

**INTER POPULATION VARIABILITY OF FROST-RESISTANCE
INPROVENANCES OF SCOT PINES (*Pinus sylvestris* L. R. *hamata* Steven)
IN TURKEY**

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Frost-resistance variability of Scotch pine (*Pinus sylvestris* L. var. *hamata* Steven) seedlings grown in nurseries conditions, originated from 10 provenances, have been analyzed. The provenances from Black Sea region, Central Anatolian region and Eastern Anatolian region in Turkey have been used in selection of seed zones. The results of frost-resistance tests indicated a strong relationship of implemented freezing degrees with injury degrees of Scotch pine needles and photosynthetic productivities. On the other hand, another significant relationship has been determined between chlorophyll fluorescence and ion leakage methods ($r=-0.801$). This result shows that those two methods can be safely used in determining the damages due to low temperatures. In frost resistance tests, Scotch pine seedlings from different provenances have been frozen at -10, -20, -30 and -40 °C. According to the Duncan test results, it has been determined that damage increased as temperature decreased. The damage level at -10°C implementation is 3.5% which can be tolerated by plants. But when the temperature has been decreased to -20°C, the level of damage has increased to 51.25%. As a result of photosynthetic analyses in this phase, it has been determined that there is a statistically significant relationship between provenances and temperature levels. Under the light of those findings, they have determined that the photosynthetic productivity has significantly decreased at temperatures between -20°C and -40°C. This situation conforms to injury index

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values determined in this study. As a result of injury index and photosynthetic productivity tests used for determining the damage after frost-resistance tests, it has been determined that the provenances of Amasya-Kunduz, Bolu-Aladağ, Düzce-Yığılca, Samsun-Vezirköprü and Eskişehir-Çatacık are more sensitive to frost than other provenances.

Keywords: Scotch pine, provenances, frost damage, injury index, photosynthetic activity.

INTRODUCTION

According to SCALTSOYANNES *et al.* (1994), pines are characterized by high total variation due to intra-population genetic variability, which points to a significant genetic differentiation in local populations and to the possibility that the same alleles are distributed throughout the entire range of the species. Thanks to the successful tree growth and development and the survival of Scots pine (*Pinus sylvestris* L) populations on the lands characterized by unfavorable soil conditions (shallow, skeletal soils of low fertility) and climate conditions (deficit and unfavorable precipitation regime over the growing season), LUČIĆ *et al.*, (2011a, b), GIERTYCH *et al.*, (1992), this specie in Turkey is used in reclamation and erosion-control afforestation. The fact this pine thrive over large areas affected by erosion processes, or on completely degraded and denuded areas on which they produce excellent results, makes it economically the most important specie used in forestry. All the above qualities, in addition to a vast natural range of distribution and a disjunctive range, led to their intensive introduction also to the sites outside their natural range. This resulted in a high number of subspecies, varieties and transitive forms. The result of this natural variation is a high genetic potential which produces the basis and the potential for successful breeding.

In Turkey, Scotch pine's natural populations are on the eastern border of the natural distribution of this species in Europe. This is the reason why gene pool and genecological characteristic of their populations differ comparing to the populations in another parts of Europe. Scotch pine forests are occurred objects rehabilitation and restoration practices which are the high altitudes and sub-alpine in places exposed to anthropogenic impact.

The main objective in the some European projects were to link physiological features of seedlings cold tolerance and bud dormancy in Scots pine and common beech seedlings with vitality during and after cold and frozen storage, and to correlate this with gene expression by utilizing genomics technology (JOONSEN *et al.*, 2006). In order to gain a better understanding of cold tolerance development plants in nurseries, a comprehensive study was performed to correlate physiological and ecological responses in Scots pine (*Pinus sylvestris* L.) seedlings.

Especially in the distribution area in Turkey as the most important type of Scotch pine forests that make up the acceptable limit. However, because of some reasons such as their hydraulic effects, their roles in fires, and providing nutrient and living place to animals, pines have important biochemical effects on ecosystem (RICHARDSON and RUNDEL, 1998).

