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Effects of some nitrogen-fixing plants on seedling growth of scotch pine

Deniz Güney[a], Erhan Seyis[b], Fahrettin Atar[a,*], Ali Bayraktar[a], İbrahim Turna[a]

Abstract: Nitrogen is one of the most important nutrients for plants, and some plants are involved in the conversion of atmospheric nitrogen to organic form. These plants are capable of nitrogen fixing by bacteria in their roots and are important in alleviating nitrogen deficiency and improving soil. The objective of this research is to determine the growth differences in Scotch pine (*Pinus sylvestris* L.) seedlings grown together with some nitrogen-fixing species. The study was conducted in The Research and Application Greenhouse at Faculty of Forestry, Karadeniz Technical University. *Alnus glutinosa, Robinia pseudoacacia* and *Vicia sativa* were selected as nitrogen-fixing species. Seeds were sown, by different sowing combinations, in special sowing crates in November. For 1-year-old scotch pine seedlings after the first growing period, seedling length, root collar diameter and sturdiness quotient were determined. The results indicated that while the average length of *Pinus sylvestris* seedlings sown alone was 3.36 cm, the average length of the seedlings belonging to *Pinus sylvestris+Vicia sativa* combination reached 6.84 cm. Similarly, mean root collar diameter was 0.47 mm greater in the *Pinus sylvestris+Vicia sativa* sowing combination. The use of higher quality seedlings obtained through sowing combination of *Pinus sylvestris+Vicia sativa* can be advantageous in areas where there is a ground cover problem or the use of quality seedlings is required because of extreme conditions in terms of altitude, climate and soil.
Keywords: Seedling morphology, *Pinus sylvestris*, Sowing combination, Nitrogen fixation

Bazı azot bağlayıcı bitkilerin sarıçam fidanlarının gelişimi üzerine etkisi

Özet: Bitkiler için en önemli besin elementlerinden biri azot olup atmosferik azotun organik forma dönüştürülmesinde bazı bitkiler görev almaktadır. Köklerinde bulunan bakteriler vasıtasıyla azot bağlama yeteneğinde olan bu bitkiler azot eksikliğinin hafifletilmesi ve toprağın iyileştirilmesi bakımından önem arz etmektedir. Araştırmada azot bağlayıcı bazı türlerle birlikte yetiştirilen sarıçam (*Pinus sylvestris* L.) fidanlarındaki gelişim farklılıklarının ortaya koyulması amaçlanmıştır. Bu çalışma Karadeniz Teknik Üniversitesi Orman Fakültesi Araştırma ve Uygulama serasında yürütülmüştür. *Alnus glutinosa*, *Robinia pseudoacacia* ve *Vicia sativa* türleri azot bağlayıcı tür olarak seçilmiştir. Tohumlar Kasım ayında özel ekim kasalarına farklı ekim kombinasyonlarıyla ekilmiştir. İlk vejetasyon dönemi sonrasında bir yaşındaki sarıçam fidanlarında, fidan boyu, kök boğaz çapı ve gürbüzlük indisi değerleri belirlenmiştir. *Pinus sylvestris* işlemine ait fidanların ortalama boyu 3.36 cm iken, *Pinus sylvestris+Vicia sativa* ekim kombinasyonuna ait fidanların ortalama boyunun 6.84 cm'ye ulaştığı tespit edilmiştir. Aynı şekilde kök boğaz çapı bakımından da *Pinus sylvestris+Vicia sativa* ekim kombinasyonunda 0.47 mm'lik bir artış meydana geldiği görülmektedir. Özellikle diri örtü probleminin olduğu ya da rakım, iklim ve toprak özellikleri bakımından ekstrem koşullar nedeniyle kaliteli fidanların kullanılması gereken alanlarda *Pinus sylvestris+Vicia sativa* ekim kombinasyonu ile elde edilecek daha kaliteli fidanları kullanmak önemli bir avantaj olabilecektir.
Anahtar kelimeler: Fidan morfolojisi, *Pinus sylvestris*, Kombine ekim, Azot bağlama

