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Morphogenetic variations of *Pinus sylvestris* L. seedlings depending on altitude

Yükseltiye bağlı olarak *Pinus sylvestris* L. fidanlarının morfogenetik varyasyonları

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Abstract

Scotch pine is a primary species that spreads from sea level to very high altitudes in Turkey and has a very wide distribution area in the world. For this reason, it is important to investigate the variations of the species depending on the altitude. The research aims to determine the morphogenetic variations of the seedling characters in populations representing the vertical distribution area of Scotch pine and to reveal the within and among population variations in the vertical distribution areas. In the study, seedlings (three, four and five-year-old) grown from the seeds collected from five populations in the altitudinal zones up to 2,250 m from sea level in the natural distribution area of Scotch pine in Trabzon region were used. The seedling's height, root collar diameter, the number of side branches and crown buds and sturdiness quotient were determined. As a result of the study, it was determined that there were statistically significant differences (p<0.01) among populations in different altitude zones. The average seedling heights increase in the transition from the 4th altitude zone to the 5th altitude zone in Zigana, while the height of the seedlings decreases in all three growth periods due to the increase in altitude in general. Also, in the transition from the 2nd elevation zone in Maçka to the 3rd elevation zone in Zigana, the mean root collar diameters increased. On the other hand, in seedlings at altitudes other than these, the root collar diameter averages decreased as the altitude increased.

Keywords: Morphology, Scotch pine, sturdiness quotient, variation

Öz

Sarıçam Türkiye'de deniz seviyesinden çok yüksek rakımlara kadar yayılan bir asli tür olup, dünyada da çok geniş yayılış alanına sahiptir. Bu nedenle, türün yükseltiye bağlı varyasyonlarının araştırılması önem arz etmektedir. Araştırma, sarıçam türünün dikey yayılış alanını temsil eden populasyonlardaki fidan karakterlerinin morfogenetik varyasyonlarını belirlemeyi ve dikey yayılış alanlarında populasyon içi ve populasyonlar arası varyasyonları ortaya koymayı amaçlamaktadır. Çalışmada Trabzon yöresinde doğal yayılış gösteren sarıçam türünün deniz seviyesinden 2.250 m yüksekliğe kadar olan bölgelerdeki beş populasyondan toplanan tohumlardan yetiştirilen (üç, dört ve beş yaşında) fidanlar kullanılmıştır. Fidanların boyu, kök boğazı çapı, yan dal ve tepe tomurcuğu sayısı ve gürbüzlük indisi belirlenmiştir. Çalışma sonucunda, farklı yükseltilere ait populasyonlar arasında istatistiksel olarak anlamlı farklılıklar (p<0,01) olduğu tespit edilmiştir. Zigana'da ikinci yükseltiden beşinci yükseltiye geçişte ortalama fidan boyları artmış, genel olarak yükselti artışına bağlı olarak her üç büyüme döneminde de fidan boyları azalmıştır. Ayrıca Maçka'da ikinci yükseltiden Zigana'da üçüncü yükseltiye geçişte ortalama kök boğazı çapları artmıştır. Bunun dışındaki yükseltilere ait fidanlarda ise yükselti arttıkça kök boğazı çap ortalamaları azalmıştır.

Anahtar Kelimeler: Morfoloji, sarıçam, gürbüzlük indisi, varyasyon

1. Introduction

Seeds and seedlings with superior genetic qualities should be used to ensure the future of forest establishment and forestation studies, which cover long periods and have high costs, and to minimize risks. In order to reveal the genetic quality of a seedling, it is necessary to know the genetic variations of the population and the trees in the population, which are the source of the material from which it is produced generatively or vegetatively. The resistance of plants to biotic and abiotic damage and their adaptation to changing climatic conditions are directly related to intraspecies genetic diversity (Filiz et al., 2011; Güney et al., 2014; Güney et al., 2019; Koç 2022a).

