

# Genetic and Conservation Research on *Pinus peuce* in Bulgaria

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**Abstract**—Macedonian (Balkan or Roumelian) pine (*Pinus peuce* Griseb.) is a five-needle pine native to the Balkan Peninsula, occupying in Bulgaria an area of 14,223 ha. Genetic investigations made in Bulgaria include determination of the monoterpene composition of oleoresins, the delineation of geographic and ecological races, detailed analysis of progeny tests and other genetic studies. Many of the natural stands have the status of national parks and reserves with a total area of 5,250 ha, including 65 seed stands with an area of 709 ha. In addition, 152 candidate-elite trees have been selected. *Ex situ* methods for conservation of the genetic resources of this species include 40 clones in seed orchards (10 ha), six half-sib progeny trial plantations (5.6 ha), five provenance trial plantations (7.2 ha), and a forest seed bank. The indigenous populations of Macedonian pine in Pirin are a valuable genetic resource available for the introduction of this species into other countries of Europe, and also North America and Asia.

**Key words:** *Pinus peuce* Griseb., genetic resources, *in situ* conservation and *ex situ* conservation.

## Species Distribution

*Pinus peuce* Griseb. is found only in the Balkan Peninsula, occurring in some of the high mountains of Bulgaria, Serbia, Macedonia, Montenegro, Albania and Greece in the range between 41° and 43° northern latitudes. In Bulgaria, the natural range of this species consists of two parts separated by the valley of the Vardar River. The eastern part is in southwestern Bulgaria and includes Pirin Mountain, Slavyanka Mountain (Ali Botush), Rila Mountain, the western Rhodopes, Vitosha Mountain, and the Central Balkan Range. The western part includes Macedonia, southwestern Serbia, southeastern Montenegro, eastern Albania, northeastern Greece and some spurs of the Dinar Alps, including Prokletija, Kom, Sekiritsa, Sar, Pelister, Kozhuh, Nidje, Korab, Rudoka, and Tsena (Dimitrov 1963).

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## Distribution in Bulgaria

The easternmost occurrence of Macedonian pine is in the Central Balkan Range. The westernmost, which is also the northernmost population, is on Sekiritsa Mountain, and the southernmost is in the Pelister, Nidje and Tsena Mountains (Dimitrov 1963). The areas occupied by the species in Bulgaria, listed by mountain, are as follows: Pirin 7,175 ha, Rila 6,230 ha, Central Balkan Range 193 ha, Western Rhodopes 170 ha, Vitosha 104 ha and Slavyanka 57 ha. Within these areas, *P. peuce* stands are scattered like islands, the most compact ones being those in the Pirin, Rila, Prokletija and Pelister Mountains.

There are two complexes of the species on Pirin Mountain, one in the northeast with an area of 3,775 ha, where the altitudinal distribution of the trees ranges from 1,600 to 2,200 m, and one in the southwest with an area of 3,400 ha with an elevation range from 1,700 to 2,200 m.

On Rila Mountain there are three *P. peuce* complexes, one in the southern part (1,635 ha in area and 1,700 to 2,000 m in elevation), one in the central part along the Rilska river (911 ha and up to 2,100 m elevation), and one in the northern part (3,684 ha with a 1,600 to 2,100 m range in elevation).

In the Central Balkan Range, two populations separated by the main ridge have been differentiated, one of 188 ha on the northern slope, from 1,500 m to 1,900 m elevation, and the other on the southern slope with an area of 5 ha and from 1,300 m to 1,400 m elevation.

On Slavyanka Mountain, *P. peuce* occurs in groups and as solitary trees, while on Vitosha Mountain and in the Western Rhodopes the species occurs mainly in plantations. On Sredna Gora it occurs only in plantations totalling 88 ha (Alexandrov 1998).

In 2000, the total wood volume of the 14,223 ha of Macedonian pine stands in Bulgaria was 4,198 000 m<sup>3</sup>, distributed by age class from I (1-20 years) to VIII (141-160 years) and following approximately the normal curve. The stands of age classes V (81-100 years) and VI (101-120 years) had the largest area, totalling 6,037 ha (42.5 percent of all stands) with a growing stock of 2,160 000 m<sup>3</sup> (51.5 percent of the total wood volume).

The overall average volume of the Macedonian pine forests in Bulgaria is 295 m<sup>3</sup>/ha, the average quality class is III (medium) and the rotation period is 160 years (Tsakov 2001). The average stand volume exceeds that of *Picea abies* (L.) Karst and is considerably higher than that of *Pinus silvestris* L. (Krastanov 1970).