1. Introduction

Nitrogen is the main ingredient of amino acids, nucleic acids and other amino compounds and polymers formed in the cells of all living creatures (Tecimen and Sevgi, 2008). Although nitrogen is among the most abundant elements in the world, it is the critical limiting element for growth of most plants because of its unavailability (Smil, 1999; Socolow, 1999; Graham and Vance, 2000). When the proportions of the elements in the structure of the plants are examined, it is seen that nitrogen is found to be lower in ratio than carbon, hydrogen and oxygen (Hasman, 1972; Haynes, 1986). While nitrogen constitutes 1.5-5% of the plant dry weight (Haynes, 1986) and is present in a very low amount in terms of quantity, it is one of the main nutrients in plant life and biochemical events of organic compounds (Haynes, 1986; Gebauer and Schulze, 1997). Nitrogen mineralization in soil in the productivity of ecosystems (Rehder, 1976; Gökçeoğlu and Rehder, 1977; Woodmansee et al., 1978; Woodmansee and Duncan, 1980; Runge, 1983; Vaughn et al., 1986; Güleryüz and Gökçeoğlu, 1994) and nitrate reductase activity in species belong to various taxonomic groups are seen as important indicators (Al Gharbi and Hipkin, 1984; Smimoff and Stewart, 1985; Lee et al., 1986; Gebauer et al., 1988; Widmann et al., 1990; Gebauer and Schulze, 1997; Güleryüz and Arslan, 1999).

Nitrogen constitutes 78% of the atmosphere with its gaseous form and has 15% share in the structure of nucleic acids, proteins and vitamins. Plants cannot use nitrogen in gaseous form. Nitrogen is converted into nitrite by nitrite

[a] Karadeniz Teknik Üniversitesi, Orman Fakültesi, Silvikültür Anabilim Dalı, Trabzon
[b] Çanakkale Onsekiz Mart Üniversitesi, Yenice Meslek Yüksekokulu, Çanakkale
[*] **Corresponding author** (İletişim yazarı): fatar@ktu.edu.tr
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bacteria, and nitrite is converted into nitrate by nitrate bacteria and made usable by plants (İmriz et al., 2014). Symbiotic nitrogen fixation plays an important role in most forest soils. This process occurs as a result of a symbiosis between leguminous plants and bacteria of the genus *Rhizobium* and between *Frankia* bacteria, a genus of *Actinomycet*, and non-leguminous plants (*Alnus*, *Myrica*, *Hippophae*, *Elaeagnus*, *Shepherdia*, *Casuarina*, *Coriaria* and *Ceanothus*) (Tilki, 2002; Pritchet and Fisher, 1987; Burris, 1988; Wheeler, 1991).

Generally, 100 to 400 kg of N/ha/year can be supplied by biological nitrogen fixation to the forest ecosystem. Nitrogen is fixed in soil with free-living bacteria (<15 kg N/ha/year), *Cyanobacteria* (7-80 kg N/ha/year), symbiotic life of *Frankia* bacteria and non-leguminous species (2-362 kg N/ha/year), and *Rhizobium* bacteria and leguminous species (24-584 kg N/ha/year) (Elkan, 1992; FAO, 1993). Some plants that are capable of nitrogen fixation through the bacteria in their roots are used for the alleviation of nitrogen deficiency and soil improvement in forestry in some countries, especially in European countries, Australia and the USA. Plants with nitrogen-fixing bacteria help improve poor soils and enrich plant nutrients (Diagne et al., 2013; Stokdyk and Herrman, 2014). These plants also contribute to the development of other species in the afforestation studies by providing nitrogen support (Miller and Murray, 1978; Voigtlaender et al., 2012; Mortimer et al., 2015).

In some studies about nitrogen fixation, it were determined that 10.6 kg/ha/year fixation in *Myrica gale* (Permar and Fisher, 1983) and 62 kg/ha/year fixation in *Alnus rubra* (Tripp et al., 1979). With reference to Pritchet and Fisher (1987) and FAO (1993) in the study of the amount of nitrogen fixation in relation to some species, Tilki (2002) stated that *Alnus glutinosa*, *Casuarina equisetifolia*, *Elaeagnus* sp., *Robinia pseudoacacia*, *Hippophae* sp. and *Wax myrtle* fix nitrogen as <56, 60-200, <15, 100-200, 2-180 and <132 kg/ha/year, respectively. In the mixed grown plantations with *Alnus rubra* in previous studies conducted by Miller and Murray (1978) and Debell and Radwan (1979), they investigated the increments of *Pseudotsuga menziesii* (Mirb.) Franco and *Populus trichocarpa* Torr. Additionally, biomass developments in *Pinus tunbergii* Parl. (Moffat, 2000), poplar (Van der Meiden, 1961) and *Pseudotsuga menziesii* plantation (Binkley et al., 1984) grown together with alder were researched. Moreover, Ashby and Baker (1968), Turvey and Smethurst (1983) studied the effects of *Robinia pseudoacacia* on the development of its mixed plantations with coniferous and deciduous species.