Forest tree populations are faced with a number of dangers due to the global climate change that has taken place in recent years. Therefore, the importance of gene protection studies is increasing and it is necessary to develop protection strategies in light of these effects. In this way, it will be an important option to identify populations with higher genetic diversity as an option against future needs (Tekin et al., 2022; Varol et al., 2022; Zeren Çetin et al., 2022, Key et al., 2022; Koç 2022b; Koç 2021).

Intraspecies genetic diversity studies are emphasized at the beginning of breeding programs (Ledig, 1986). As a genetic resource in tree breeding, primarily natural stands are used, and it is stated that the best way to determine the genetic variation in a species is by comparing the populations in different habitats (Chmura and Rozkowski, 2002). According to selection theory in breeding, species with a large gene pool and therefore rich in genetic diversity have a wide natural distribution area, and these species can adapt more easily to different habitats (Yahyaoğlu and Genç, 2007; Kurz et al., 2022). Heterogeneity of biotic and abiotic factors in habitats and temporal and spatial changes of ecological characteristics affect the vital characteristics of plants (Işınkaralar et al., 2022; Çobanoğlu et al., 2022; Atar and Güney, 2021; Matesanz et al., 2010; Herrera and Bazaga, 2013).

The variables in the forest ecosystem change the morphological, physiological and anatomical features of the plants, and also affect the adaptation characteristics in the areas where plants are used (Nascimbene and Marini, 2015). Altitude is one of the most important factors among ecological variables, and depending on the change in altitude, many ecological features also differ. It is stated that the variations in ecological characteristics, especially climate variables, due to the increase in altitude are related to the advanced life history and evolutionary responses of plants (Turna and Güney, 2009; Aragon et al., 2012; Leingartner et al., 2014; Güney et al., 2019; Atar and Güney, 2021).

The developments seen in forest establishment studies (afforestation or reforestation) have also accelerated the studies in the field of tree breeding. At this stage, determining the breeding values of populations for seed tree selection is a very important phenomenon in terms of growing forests that are suitable for their growing environment and have high yield strength. In addition, the percentage of quality seedlings to be grown in nurseries primarily depends on the quality of the seed used. The most critical problems in this regard are from which region, altitude, and trees the quality seeds will be obtained. For this reason, it will be possible to determine the populations in which superior trees are found and to reach increasingly superior values by breeding these populations. Rational use of existing forest areas in terms of quality and quantity should be increased (Ürgenç, 1982; Şimşek, 1993; Turna and Güney, 2009).

Widespread species have a great deal of geographic variation and local races (Hume and Cavers, 1982). The rugged geographical structure and changing climate and soil characteristics of Türkiye in short distances encourage the formation of local races in forest tree populations even at short distances (Işık, 1988).

The fact that Scotch pine (*Pinus sylvestris* L.) has a very wide distribution, both horizontally and vertically, is an indication that it may have genetic variations. Especially in Türkiye, the extreme distribution of this species in altitude and its vertical distribution from sea level to 2700 m altitude makes it even more important to determine the variations it will show depending on the altitude. In breeding studies that are started with populations with a large genetic base, it is easier to find suitable breeding material and risk-free, and therefore the chance of success is higher (Turna and Güney, 2009; Şevik et al., 2010; Bilgen et al., 2011, Güney et al., 2014; Güney et al., 2019).

Scotch pine, which has the widest geographical distribution among the existing pine species, has a vast natural distribution area of approximately 3700 km in width and 14700 km in length in Europe and Asia. The northern border forms the forest border of conifers together with the *Larix sibirica* Ldb. in the Siberian steppes, in the north of Scotland, Norway, Sweden and Finland up to the 70th degree of latitude. Its southern border is in the high parts of the Pyrenees in Spain, in the Alps, in the Carpathians, in former Yugoslavia and Bulgar-

ia as scattered, in Anatolia, Crimea, and the Caucasus. Scotch pine starts from Yeşildağ in the west of Eşkişehir in our country and covers the high parts of the North Anatolian mountains towards the east, passing through Kars-Sarıkamış to the Caucasus. Kayseri-Pınarbaşı is the last place where the species descends to the south. The vertical distribution of Scotch pine, which has such a wide distribution in Türkiye, rises from sea level near Trabzon-Sürmene (Çamburnu) and Artvin-Arhavi to 2700 meters in Kars-Sarıkamış. In addition, it spreads purely and mixed with other species on average between 1000-2500 meters (Anşin and Özkan, 1997; Anonymous, 2001; Turna, 2003).