Pines have regional or global-scaled effects on climate. For example, the forests in northern hemisphere prevent the reflection of sunlight from snow cover below them. Through this prevention, a warmer winter can be provided in forest lands than in treeless lands (BONAN *et al.*, 1992). Pines are the most important tree species in world from the ecological and economical aspects, and for this reason, they have wide distribution around the world, and have been taken into cultivation out of their natural distribution regions.

Having wide geographical distribution on the world, the Scotch pine has the 5th widest distribution range in Turkey with its distribution over 1.5 million ha (ANONYMOUS, 2012). Scotch pine is generally distributed over high zones of Black Sea and Eastern Anatolian regions and in mountains' inner shoulders (YALTIRIK, 1993; ATA, 1995; GENÇ, 2004). Because of distribution of Scotch pine through those regions, they are exposed to hot and drought climate in summers and cold and rainy climate in winters. That's why; natural Scotch pine stands are exposed to severe frost damages. Significant losses are seen in natural regeneration efforts in seed germination and sapling growth periods due to frost. The Scotch pine foresting region is the 5th widest region in Turkey in terms of surface area. As of the year of 2013, totally 658.000 ha of Scots pine foresting effort has been executed in the country (ANONYMOUS, 2012). Because of its valuable wood, it has wide usage area, and is affected from frost and drought in foresting efforts in different regions negatively. In order to improve the success of foresting in cold regions, it is one of the most important criteria to choose tree species and origins which are the most frost-resistant (SAATÇIOĞLU, 1976; ÜRGENÇ, 1998).

The global warming and consequently the climate change are the most important environmental problems threatening the lives. That's why; the increase and severity of sudden and extreme weather events such as floods and low and high temperatures should be taken into account. For this purpose, the utilizing and choosing the species and origins resistant to stresses such as frost, drought and saltiness will be more important in future. The execution of Scotch pine forestation in ecosystems of Turkey such as Eastern Anatolian region and artificial timberline areas in Black Sea region and Central Anatolian where the severe winter and frost events are seen makes the selection and utilization of appropriate species and origins more important. That's why; the comparison of frost-resistance levels of the origins taken from natural distribution region of Scotch pine will make their utilization in artificial regeneration efforts in dense-frost regions easier. The main tasks of these investigations are to analyzed genetics and ecological base of the frost-resistance Scotch pine seedlings, originated from this part of Turkey. Results of such analyzes are of especially important in future target seedlings production for afforestation in high mountain region.

MATERIALS AND METHODS

Material

Frost-resistance variability of Scotch pine seedlings grown in nurseries conditions, originated from 10 provenances, have been analyzed. Seedlings were produced using the seed from selected seed zones, located in provenances - from Black Sea region, Central Anatolian region and Eastern Anatolian region. The physiographic and climatic data of the seed zones are presented in Table 1.

The seeds used in experiments have been obtained from different seed stands. In May 2014, the seeds have been sowed into containers in greenhouse at Forest State Nursery in Erzurum, Turkey. At the beginning of the study, 8 seeds have been sowed into each of containers. After the seeds germinated, only one sapling has been left in each of containers by removing the rest of them.

Table 1. The physiographic and climatic data of provenances

Origin	Climate type	Altitude (m)	Exposure	Annual mean precipitation (mm)	Mean temperature (°C)	Minimum temperature (°C)
Amasya-Kunduz	Black Sea	1100	NW	456.7	15.2	-21.3
Bolu-Aladağ	Black Sea	1370	NE	628.3	6.5	-25.6
Düzce-Yığılca	Black Sea	1300	NW	610.9	7.2	-22.7
Eskişehir-Çatacık	Marmara Temporary	850	N-NW	409.6	11.4	-18.4
Erzincan-Refahiye	Rigid Continental	1300	NE	385.2	8.6	-34.6
Erzurum-Oltu	Rigid Continental	1470	N-NW	410.8	4.3	-35.6
Erzurum-Olur	Rigid Continental	1450	NE	415.6	5.8	-23.4
Samsun-Vezirköprü	Black Sea	640	N	530.8	12.3	-8.9
Kastamonu-Daday	Black Sea	1380	N	622.5	14.5	-19.4
Karabük-Eskipazar	Continental Temporary	1150	N-NW	571.7	18.7	-21.3