Symbiotically fixing nitrogen can be transformed into available form for plants, thus helping reduce nitrogen fertilizer costs and prevent environmental pollution during the use of chemical fertilizers. The aim of this study was to determine the growth differences in *Pinus sylvestris* L. seedlings grown together with some nitrogen-fixing species.

2. Material and methods

2.1. Study area

The present study was carried out in The Research and Application Greenhouse at Faculty of Forestry, Karadeniz Technical University. *Alnus glutinosa* (A.glu), *Robinia pseudoacacia* (R.pse) and *Vicia sativa* (V.sat) were selected as nitrogen-fixing species. Seeds of the species used in the study were obtained from Trabzon region (Figure 1), and sown in open field conditions. Additionally, the climate data between 1927 and 2018 belonging to Trabzon province, where Karadeniz Technical University is located, are given in Table 1. According to the long-term climate data of the area where the open field nursery is located, the annual average temperature is 14.7 ℃ and the total annual precipitation is 820.7 mm.

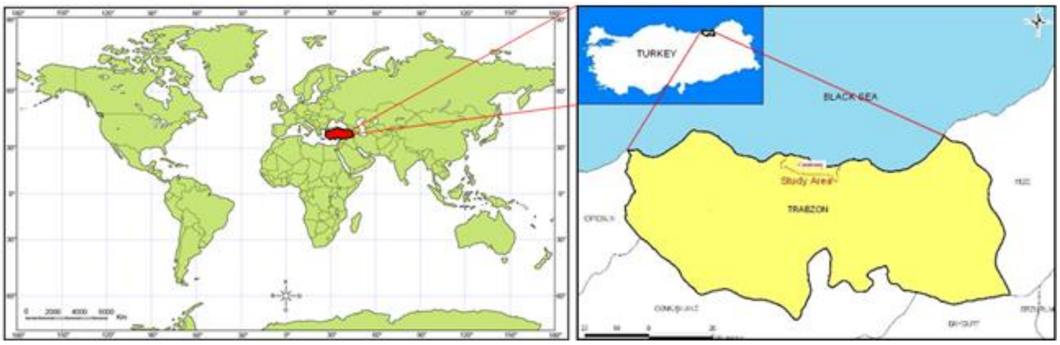

Figure 1. Geographical location of study area in Karadeniz Technical University

Table 1. The average meteorological values of the study area

	Climate Period (1927-2018)											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	7.3	7.3	8.4	11.7	16.0	20.3	23.1	23.5	20.3	16.6	12.8	9.5
2	10.7	10.8	11.9	15.5	19.1	23.1	25.9	26.5	23.7	20.0	16.5	12.9
3	4.6	4.3	5.4	8.7	12.9	17.0	19.9	20.4	17.3	13.6	10.0	6.7
4	2.7	3.3	3.4	4.4	5.5	7.1	5.9	5.6	4.9	4.5	3.7	2.7
5	12.5	12.4	13.3	12.9	12.8	10.8	8.0	8.8	11.2	12.6	12.1	12.7
6	82.4	63.7	58.7	56.8	51.8	50.1	35.4	44.9	79.5	115.0	98.9	83.5

1. Avg. Temperature (°C); 2. Avg. Highest Temperature (°C); 3. Avg. Lowest Temperature (°C); 4. Avg. Sunshine Time (hour); 5. Avg. Number of Rainy Days; 6. Monthly Total Precipitation Avg. (mm)

2.2. Experimental design and nursery treatment

Sowing of seeds was carried out in open field conditions of the research area. For the seeds sown in November, completely randomized design was used. Special sowing crates were prepared in order to eliminate soil effects in the external environment in the process of sowing of seeds and to determine the effects of nitrogen-fixing species on the growth of scotch pine seedlings. These were prepared as 1.2x0.6 m sizes and 30 cm deep. Control (only sowing of scotch pine) and four different sowing combinations were conducted as three repetitions in sowing crates. As sowing medium, the coarse gravel was laid on the bottom of the crates where the seeds were sown and then fine grained gravel was laid on top of it. Additionally, the unsieved forest soil was cleaned from foreign materials and laid on the fine gravel and finally, covered with sieved forest soil and made ready for sowing. Sowing in row of seeds were conducted by opening the lines. The schematic representation of the trial design used for sowing seeds is given in Figure 2.