In this study, it was aimed to determine the morphogenetic variations of the seedling characters in populations representing the vertical distribution area of Scotch pine in Trabzon and to reveal the within and among population variations in the vertical distribution areas.

2. Material and Method

2.1. Material

In the study, seedlings grown from the seeds collected from five populations in the altitudinal zones up to 2250 m from sea level (0 m) in the natural distribution area of Scotch pine in Trabzon region were used. Some characteristics of the populations are given in Table 1, and their geographical locations are given in Figure 1.

Table 1. Description of the Scotch pine populations sampled
Tablo 1. Örneklenen sarıçam populasyonlarına ait tanımlayıcı bilgiler

Population Name	Altitudinal Zone (Spacing)	Latitude	Longitude
Çamburnu	1 (0-450 m)	4531000-4530000	603000-602000
Maçka	2 (450-900 m)	4527500-4527000	557250-556750
Zigana-1	3 (900-1350 m)	4498500-4496000	529500-527000
Zigana-2	4 (1350-1800 m)	4502750-4501000	535500-534000
Zigana-3	5 (1800-2250 m)	4501000-4499000	535000-533000

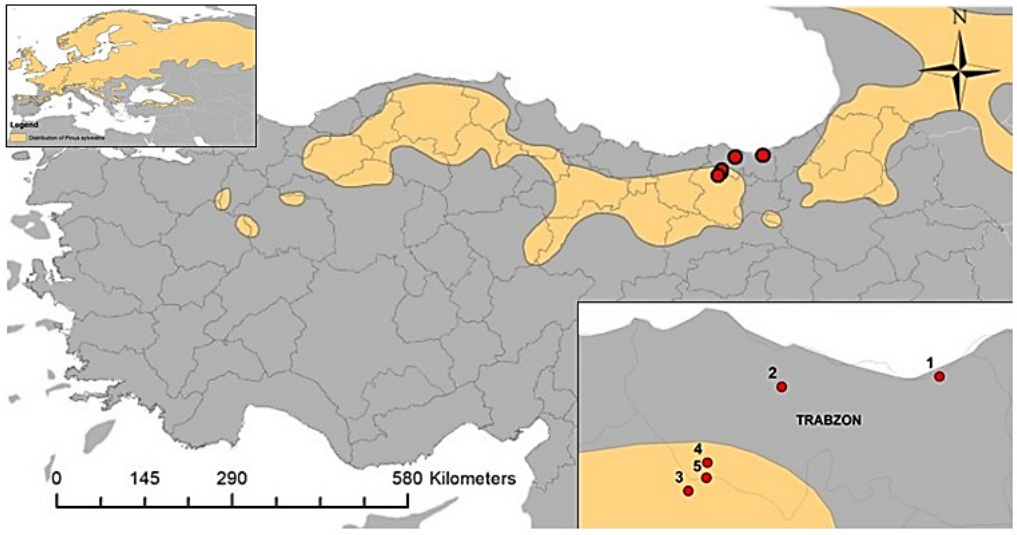

Figure 1. Geographic locations of populations at different altitudes
Şekil 1. Farklı yükseltilerdeki populasyonların coğrafi konumları

2.2. Method

Seeds were collected from a total of 150 trees (5 altitude levels × 30 trees) and separated on a tree-by-tree basis with the labels of the sampled trees. Seeds of each tree and population were sown using the line sowing method by a randomized block design in outdoor nursery seedbeds in the Research and Application Greenhouse, Faculty of Forestry, Karadeniz Technical University in Trabzon.