## Genetic Research

Genetic studies of the Macedonian pine in Bulgaria, which were performed during the last 10 years, included a seed stand in each of the following areas, except for the larger number of stands in the Pirin and Rila regions, as indicated:

1. Pirin (3 Forestry Estates) – 1,900 m altitude, 10 seed stands
2. Gotse Delchev Forestry Estate – 1,800 m alt.
3. Bansko Forestry Estate – 1,700, 1,800, 1,900, 2,000 m alt.
4. Razlog Forestry Estate – 1,700, 1,900, 2,000, 2,100 m alt.
5. Rila (7 Forestry Estates) – 1,800, 2,000 m alt., 9 seed stands
6. Belitsa Forestry Estate – 1,900 m alt.
7. Yakoruda Forestry Estate – 2,000 m alt.
8. Rila Monastery Forestry Estate – 1,800 m alt.
9. Kostenets Forestry Estate – 1,900 m alt.
10. Samokov Forestry Estate – 1,800 m alt.
11. Doupnitsa Forestry Estate – 1,800, 1,900 m alt.
12. Central Balkan (Ribaritsa Forestry Estate) – 1,700 m alt.

Analyses were made of variation in monoterpene composition and in morphological and physiological characteristics.

## Monoterpene Variation

Monoterpene composition was determined from apical buds, 2-year-old needles, wood samples and bark from 2-year-old branches collected from representative Macedonian pine populations in the northern Pirin Mountains, the northern Rila Mountains and the northern slopes of the Central Balkan Mountain. Twelve monoterpenes were identified, eight of them ( $\alpha$ -pinene, camphene,  $\beta$ -pinene,  $\Delta$ -3-carene, myrcene, limonene,  $\beta$ -phellandrene and terpinolene) having relative proportions above 0.5 percent, regardless the origin of the samples or the investigated tissue. It was shown that the populations studied differ statistically in their monoterpene compositions. This made possible the division of the Macedonian pine populations from Northern Pirin, Rila and Central Balkan into separate geographical races based on the monoterpene composition of the oleoresins (Dobrev 1992).

## Variation in Morphological and Phenological Characteristics

Measurements were made on cones, seeds and seedlings. On the basis of these results, the Macedonian pine population from Southern Pirin could be distinguished as a separate geographic race. It was established that the repeatability coefficients for these traits, i.e. repeatability in different areas, are relatively high for the origins from the central parts of the species natural range in Bulgaria, whereas the reproductive materials (cones and seeds) from the marginal parts of the natural range have lower coefficient values (Dobrev 1995).

With respect to 20 characteristics reflecting the morphology of the cones, cone scales, seeds and the sizes, morphology and phenology of 1-year-old seedlings in half-sib progeny trials, a phenotypic similarity was established between mature

trees of 13 representative provenances of Macedonian pine from Pirin, Rila and Central Balkan Range. Based on of the calculated similarity matrix and the dendrogram of grouping pattern of populations from these mountains, the taxonomic distances were shown to be large and not proportional to the geographic distances between them. The results were comparable with those from preliminary studies of monoterpenes from sample trees in several *P. peuce* populations in Bulgaria (Dobrev 1996).

## Identification of Geographic/Ecological Races

From analysis of 44 morphological, growth, phenological and chemical traits characterizing the populations of Macedonian pine and their progenies, it was possible to distinguish five geographic and ecological races of this species in Bulgaria, as follows: Central Balkan, Rila, Southern Pirin, and Northern Pirin (where one middle mountain and one high mountain ecotype could be separated).

In an evaluation of growth rate differences, as determined from total tree height, statistically significant differences ( $p < 0.01$ ) were found among six-year-old families from 13 Bulgarian seed sources in five half-sib progeny trials distributed over a diversity of sites. Test locations included Stara Reka Forestry Estate at 1000 m elevation, Sliven Forestry Estate at 1000 m elevation, Yakoruda Forestry Estate at 1450 m elevation, Belitsa Forestry Estate at 1650 m elevation and Kostenets Forestry Estate at 1850 m elevation. Family heritability estimates for height growth were statistically significant, varying with site from 0.220 to 0.574 (Dobrev 1998).

A 10th-year evaluation of these 13 progenies growing in four of these locations (excluding Kostenets) showed that the fastest-growing trees came from the Northern Pirin region at 1,900 m elevation. This population was consistently superior in growth in different tests at elevations from 1,000 to 1,450 m.

## Genetic Considerations in Reforestation and Afforestation

Macedonian pine is one of the species most suitable for restoring the upper forest zone below the tree limit, which, in many mountains, has been moved down as a result of human interference. The trial plantations of *P. peuce*, which have been successfully established in the high parts of the mountains, provide a reason for expanding these plantings. However, satisfactory growth is possible only if transfer of genotypes is from lower to higher altitudes, with a maximum vertical seed transfer distance of 300 m (Alexandrov 1998).