In this study, a total of 2100 seeds were sown to seedbed using 300 seeds for each species in each treatment. As specified in the trial design in Figure 2, 300 seeds for P.syl, 600 seeds for P.syl+A.glu, 600 seeds for P.syl+R.pse and 600 seeds for P.syl+V.sat were used.

For 1-year-old seedlings grown in the crates after first vegetation period, seedling length (SdL), root collar diameter (RCD) and sturdiness quotient (SQ) were determined. 1350 seedlings (3×50 seedlings from each treatment) were used for measurements (Figure 3). The sturdiness quotient attributes to the ratio of the length of the seedling to the root collar diameter and expresses the vigour and robustness of the seedling (Thompson, 1985; Aldhous, 1994; Jaenicke, 1999).

2.3. Data analysis

Data were analysed using the SPSS 23.0 statistical program. The analyses included ANOVA and Bonferroni Test. One-way analysis of variance is used to test the equality of k independent groups averages taken from k population showing the normal distribution (Ercan, 1997; Özdamar, 1999; Özkan, 2003). In addition, the statistical significance of the differences between the results of different sowing combinations with the Bonferroni test was demonstrated.

3. Result and discussion

The results of the analysis of variance, mean and standard deviation related to sowing combinations are given in Table 2. According to the results of the analysis of variance, there were statistically significant differences ($P<0.01$) among the sowing combinations in terms of all measured morphological characters. The average seedling length for P.syl was determined as 3.36 cm, while the average values for, P.syl+A.glu, P.syl+V.vat and P.syl+R.pse occurred as 6.25 cm, 6.84 cm and 5.53 cm respectively. On the other hand, while the average root collar diameter for P.syl seedlings was 1.29 mm, the average values for P.syl+A.glu, P.syl+V.vat and P.syl+R.pse were determined as 1.46 mm, 1.76 mm and 1.15 mm respectively. The sturdiness quotient, was 2.67 for P.syl, 4.38 for P.syl+A.glu, 3.98 for P.syl+V.vat and 4.91 for P.syl+R.pse. The differences between SdL, RCD and SQ values depending on the sowing combinations are shown in Figure 4.

Pinus sylvestris seedlings obtained by sowing combinations had high growth than the seedlings acquired by control seeds, especially in terms of seedling length. Compared to seedlings in control, more seedling length values were acquired to be 103.6% for seedlings obtained from P.syl+V.vat sowing combination, 86.0% for P.syl+A.glu and 64.6% for P.syl+R.pse. RCD occurred as lower for the seedlings from P.syl+R.pse sowing combinations compared to P.syl seedlings in control, while it was higher for the seedlings from P.syl+A.glu and P.syl+V.vat sowing combinations. Also, it was determined that the seedlings from all mixed sowing combinations produced a higher ratio than the seedlings in control in terms of sturdiness quotient (Table 2).

The significance of differences between sowing combinations related to measured morphological characteristics was determined by Bonferroni test and the results are shown in Table 3.

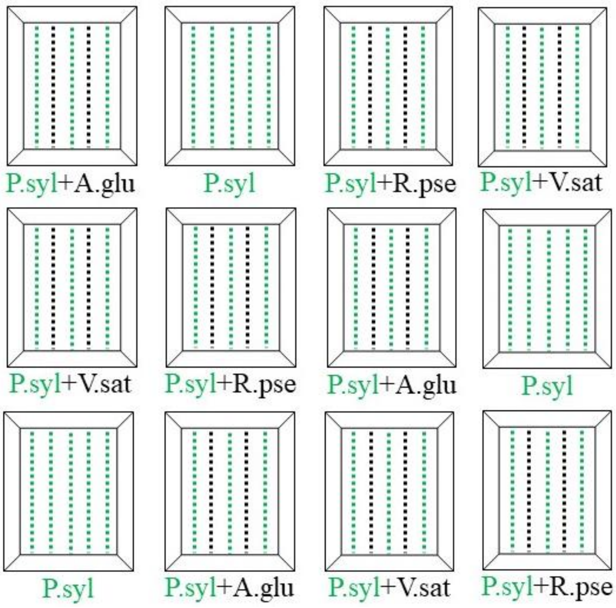

Figure 2. Trial design related to sowing combinations

Table 2. ANOVA results related to sowing combinations

	SdL (cm)	RCD (mm)	SQ
P.syl	3.36 ± 0.86	1.29 ± 0.31	2.67 ± 0.72
P.syl+A.glu	6.25 ± 1.32	1.46 ± 0.28	4.38 ± 1.01
P.syl+V.vat	6.84 ± 1.56	1.76 ± 0.45	3.98 ± 0.86
P.syl+R.pse	5.53 ± 1.40	1.15 ± 0.29	4.91 ± 1.25
F	51.471	48.770	38.713
P	0.000	0.000	0.000

