grown at low-altitude had significantly greater average ring width than saplings grown at high-altitude ($F_{1, 48}$ = 49.68 $p < 0.05$). The average ring width was 0.56 cm in saplings grown at high-altitude and was on average 0.91 cm in saplings grown at low-altitude so ring width was almost 2 times greater at low-altitude (Figure 2b). The decrease in ring width could be related to differences in temperature and environmental conditions since the temperature is colder and rainfall is lower at higher altitudes than lower altitudes therefore narrower rings occur at the higher altitude. This is the adaptive response of saplings to changes in environments. The findings of this study are in agreement with previous studies. Previous studies also suggested that the annual ring width decreases with increasing altitude (Tranquillini, 1979; Gindl et al., 2001; Arx et al., 2006; Peters, 2013; Dulamsuren et al., 2014).

In each sapling, different cell properties were also determined and compared between two altitudes. The cell characteristics between the two altitudes are shown in Table 2.

Rays were exclusively uniseriate and composed of straight and squared cells; the height of rays (RH) varied between three to six cells (Figure 3) The maximum ray number (RN) was found in the saplings grown at low-altitude and was 1.2 times higher (mean 22.1) than the saplings grown at high-altitude (mean 17.9) (Table 2). One-way ANOVA results indicated a significant difference in RN between two altitudes ($p < 0.05$). The saplings grown at high-altitude had a mean height of ray 558 μm and saplings grown at low-altitude had a mean height of ray 529.9 μm. However, no significant differences were found in RH values between two altitudes ($p > 0.05$). The saplings grown at high-altitude had a mean width of ray (RW) 126.8μm, which was significantly greater than that of the saplings grown at low-altitude (mean 100.5 μm) ($p < 0.05$).

As for the tracheids, the saplings grown at low-altitude had a mean length of tracheid (TL) 1256 μm and mean width of tracheid (TW) 31.1 μm, which were greater than that of the saplings grown at high-altitude (mean 1246 μm TL and 26.3 μm TW) (Table 2). One-way ANOVA however did not find statistically significant differences in the TL and TW between two altitudes ($p > 0.05$). Analysis of tracheid anatomy also indicated that tracheid lumen width (TLW) and tracheid wall thickness (TWT) was higher in the saplings grown at high-altitude (Figure 4) One-way ANOVA found significant differences in TWT between two altitudes ($p < 0.05$); TWT values in the saplings grown at high-altitude were larger than the saplings grown at low-altitude (mean 2.7 μm at high-altitude, mean 1.8 μm at low-altitude).

There was also a significant relationship between wood density and TWT (Figure 5). Linear regression analysis showed that wood density was positively

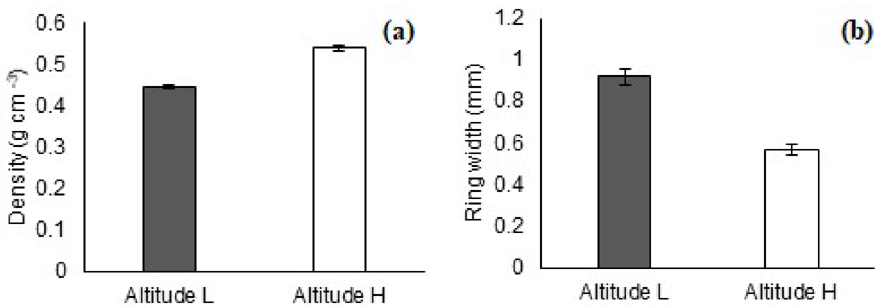

FIGURE 2 Wood density and anatomical properties of saplings of Trojan fir grown at two different altitudes. Altitude L means saplings grown at relatively low-altitude (795 m a.s.l.) and Altitude H means saplings grown at relatively high-altitude (1350 m a.s.l.). A standard error is shown by the error bars.

TABLE 2 The anatomical characteristics of Trojan fir saplings grown at two different altitudes. Altitude L means saplings grown at relatively low-altitude (795 m a.s.l.) and Altitude H means saplings grown at relatively high-altitude (1350 m a.s.l.). The values are the means of measured cells. RN: ray number per mm^2; RH: uniseriate ray height (μm); RW: uniseriate ray width (μm); TL: tracheid length (μm); TW: tracheid width (μm); TLW: tracheid lumen width (μm); TWT: tracheid wall thickness (μm).