## Conservation of Genetic Material

### *In situ* Conservation

National parks, nature parks, reserves, seed stands and plus trees provide *in situ* conservation of genetic resources of Macedonian pine. The total area of the natural forests,

which are included in Pirin, Rila and Central Balkan National Parks and Bayuvi Dupki – Djindjiritsa, Yulen, Rilomanastirska Gora, Parangalitsa, Ibar, Tsarichina and Central Rila Reserves, amounts to 5,250 ha. The gene pool of this species as a whole is preserved through the genetic diversity inherent in these different ecological regions.

The preservation of gene resources in permanent seed stands provides the basis for implementing a breeding programme. There are 65 *P. peuce* seed stands in Bulgaria, totalling 709 ha, or approximately five percent of the Macedonian pine forests. These seed stands are sufficient to meet anticipated needs for the species (Alexandrov 2000). In addition, the 152 candidate-elite Macedonian pine trees showing phenotypically superior growth, form and resistance (trees showing no damage from insects and diseases) have been selected by the Forest Seed Testing Stations in Sofia and Plovdiv. This *in situ* selection in natural stands provides a base of information and material for future genetic investigations, breeding improvement and greater utilization of *P. peuce* for reforestation and afforestation.

### Ex situ Conservation

The genetic resources of Macedonian pine are also being preserved *ex situ* in Bulgaria through provenance testing plantations, progeny trial plantations, seed orchards and gene banks for seeds. Provenance trials at ages of 28-31 years include plantations on Rila Mountain at 2,050 m, the Western Balkan Range at 1,650 m and 1,700 m, and the Rhodopes at 2,050 m and 2,100 m, with a total area of 7.2 ha (Dakov and others 1980).

There are six half-sib progeny trial plantations, age 12 at the time of writing of this paper, distributed in various parts of the species range at elevations from 1,000 to 1,850 m. In all, 170 half-sib families from 13 provenances are being tested in these plantations on a total of 5.6 ha.

A 32-year-old, 10 ha clonal seed orchard in the Western Rhodopes (1450 m elevation) includes 40 clones (Bogdanov 1970).

Assessing the relative advantages of *in situ* and *ex situ* conservation of the genetic resources of Macedonian pine, the first seems to be a more reliable method for Bulgaria, because of the growth and health of the species under a diversity of ecological conditions. Our results indicate that the native populations of Macedonian pine in Pirin are an especially valuable genetic resource for the introduction of this species into many countries of the Northern Hemisphere.

### References

- Alexandrov, A. 1998. *Pinus peuce* Griseb. – In Enzyklopädie der Holzgewächse, ECOMED, Landsberg, Germany, III-1:1-10.
- Alexandrov, A. 2000. Genetic conservation of conifers in Bulgaria – In: The Balkan Ecology 3:5-10.
- Bogdanov, B. 1970. Seed Orchards ? from *Pinus peuce*. -In: Proc. Symp. on the Macedonian pine, Skopje, 211-220 (in Serb.).
- Dakov, M., I. Dobrinov, A. Iliev, V. Donov, S. Dimitrov. 1980. Raising the upper forest limit. Zemizdat, Sofia, 220 pp (in Bulg.).
- Dimitrov, T. 1963. Roumelian pine (*Pinus peuce* Griseb.), Sofia, 116 pp (in Bulg.).
- Dimitrov, M. 1980. The Macedonian pine (*Pinus peuce* Griseb.). Zemizdat, Sofia, 180 pp (In Bulg.).
- Dobrev, R. 1992. Monoterpene composition of the essential oil of some populations of Macedonian pine (*Pinus peuce* Griseb.) in Bulgaria. Forest Science (Sofia) 2:8-16 (In Bulg.).
- Dobrev, R. 1995. Intraspecific variation in *Pinus peuce* in Bulgaria: cone characteristics. In: Caring for the Forest: Research in a Changing World. Abstracts of Invited Papers. IUFRO XX World Congress, Tampere, Finland, p. 146.
- Dobrev, R. 1996. Phenotypic similarities of representative populations of Macedonian pine (*Pinus peuce* Griseb.) in Bulgaria. Forest Science (Sofia) 4:16-23 (in Bulg.).
- Dobrev, R. 1998. Variance, genotypic stability and family mean heritability of the growth in height of 6-year-old seedlings of Macedonian pine (*Pinus peuce* Griseb.) in a series of half-sib progeny trial plantations. Forest Science (Sofia) 1-2:5-23. (in Bulg.).
- Krastanov, K. 1970. Wachstum, Leistung und Technisches Haubarkeitsalter von *Pinus peuce* - Bestände in Bulgarien. Berichte. Symposium über *Pinus peuce*, Skopje, pp 277-289 (In Serb.).
- Tsakov, H. 2001. Composition and productivity of natural Macedonian Pine endrocoenoses in Northern Pirin Mountain and created anthropogenic ones in Vitosha and Sredna Gora Mountains. D.Sc. Thesis, Sofia, 137 pp. (in Bulg.).