Morphological characteristics of three, four and five-year-old seedlings were measured on a total of 4500 seedlings to be 5 populations × 30 trees × 30 seedlings at the end of each growth period. Seedling height (SH), root collar diameter (RCD), number of side branches (NSB), and number of crown buds (NCB) were measured. Also, the sturdiness

quotient (SQ), which expresses the ratio of seedling height (cm) to root collar diameter (mm), was determined (Aphalo and Rikala, 2003). The sturdiness quotient expresses the vigor and robustness of the seedling. The seedlings were classified as quality seedlings (SQ<50), medium-quality seedlings (50<SQ<60) and low-quality seedlings (SQ>60) by using the SQ values (Yahyaoğlu and Genç, 2007). In addition, the coefficient of variation of seedling morphological characteristics was also evaluated depending on the altitude. The coefficient of variation (CV) was calculated as follows (Bland and Altman, 1996):

$$CV\% = (\sigma / \mu) \times 100$$

In this equation, σ is standard deviation and μ is the overall mean.

2.3. Statistical analysis

The obtained data were analyzed using the SPSS 26.0 statistical software program. In the study, variance analysis (one-way ANOVA) was performed to reveal the statistical significance (p<0.05) of the differences in the morphological characteristics of seedlings among and within populations depending on different altitudes. In case of significant differences as a result of analysis of variance, the groups formed by the altitudes were determined by Duncan's test (Özdamar, 1999).

3. Results

The mean values for the morphological characteristics of three, four- and five-year-old seedlings, and the results of the analysis of variance and Duncan's test are given in Table 2. It was determined that there were statistically significant (p<0.01) differences among populations in different altitude zones depending on the morphological characteristics measured in the seedlings of three different ages. The values of SH varied between 20.3-35.5 cm in 3-year-old seedlings, 28.6-47.4 cm in 4-year-old seedlings, and 36.8-65.2 cm in 5-year-old seedlings. Considering the change in the height of the seedlings according to the altitude, it is understood that the average SH increase in the transition from the 4th altitude zone (1350-1800 m) to the 5th altitude zone (1800-2250 m) in Zigana, while the height of the seedlings decreases in all three growth periods due to the increase in altitude in general.

The RCD of Scotch pine seedlings varied between 5.0-6.1 mm at 3 years old, 8.1-10.4 mm at 4 years old, and 10.0-13.1 mm at 5 years old. When the change of RCD according to the altitude is examined, in the transition from the 2nd elevation zone (450-900 m) in Maçka to the 3rd elevation zone (900-1350 m) in Zigana, the mean RCD increased. On the other hand, in all the 3-, 4-, and 5-year-old seedlings at altitudes other than these, it was de-

Table 2. The results of mean, variance analysis and Duncan's test for seedling characters
Tablo 2. Fidan karakterlerine ait ortalama değerler, varyans analizi ve Duncan testi sonuçları