Method

The methods used in frost-resistance test

The saplings of 10 provenances to be used in winter experiments have been placed into climate chamber for cold climate implementation, and kept there at +5°C for 90 days. In order to replicate the shortening sunbathing environment of winter in climate room, the 120 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ fluorescence lamps have been utilized. The enlightening has been executed in “8 hours day and 16 hours night” form like other studies (RYYPO *et al.*, 1997). The relative moisture has been kept between 75 and 80%. The soils of saplings have been watered in the way providing field conditions. The 3M Climacell air conditioner has been utilized for these processes.

It has been determined that the temperature levels to be used in winter frost treatments should be -10, -20, -30 and -40°C as in previous studies (SEMERCİ *et al.*, 2008). 90 saplings have been taken into control group by keeping them unfrozen. Other saplings have been transferred to cooling rooms, and the temperature has been decreased 5°C each hour, and set to various temperature levels. The saplings in low temperature levels have been waited for approximately 18 hours, and the frozen saplings have been slowly heated 5°C each hour in order to increase their temperature to room conditions. The ion loss and chlorophyll measurements have been executed on saplings.

Determining the Damage Due to Frost Event

One of the methods used for determining the damage after frost-resistance experiments is “ion loss (leakage)” method (TINUS, 2002). The relative conductivity of control groups ($RC_{Control}$) and frozen groups (RC_{Frozen}) have been measured and calculated in leaf sections via conductivity meter. The “Relative Conductivity” values have been used in determining the damage. According to the injury index which SEMERCI *et al.* (2008) suggested, the level of injury has been calculated in percentage. The injury index (I_t) is represented below. in this method, higher injury index values indicate more injuries, while lower values indicate less damage (SEMERCI *et al.*, 2008; KACAR *et al.*, 2010; ATALAY and EFE, 2012).

$$RC_{Control} = (\text{First Reading/Last Reading}) \times 100$$

$$RC_{Frozen} = (\text{First Reading/Last Reading}) \times 100$$

$$I_t = (RC_{Frozen} - RC_{Control}) / (1 - (RC_{Control}/100))$$

The second method used in determining the frost damage is “chlorophyll a fluorescence” measurement. In order to determine the damages due to frost, the saplings treated for adaptation to cold and the saplings frozen in frost-resistance tests have been kept at room temperature conditions for 7 hours under $560 \mu\text{mol m}^{-2}\text{s}^{-1}$ light. After this process, the needles have been kept at darkness for 2 hours. The fluorescence measurements have been performed with J-815 model fluorescence measurement device in Plant Physiology Laboratory of Berlin Technical University.

The photochemical productivity (F_v/F_m) has been calculated according to the formula stated below. The decrease in F_v/F_m rate is a good indicator or decrease in photosynthetic activity. Being approximately 0.832 in healthy plants, indicates an environmental stress when decreases below 0.8 (SEMERCI *et al.*, 2008; KACAR *et al.*, 2010).

$$F_v/F_m = [(F_m - F_0) / F_m]$$

Statistical Analyses

Factorial Experiment Pattern has been used in winter and spring frost experiments. Using the saplings of 10 origins, the experiments have been repeated 5 times containing 5 saplings each treatment. According to that; 1000 saplings (10 provenances x 4 temperature levels x 5 repeated x 5 saplings = 1000) have been exposed to low temperature in each season. The effects of resistance difference and temperature levels on origins have been determined through variance analyses (ANOVA) among the origins. The relationships between measured variables have been evaluated through Pearson correlation analysis.

RESULTS

Saplings of 10 provenances have been frozen at -10, -20, -30 and -40°C. The correlations between damage level in needles of saplings with ion loss (I_t) and photochemical activity (F_v/F_m) at those temperatures are given in Table 2. According to the correlation analysis performed, there is a significant-negative relationship between low temperature and injury index ($p < 0.01$) (Table 2).