Figure 3. Different sowing combinations and morphological measurements of seedlings

Table 3. The results of Bonferroni test with respect to sowing treatments

Tree species		Mean difference		
		SdL (cm)	RCD (mm)	SQ
P.syl	P.syl+A.glu	-2.89*	-0.17	-1.71*
	P.syl+V.vat	-3.48*	-0.47*	-1.31*
	P.syl+R.pse	-2.17*	0.14	-2.23*
P.syl+A.glu	P.syl	2.89*	0.17	1.71*
	P.syl+V.vat	-0.58	-0.30*	0.40
	P.syl+R.pse	0.72*	0.31*	-0.53*
P.syl+V.vat	P.syl	3.48*	0.47*	1.31*
	P.syl+A.glu	0.58	0.30*	-0.40
	P.syl+R.pse	1.31*	0.61*	-0.93*
P.syl+R.pse	P.syl	2.17*	-0.14	2.23*
	P.syl+A.glu	-0.72*	-0.31*	0.53*
	P.syl+V.vat	-1.31*	-0.61*	0.93*

*P<0.05 (There is a statistically significant difference.)

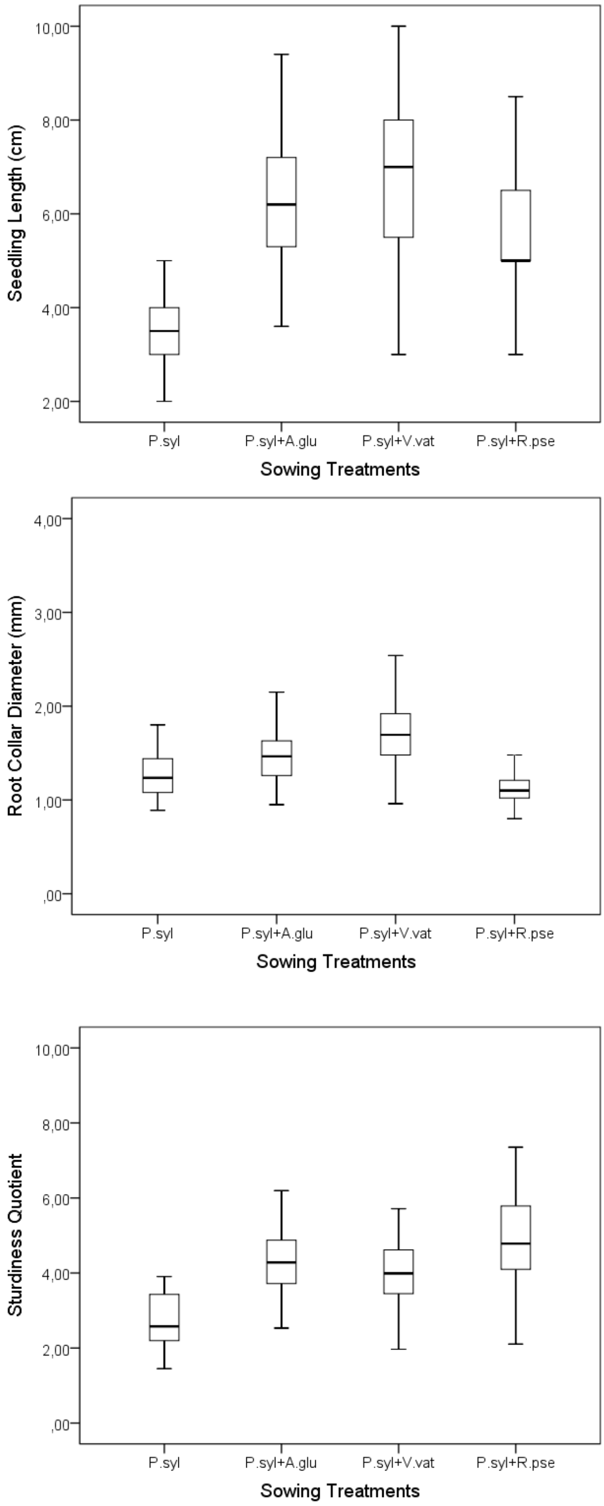

Figure 4. The results of SdL, RCD and SQ according to sowing combinations

The maximum length difference (3.48 cm) in terms of seedling length occurred between P.syl and P.syl+V.vat sowing combinations. While especially the average length of the seedlings belonging to P.syl is 3.36 cm, it is noteworthy that the average length of the seedlings belonging to P.syl+V.vat sowing combination reaches 6.84 cm. Similarly, an increase of 0.47 mm was observed in the P.syl+V.vat sowing combination in terms of the root collar diameter. For the P.syl+A.glu sowing combination, it was found that the average seedling length was 2.89 cm, the average root collar diameter was 0.17 mm more than the values of P.syl. In the previous studies, it was determined that the better increments occurred as 59% for *Pseudotsuga menziesii* (Mirb.) Franco (Miller and Murray, 1978), as 50% for *Populus trichocarpa* Torr. and Gray (DeBell and Radwan, 1979) in mixed grown plantations with *Alnus rubra* Bong. compared to the purely grown plantations. In other studies, that were investigated the effects of alder on the species grown together with it thanks to nitrogen-fixing ability, it was reported that biomass development increased in *Pinus tunbergii* Parl. (Moffat, 2000), poplar plantation (Van der Meiden, 1961) and *Pseudotsuga menziesii* plantation (Binkley et al., 1984). Instead of pure plantation of *Populus trichocarpa*, as a result of grown together of *P. trichocarpa* and *Alnus rubra* species were accomplished more yield (Tarrant, 1983; Binkley et al., 1984). As a result of growing together with *Pseudutsuga menziesii* of *Alnus rubra*, together with coniferous and deciduous species of *Alnus glutinosa*, it is stated that the amount of nitrogen in the soil and the growth rate in parallel increase (Permar and Fisher, 1983; Turvey and Smethurst, 1983; Wheeler and Miller, 1990). In a study conducted by Miller and Murray in 1978, *Alnus rubra* was planted to 4-year-old *Pseudotsuga menziesii* plantation which tree growth is limited due to insufficient available nitrogen. As a result of the study, the total volume of the