Population	Seedling Age	SH (cm)	RCD (mm)	SQ	NSB	NCB
1st Altitude	3	35.5±7.1 a	6.1±1.7 a	60.6±13.7 b	4.3±1.8 b	3.8±1.1 ab
	4	47.4±10.0 a	10.4±2.2 a	46.4±10.6 b	6.3±2.5 b	5.0±2.1 a
	5	65.1±12.2 a	13.1±2.9 a	51.0±10.4 b	9.3±3.1 bc	5.5±2.5 a
2nd Altitude	3	33.5±7.4 b	5.4±1.5 c	64.7±15.9 a	3.8±1.8 a	3.9±1.2 a
	4	44.1±10.6 b	9.1±2.0 c	49.7±10.2 a	6.0±2.6 bc	5.3±2.2 a
	5	59.3±12.4 b	11.3±2.8 b	54.4±12.6 a	9.9±3.9 ab	5.6±2.6 a
3rd Altitude	3	22.3±6.3 d	5.6±1.6 bc	40.7±10.1 d	4.6±2.2 ab	3.6±1.1 bc
	4	35.8±8.2 c	9.9±2.2 b	37.2±7.9 d	7.0±2.2 a	4.9±2.0 a
	5	41.2±9.5 c	11.2±2.6 b	37.7±8.6 c	10.1±3.4 a	5.0±2.4 b
4th Altitude	3	20.3±5.1 e	5.0±1.3 e	42.1±10.5 cd	4.8±1.7 a	3.0±1.0 d
	4	28.6±6.1 c	8.1±1.8 e	36.2±7.2 d	6.1±2.3 bc	4.3±1.7 b
	5	36.8±9.7 d	10.0±2.3 c	37.5±7.7 c	9.2±3.2 c	4.1±2.1 c
5th Altitude	3	24.1±4.9 c	5.7±1.5 b	44.1±10.1 c	3.9±1.7 a	3.6±0.9 c
	4	33.6±6.7 d	8.4±1.9 d	40.6±7.1 c	5.8±2.4 c	4.5±1.7 b
	5	38.5±8.9 d	10.1±2.4 c	38.9±7.2 c	9.3±3.2 bc	4.7±2.3 b
Mean	3	27.1±8.6	5.5±1.5	50.7±15.9	4.3±1.9	3.6±1.1
	4	37.9±10.9	9.1±2.2	42.3±10.2	6.2±2.5	4.8±1.9
	5	47.6±15.6	11.1±2.8	43.7±11.9	9.5±3.4	5.0±2.4
F	3	349.9**	21.6**	253.2**	16.2**	31.8**
	4	202.2**	56.2**	117.8**	9.8**	11.2**
	5	356.0**	54.8**	173.5**	3.8**	14.8**

** (There is a statistically significant difference at 99% confidence level)
Letters (a, b, c, etc.) represent the groups formed as a result of the Duncan test.

termined that the RCD averages decreased as the altitude increased.

SQ values ranged between 40.7 and 64.7, 36.2 and 49.7, and 37.5 and 54.4, respectively, in 3-, 4- and 5-year-old seedlings. It was determined that the SQ values of the seedlings of all three ages, which were measured, generally decreased with the increase in altitude, and that the populations located at high altitudes had a better SQ value. The average values of NSB and NCB increased depending on the age of the seedlings.

There were also statistically significant differences between trees within the populations regarding morphological characteristics in all measured characters, except for RCD in 4-year-old seedlings, and the results are given in Table 3. These differences emerged in all three growth periods, and different groups ranging from 3 to 12 occurred depending on Duncan's test.

Table 3. Variance analysis results for seed and seedlings within populations
Tablo 3. Populasyonlar içi tohum ve fidanlara ilişkin varyans analizi sonuçları