Table 2. The result of correlation analysis between Temperature and F_v/F_m and I_t

Variables	Temperature (°C)	F_v/F_m	I_t
Temperature (°C)	1.000	0.940**	-0.834**
F_v/F_m		1.000	-0.810**
I_t			1.000

** : significant at $p < 0.01$ level

As a result of performed variance analysis (Table 3), it has been determined that the temperature levels implemented ($p<0.001$) and origins ($p<0.01$) have statistically significant effects on needle injury index.

Table 3. The variance analysis result of injury index (I_i) of saplings of 10 origins at different freezing degrees

Variation Source	Freedom Level	Sum of Squares	Average of Squares	F	P
Temperature	3	188562.05	67356.4	246.3	$P<0.001$
Origin	9	5463.6	518.4	2.35	$P<0.001$
Failure	127	2145.4	115.6		

According to the results of Duncan test, it has been determined that the level of injury increased as temperature decreased. The level of injury as a result of implementation of -10°C temperature is 3.5%, and this level can be tolerated by plants. But it has been determined that the level of injury increased to 51.25% when the temperature decreased to -20°C . The injury level of needles was calculated more than 50% indicates that there is a significant damage on needle tissue and they cannot execute their physiologic activities.

Comparing the mean injury values in Table 4, it has been determined that the most effected origins are Amasya-Kunduz, Bolu-Aladağ, Düzce-Yığılca, Eskişehir-Çatacık and Samsun-Vezirköprü origins. From the aspect of temperature levels, 4 groups have been created according to injury index. For this reason, the lowest damage occurs at -10°C , while the highest damage occurs at -40°C . At -20 and -30°C temperature levels, no statistically significant difference could be determined between various Scotch pine origins' needles in terms of damage (Table 4).

Table 4. Injury index (I_i) values of Scotch pine provenances at different freezing degree levels

Origin	Temperature Levels ($^{\circ}\text{C}$)				Mean
	-10	-20	-30	-40	
Amasya-Kunduz	4.45	68.56	73.75	93.68	60.11a
Bolu-Aladağ	2.78	63.24	71.43	90.18	56.92a
Düzce-Yığılca	2.44	61.28	59.37	85.34	52.10ab
Eskişehir-Çatacık	2.38	58.17	57.21	81.36	49.80ab
Samsun-Vezirköprü	2.21	47.86	53.14	75.33	44.64ab
Kastamonu-Daday	1.85	42.39	50.28	71.47	41.50b
Karabük-Eskipazar	1.83	41.23	48.75	65.69	39.38bc
Erzincan-Refahiye	1.81	40.34	42.67	61.25	36.52c
Erzurum-Oltu	1.35	38.21	40.54	57.13	34.31cd
Erzurum-Olur	1.23	35.47	36.52	48.22	30.40d
Mean	2.23A	49.68B	53.37B	73.00C	44.60

The variance analysis (Table 5) performed for investigating the effects of low temperature and origin factors on F_v/F_m indicated that the temperature and origin factors are effective on photosynthetic productivity ($p<0.01$).

Table 5. The results of variance analysis on photosynthetic productivity (F_v/F_m) of saplings of 10 provenances at freezing degrees

Variation Source	Freedom Level	Sum of Squares	Average of Squares	F	P
Temperature	3	25.9845	7.3452	228.14	$P<0.001$
Origin	9	2.2364	1.2123	5.37	$P<0.001$
Failure	127	4.6589	0.0214		

The photosynthetic productivity (F_v/F_m) decreased as the severity of frost increases. According to the results of variance analysis, no statistically significant difference has been determined among provenances between -10°C and -20°C temperature levels in terms of photosynthetic productivity. But after the level of -30°C , the statistically significant differences among provenances in terms of photosynthetic productivity occurred. According to the Duncan test results, it has been determined that photosynthesis activity was about to end in Amasya-Kunduz, Bolu-Aladağ, Düzce-Yığılca and Samsun-Vezirköprü Scotch pine provenances. The photosynthetic activity has significantly decreased in Eskişehir-Çatacık, Kastamonu-Daday and Karabük-Eskipazar provenances, and the Erzincan-Refahiye, Erzurum-Oltu and Erzurum-Olur provenances are more resistant than other provenances in terms of photosynthetic activity (Table 6).