Anatomical Parameters	Altitudes	
	Altitude L (mean ± se)	Altitude H (mean ± se)
RN	22.1 ± 1.56	17.9 ± 0.85*
RH	529.9 ± 29.1^{ns}	558 ± 20.6
RW	100.5 ± 7.4**	126.8 ± 4.5
TL	1256 ± 54.5	1246 ± 56.9^{ns}
TW	31.1 ± 3.1	26.3 ± 1.5^{ns}
TLW	4.36 ± 0.18^{ns}	4.62 ± 0.23
TWT	1.8 ± 0.11***	2.7 ± 0.11

Values indicate the mean ± standard error (SE). The significance is indicated as *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ and ns (i.e., not significant).

correlated to TWT ($r = 0.50$ $R^2 = 0.25$, $p < 0.05$). The results of this study are in agreement with the findings of Yasue et al. (2000) which showed increasing cell wall thickness make the wood of *Picea glehnii* (F.Schmidt) Mast. denser. However, the findings of current study do not support the previous study by Gindl et al. (2001) who found that tracheid wall thickness and cell division of Norway spruce were greater at the low-altitude than high-altitude. In this study, although average tracheid length and width were higher at low altitude, tracheid wall thickness was greatest at high-altitude. Previous studies suggest that cell wall thickness could be due to

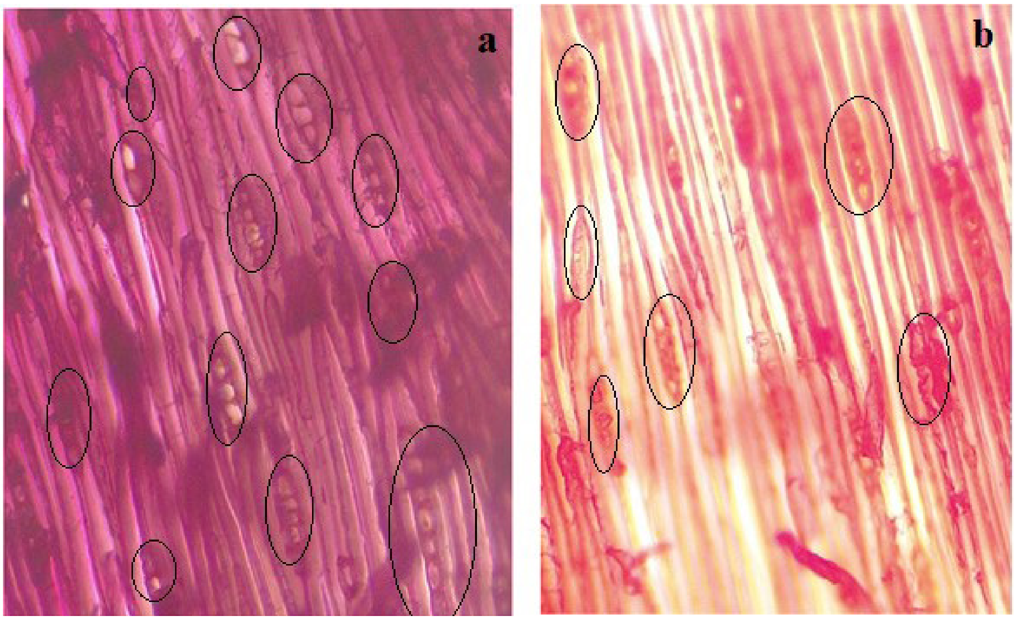

FIGURE 3 Showing ray frequency in the tangential direction (a) ray frequency at low-altitude and (b) ray frequency at high-altitude.