Seedling Age	Morphological Characters		1st Altitude	2nd Altitude	3rd Altitude	4th Altitude	5th Altitude
3	SH	F-value	3.404	12.649	10.539	5.383	4.733
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	7	11	7	9	9
	RCD	F-value	3.434	2.166	2.961	2.309	3.936
		p-value	0.00	0.01	0.00	0.00	0.00
		Gn	8	4	6	5	9
	SQ	F-value	4.927	5.552	4.447	6.583	3.458
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	8	10	8	7	10
	NSB	F-value	4.927	4.669	2.866	1.525	3.673
		p-value	0.00	0.00	0.00	0.04	0.00
		Gn	8	11	5	3	7
	NCB	F-value	4.927	2.667	1.877	3.149	1.745
		p-value	0.00	0.00	0.00	0.00	0.01
		Gn	8	7	4	7	5
4	SH	F-value	4.479	6.715	6.408	3.652	4.778
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	7	12	9	6	10
	RCD	F-value	1.480	1.798	3.505	3.565	2.262
		p-value	ns	0.00	0.00	0.00	0.00
		Gn	2	5	8	4	5
	SQ	F-value	4.553	9.372	6.720	5.030	1.773
		p-value	0.00	0.00	0.00	0.00	0.01
		Gn	7	12	9	7	6
	NSB	F-value	2.150	1.919	1.529	2.421	2.520
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	5	7	4	4	6
	NCB	F-value	2.775	2.032	3.298	2.149	2.174
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	6	3	8	6	5
5	SH	F-value	2.632	6.814	5.654	3.088	3.729
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	4	11	8	8	9
	RCD	F-value	1.745	1.911	2.955	2.075	2.902
		p-value	0.02	0.00	0.00	0.00	0.00
		Gn	3	4	5	4	4
	SQ	F-value	3.785	5.092	4.521	4.427	3.308
		p-value	0.00	0.00	0.00	0.00	0.00
		Gn	6	9	7	7	8
	NSB	F-value	1.588	3.538	2.785	3.233	2.972
		p-value	0.04	0.00	0.00	0.00	0.00
		Gn	3	7	4	7	8
	NCB	F-value	1,954	2.246	1,972	2.116	1.654
		p-value	0.00	0.00	0.00	0.00	0.02
		Gn	3	4	4	4	4

Gn: Number of groups formed according to Duncan's test, ns: No significance between trees in the population

The coefficients of variation determined for each age and altitude zone depending on the morphological characters measured in Scotch pine seedlings are given in Figure 2. Accordingly, the highest coefficient of variation generally appeared in the populations in the 3rd altitude zone in 3-year-old seedlings. However, the coefficients of variation in the 2nd altitude zone were higher in 4- and 5-year-old seedlings.

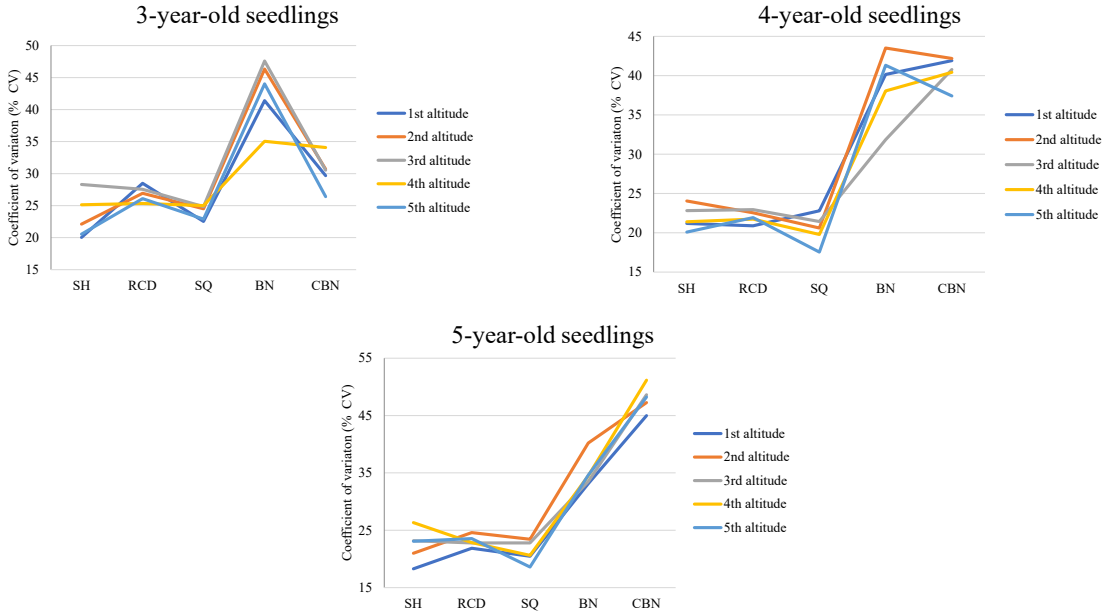

Figure 2. Coefficients of variation (%CV) in three-, four- and five-year-old seedlings
Şekil 2. Üç, dört ve beş yaşındaki fidanlara ait varyasyon katsayıları