Table 6. Photosynthetic productivity (F_v/F_m) values of Scotch pine origins at different freezing degree levels

Origins	Temperature Levels ($^{\circ}\text{C}$)				Mean
	-10	-20	-30	-40	
Amasya-Kunduz	0.812	0.563	0.459	0.325	0.539a
Bolu-Aladağ	0.842	0.568	0.462	0.332	0.551a
Düzce-Yığılca	0.856	0.615	0.467	0.337	0.569a
Samsun-Vezirköprü	0.873	0.623	0.471	0.345	0.578a
Eskişehir-Çatacık	0.876	0.674	0.475	0.358	0.596ab
Kastamonu-Daday	0.881	0.773	0.483	0.361	0.625b
Karabük-Eskipazar	0.887	0.778	0.488	0.378	0.633b
Erzincan-Refahiye	0.892	0.814	0.492	0.384	0.646bc
Erzurum-Oltu	0.895	0.821	0.496	0.392	0.651c
Erzurum-Olur	0.897	0.828	0.510	0.395	0.658c
Mean	0.871A	0.706B	0.480C	0.361D	0.605

DISCUSSION

The characteristic of the Scotch pine's edge and marginal distribution is a distribution area in Turkey. Especially in the distribution area in Turkey as the most important type of Scotch pine forests that make up the acceptable limit. However, Scotch pine forests are occurred objects rehabilitation and restoration practices which are the high altitudes and sub-alpine in places exposed to anthropogenic impact. In this study; the resistant to low temperatures Scotch pine populations were determined for many years in high places in the reforestation and restoration of mountain and subalpine degraded Scotch pine forest. In this context, the results of frost-resistance tests indicated that there is a significant relationship between freezing degrees and injury index

values of Scotch pine needles and their photosynthetic productivities (Table 2). On the other hand, another significant relationship has been determined between chlorophyll fluorescence and ion leakage methods ($r = -0.801$). This result indicates that those two methods are reliable methods for determining the damages of low temperatures (LINDGREN and HALLGREN, 1993; SEMERCI *et al.*, 2008). Hence, other studies on this topic through various forest tree species indicated that those two parameters can be reliably used in determining the early and post-frost damages (KACAR *et al.*, 2010; BINDER and FIELDER, 1996; KANDEMİR, 2002; PAVICIC *et al.*, 2004).

In frost-resistance tests, Scotch pine saplings from various provenances have been frozen at -10, -20, -30 and -40°C. According to the Duncan test results, it has been found that injury level increased as temperature decreased. The damage level was calculated based injury index values at -10°C implementation is 3.5% which might be tolerated by plants. But when the temperature has been decreased to -20°C, the level of damage has increased to 51.25% (Table 3). This result indicates that the level of Scotch pine injury increases as duration and severity of frost stress increase. In a study conducted on various provenances of European beech which is sensitive to outdoor conditions, it has been stated that the duration and severity of frost significantly decrease especially the photosynthetic productivity (GOMORY *et al.*, 2011). Also, the 5.25% injury ratio was calculated by used injury index values at -20°C in Scotch pine saplings is in harmony with results obtained by LINDGREN and HALLGREN (1993). According to that, it has been determined that the significant frost damage occurs and almost half of population died is -20°C.

In this research, it has been determined as a result of photosynthetic investigations that there is a statistically significant relationship between origins and temperature (Table 6). According to that, it has been revealed that photosynthetic productivity significantly decreased between -20°C and -40°C. This situation closely corresponds with injury index values obtained in study (Table 4). In this topic, it has been determined that our results corresponds with those obtained in a study which has been conducted in Catalan region of Spain on relationship of photosynthetic productivity and injury index values of Scotch pine origins. Increases have been observed after -30°C in especially needles and summer woods, and on the other hand, it has been determined that photosynthetic productivity has decreased by 50.23% (MARTIN *et al.*, 2010).