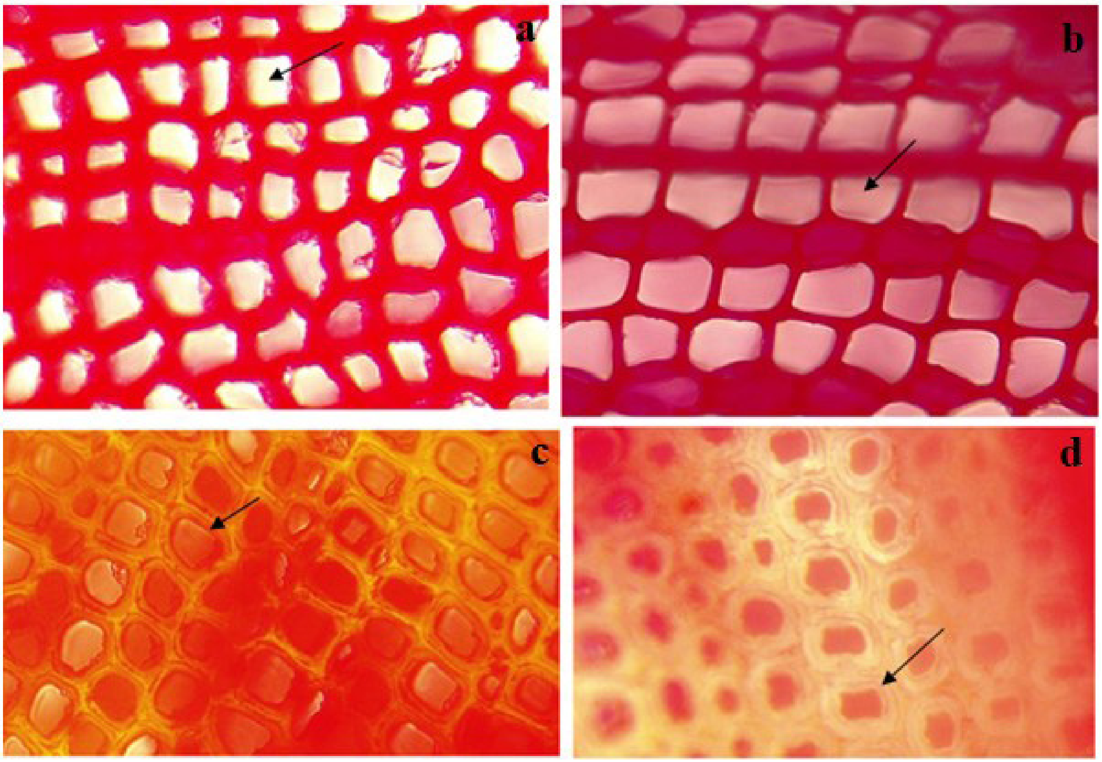

FIGURE 4 Showing cell properties in the radial direction (a) is tracheid lumen at low-altitude (b) is tracheid lumen at high-altitude (c) is the tracheid wall thickness at low-altitude (d) is tracheid wall thickness at high-altitude.

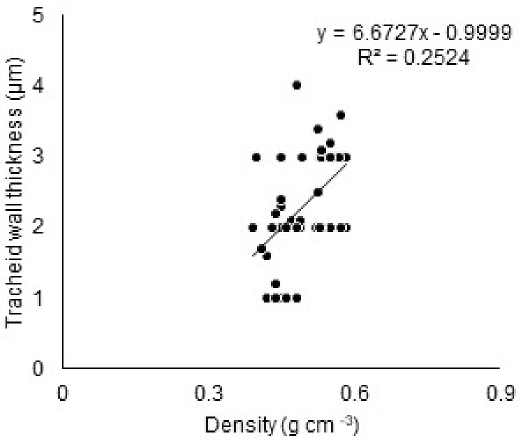

FIGURE 5 Relationships between wood density and tracheid wall thickness in the saplings of Trojan fir saplings.

variations caused by the cell distribution, wood density, and tracheid sizes (Thomas et al., 2006; Gibson, 2012). Previous studies also found that density is related to cell division, enlargement (diameter or radial growth), and cell wall thickening during the growing period (MacDonald and Hubert, 2002; Thomas et al., 2006). Similarly, in this study, tracheid wall thickness was found to be significantly associated with wood density since wood was denser at high-altitude so indicated thicker cell walls. The variations in the results with previous findings could be related to the life stage of trees since trees at the sapling stage were used in this study and the functional traits of saplings could show alterations depending on the growth requirements.

The relationships were analyzed by linear regression. However, the differences in TLW values between the two altitudes did not differ significantly ($p > 0.05$) (4.62 μm in high-altitude and 4.36 μm in low-altitude) (Fig. 4)Previous studies showed that tangential vessel diameter, radial vessel diameter, ray number, ray height, tracheid length and diameter, fibre length values decreased with increasing altitude, whereas vessel number, fibre width, fibre lumen width, and fibre wall thickness increased with increasing altitude (van der Graff and Baas, 1974; Noshiro et al., 1994; Topaloğlu et al. 2016; Lopez-Mata et al., 2017).The anatomical findings of this study are in line relatively with the previous findings since the number of rays and tracheid length were found to be relatively larger at low-altitude than high-altitude. However, further studies need to be carried out to validate those findings.

CONCLUSION

This study showed how two different altitudinal steps affected the morphological, anatomical, and wood density properties of Trojan fir saplings of similar age. Stem height, diameter, node number, xylem area, wood density, ray traits and tracheid wall thickness increased with increasing altitude. The results also showed that saplings grown on high-altitude had greater wood density due to higher tracheid wall thickness at high-altitude. This study also suggested that saplings grown at high-altitude had greater radial growth to make stems stronger to changes in environmental conditions. The results of this study suggest that high-altitude provides more suitable and ideal conditions for the growth and development of fir saplings since saplings grown on high-altitude showed greater morphological, anatomical and density traits than at-low altitude. The findings of this study therefore could provide essential ecological knowledge about the Trojan fir for their sustainable management and plantations. The effective plantations of endemic species could conserve tree genetic resources. However, further studies should be carried out at various altitudinal steps to determine how Trojan fir saplings grow and develop under different altitudinal conditions.

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