The relationships between the SH and the RCD obtained by taking the general average of all populations in Scotch pine seedlings were evaluated separately for each of the three seedling ages. Accordingly, the correlation coefficient between SH and RCD was determined as $R^2 = 0.22$, $R^2 = 0.35$ and $R^2 = 0.37$ for seedlings aged 3, 4, and 5 years, respectively (Figure 3).

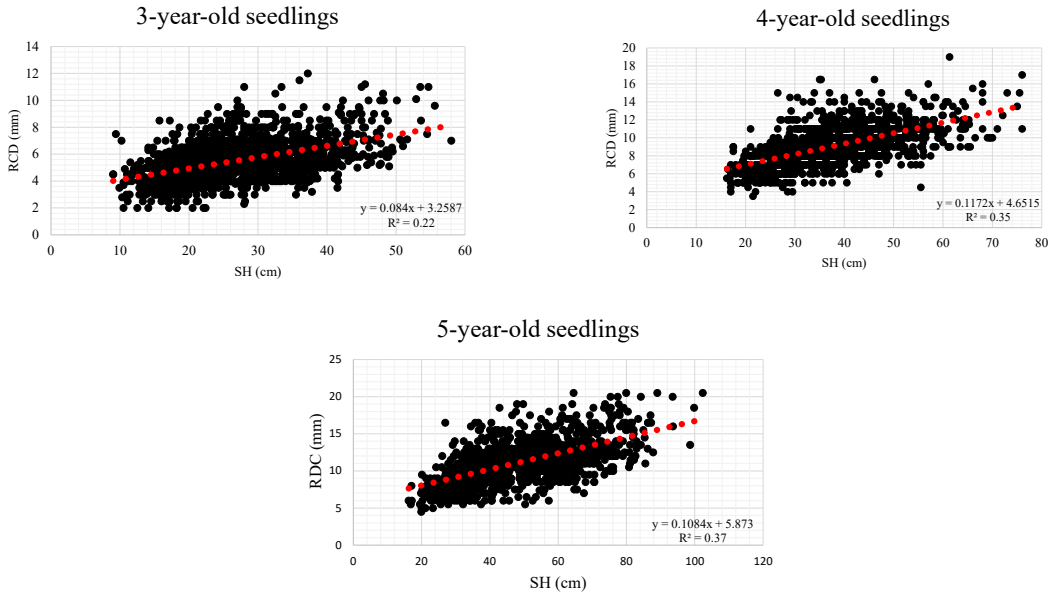

Figure 3. The relationship between RCD-SH in Scotch pine seedlings
Şekil 3. Sarıçam fidanlarında kök boğaz çapı-fidan boyu arasındaki ilişki

4. Discussion and Conclusion

SH, RCD, NSB, and NCB were measured and the SQ was determined in the seedlings, which were examined in three growth periods on the basis of population and tree, depending on the altitude. Considering the average of all populations, it was determined that the average height of the seedlings was 27.1, 37.9, and 47.6 cm in 3-, 4-, and 5-year-old seedlings, respectively. It is seen that the annual height increments of the seedlings are close to each other.

In a study conducted on Scotch pine, Turna and Güney (2009) reported that the average hypocotyl length in the seedling measured 45 days after germination was 1.8 cm, and the average 1-year-old SH was 6.4 cm. Considering the annual growth rate of Scotch pine after the age of three, it can be said that the seedlings of Scotch pine show a rapid growth course at young ages. However, depending on the results of our study, it is understood that the average SH decreased in general due to the increase in altitude on Scotch pine seedlings.