As a result of injury index and photosynthetic productivity tests used for determining the damage after frost-resistance tests, it has been determined that the provenances of Amasya-Kunduz, Bolu-Aladağ, Düzce-Yığılca, Samsun-Vezirköprü and Eskişehir-Çatacık are more sensitive to frost than other origins (Table 4, 6). This situation indicates that, despite low temperature values are observed in their natural distribution regions in Amasya, Bolu, Düzce, Samsun and Eskişehir, those regions profit from moderating effect of the sea more than Erzurum where the study has been carried out. Because it has been determined in this study that the local provenances of Erzurum province are the most resistant to severe winter conditions than other provenances in terms of both of injury index and photosynthetic activity because they have been exposed to lower temperatures

According to those results obtained from study, it has been determined that Erzurum-Oltu, Erzurum-Olur provenances, taken from Eastern Anatolian region, among 10 provenances used in study and Kastamonu-Daday and Karabük-Eskipazar origins taken from Western Black Sea region are resistant to frost more than other provenances. That's why; in artificial regeneration and foresting efforts to be performed in high-frost-risk regions such as Eastern Anatolia region, those origins will be useful for the success of efforts. The results of this study

should be taken into the practical application value both silvicultural practices (afforestation, restorations, artificial regeneration) and breeding strategy (breeding populations, gene conservation areas, seed breeding zones, seed sources and seed orchards, etc.).

CONCLUSION

Scots pine has active mechanisms, genetically controlled, to carefully adjust and tune their tolerance of cold temperatures to minimize damage and energy expended. These cold tolerance mechanisms are reviewed here to help tree health care professionals and tree owners to better understand cold damage.

The Scotch pine's natural populations in Turkey are on the marginal and peripheral distribution comparing to the total area of this species in Europe. This is the reason why genetic-ecological characteristic of their populations differ comparing to the populations in another parts of Europe.

Scots pine seedlings in nurseries may experience freezing temperatures outside during the winter or may be protected by cold or frozen storage in a controlled environment. In each of the above cases, the ability to withstand freezing and the overall stress resistance associated with cold hardening of seedlings make cold hardiness an important attribute for nursery stock production.

The study of Scots pine **inter-population variability of frost-resistance** seedlings grown in nurseries conditions in Turkey is multidisciplinary, it applies ecological, and physiological analyses. The applied study methods contributed to a better understanding of genetic-ecological characteristics of Scots pine populations in a part of its range in Turkey, which is the base for further directed utilization of the total biological potential of this species.

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**INTERPOPULACIONA VARIJABILNOST REZISTENTNOSTI NA MRAZ
PROVENIJENCIJE ŠKOTSKO BORA ((*Pinus sylvestris* L. r. *hamata* Steven) U TURSKOJ**

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Izvod

Vršena je analiza varijabilnosti rezistentnosti na mraz u kontrolisanim uslovima, sejanaca škotskog bora (*Pinus sylvestris* L. var. *hamata* Steven), poreklom iz 10 provenijencija. Rezultati testa rezistentnosti su ukazali na jaku zavisnost stepena hlađenja i stepena oštećenosti iglica i produktivnosti fotosinteze. Utvrđena je značajna zavisnost metoda protoka jona i fluorescencije hlorofila ($r=-0.801$). Dobijeni rezultati pokazuju da ove dve metode mogu da budu sigurno korišćene u određivanju oštećenja niskim temperaturama. Zamrzavanje sejanaca je vršeno na temperaturama -10, -20, -30 i -40 °C. Primenom Duncan testa utvrđeno je da se oštećenja povećavaju snižavanjem temperature. Nivo oštećenja na -10 °C biljke mogu tolerisati dok se oštećenje pri -20 °C povećavaju do 51, 25 %. Utvrđena je statistički značajna zavisnost provenijencija i nivoa temperature kada su vršene analize procesa fotosinteze. Značajno je smanjena produktivnost fotosinteze snižavanjem temperature između -20°C i -40°C. Situacija odgovara vrednosti indeksa oštećenja utvrđenog u ovim istraživanjima. Indeksom stepena oštećenja i produktivnost fotosinteze utvrđeno je da su provenijencije Amasya-Kunduz, Bolu-Aladağ, Düzce-Yığılca, Samsun-Vezirköprü i Eskişehir-Çatacık osetljivije na zamrzavanje u odnosu na druge ispitivane u ovim istraživanjima.

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