mixed plantation was nearly twice compared to the pure *Pseudotsuga menziesii* plantation. However, there are also studies indicating that the naturally grown *Alnus rubra* adversely affect the individuals in plantation area depending on the intensity of the species and competition occurring in the area (Radosevich et al., 2006). In another study, it was reported that both alder percentage and distance between species were effective in height growth for poplars in short-rotated cultures where alder-poplar mixture is dense. It was determined by regression analysis that there was a significant increase in the poplar height of 3 years with the increase of alder in the mixture and decrease in the height with the increase in the planting distance between poplar and alder (Hansen and Dawson, 1982).

The seedlings belonging to P.syl+R.pse sowing combinations had more height increments with 2.17 cm, compared to the seedlings obtained from P.syl in this study. *Robinia pseudoacacia* is used to stabilize nitrogen-poor areas, eroded lands and coal residues in Europe (Sprent and Sprent, 1990). Similarly, *Acacia cyanophylla* has been used in Greece for sand dune stabilization, *Acacia holoserica* has been used in Australia for the improvement of coal residual areas (Nakos, 1977; Langkamp et al., 1979). Ashby and Baker (1968), Turvey and Smethurst (1983) reported that *Robinia pseudoacacia* has a positive effect on the development of its mixed plantations with coniferous and deciduous species, it also increases especially height growth of *Juglans nigra*, *Liriodendron tulipifera*, *Acer saccharinum*, *Quercus rubra* and *Maclura pomifera*. However, considering the fact that *R. pseudoacacia* is a fast growing species, it was stated that it would be beneficial to bring under the coniferous species in order not to adversely affect the coniferous species in which mixed planting is done, or to grow them together with fast growing deciduous species (Turvey and Smethurst, 1983). In another study used of *Alnus glutinosa* and *Robinia pseudoacacia* as nitrogen fixing species in order to investigate growth performance of 14-year-old *Juglans nigra* plantation, it was found that both the amount of soil nitrogen concentration and, the diameter and height of *Juglans nigra* increased (Friedrich and Dawson, 1984).

From this point of view, it can be a significant advantage to use quality seedlings to be obtained with sowing combination of *Pinus sylvestris* and *Vicia sativa* in extreme areas, especially where there is a ground cover problem or extreme ecological conditions. For the further researches, it can be suggested to grow *Pinus sylvestris* plantations with annual nitrogen-fixing plants such as *Vicia sativa* in areas where ecological conditions are difficult.

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