The average RCD were determined as 5.5, 9.1 and 11.1 mm for seedlings aged 3, 4, and 5 years, respectively. In a study carried out on Scotch pine, the average RCD of 1-year-old seedlings was 2.7 mm (Turna and Güney, 2009). Similar to the height of the seedlings, the RCD also tended to decrease due to the increase in altitude. The seedlings obtained from the populations in the 1st (0-450 m) and the 2nd (450-900 m) altitude zones had the highest averages in terms of both SH and RCD. In terms of the NCB, the populations in the 2nd and 1st altitudes, respectively, had the highest values at all three seedling ages. Although there are statistical differences in the NSB, a change parallel to the altitude was not observed.

Morphogenetic characters can vary at the level of origin and clone, as well as within families, and the determination of these variations is important for the success of plantation and artificial regeneration studies as well as the adaptability of the species (Tunçtaner, 2007). In the present study, there were variations depending on statistical analyzes both between the populations and the trees within the populations in all three different age groups of the seedlings grown from five different altitude zones in the distribution area represented from sea level to 2250 m altitude belt. Similarly, there are studies that determine the variations according to the populations in species such as Turkish pine (*Pinus brutia*: Işık, 1986; Işık, 1994; Işık and Kara, 1997), oriental spruce (*Picea orientalis*: Atasoy, 1996; Güney et al., 2019), Kazdagi fir (*Abies nordmanniana* spp. *equi-tojani*: Velioğlu et al., 1999), maritime pine (*Pinus pinaster*: Şimşek et al., 1985), umbrella pine (*Pinus pinea*: Yahyaoğlu et al., 2012), oriental beech (*Fagus orientalis*: Güney et al., 2016), common hornbeam (*Carpinus betulus*: Güney et al., 2015; Atar et al., 2017; Atar, 2021), and oriental hornbeam (*Carpinus orientalis*: Güney et al., 2013; Atar et al., 2014; Atar and Güney, 2021).

In our study, considering the coefficients of variation determined based on morphological characteristics, the highest coefficients of variation were found in the 3rd altitude zone in 3-year-old seedlings and the 2nd altitude zone in 4- and 5-year-old seedlings. When geographic variation is evaluated with respect to altitude, an altitude difference of 1000 meters will often bring more variation than the variation in climate that can occur in a few hundred kilometers of a flat country. As a result of this, such a natural selection occurs that the individuals of the high and the low region are completely different from each other. Studies on Scotch pine in Sweden and ponderosa pine (*Pinus ponderosa*) in California, USA can be given as examples to reveal altitude-related differences. In both tree species, genetic structures change with the changes in altitude levels (Ürgenç, 1982; Şimşek, 1993).

Altitudinal variations have been revealed in some studies carried out on Scotch pine (Turna, 2003; Turna and Güney, 2009; Şevik et al., 2010; Bilgen et al., 2011). Along with these, in Scotch pine, variations have been studied on the needle, pollen, cone, seed, seed wing, the number of cotyledons, seedling characteristics (Eliçin, 1971; Lučić et al., 2012), anatomical seed maturity (Harju et al., 1996), isoenzyme and DNA analysis (Prus-Glowacki and Stephan, 1994, Vidyakin et al., 2015; Tereba et al., 2021; Tikhonova et al., 2021) made by using seed samples.

Scotch pine with a wide vertical distribution showed significant variations at different altitude zones. For this reason, it is important for success to use seedlings to be obtained from altitude zones suitable for the desired characteristics in both breeding and genetic studies. The genetic structures of forest trees contain all kinds of information necessary for them, and it is extremely important to know the genotypic characteristics in tree breeding studies. Forest trees are sometimes affected by natural or artificial environmental changes, and as a result, genotypic reductions occur in the stands formed by these trees. All biological, ecological and technical measures should be taken to protect genetic resources in forestry, and the genetic diversity of forest trees should be transferred to future generations (Yahyaoğlu and Genç, 1990; Yahyaoğlu et al., 1993).

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