Selection and genetic aspects of reforestation in the Republic of Bashkortostan

V F Konovalov, R R Baiturina and E R Khanova

Bashkir State Agrarian University, 50-letnia Oktiabria Street 34, Ufa 450001, Republic of Bashkortostan, Russian Federation

*Corresponding email: aspirant_bsau@mail.ru

Abstract. The paper describes reforestation issues based on selection and genetic approaches to forest cultivation and seed production. It also deals with the structure of the existing objects of the unified genetic and breeding complex of the main forest-forming species, the state and prospects for developing selective seed production to increase the volume of high-quality harvesting seeds. The state and growth regularities of Scots pine plus trees are analyzed. The best individual trees with high indicators for creating forest seed plantations and further breeding are identified. The paper presents the study of the genetic variability of Scots pine trees carried out on forest-seed plantations. ISSR markers were used to identify the existing gene pool and confirm the true origin of forest-seed plantations of this species.

1. Introduction

Increasing the productivity of the forests is one of the urgent tasks of forestry activities. The introduction of modern scientific selection and genetic achievements in reforestation is an important basis for improving artificial plantings' quality. The increasing volumes of forest stand exploitation and insufficient natural renewal of forest-forming species significantly impoverish their valuable gene pool. Therefore, special attention should be paid to preserving the most valuable objects such as the best natural populations, plus plantings and trees [1, 2]. A successful solution to creating high-quality plantings is possible if seeds with improved hereditary properties are used for the planting material cultivation. Such seeds are harvested from plus trees and plantations of various tree species [3]. The use of selection-valuable seeds for planting material will allow the development of high-quality forest plantations and transferring them to the forest area category at an earlier time [4]. Along with the above, it is important to pay attention to the prospects for the development of population genetics [5, 6], the study of the genetic determination of the variability of economically valuable traits and properties of tree species to establish their inheritance level and assess the adaptive ability of plants to extreme environmental conditions and high-quality natural reforestation [7, 8]. Currently, it is becoming increasingly important to identify the origin of objects of a single genetic breeding pool of forest tree species using microsatellite DNA loci [9, 10]. Molecular genetic markers are particularly effective for assessing genetic diversity [11, 12]. The following types of DNA markers based on polymerase chain reaction with arbitrary primers have found the greatest application in forestry practice: RAPD (Random amplification of polymorphic DNA); ISSR (Inter Simple Sequence Repeats), and DAF (fingerprinting of amplified DNA). Outstanding features of the DNA analysis technology are its high informative value, no need for genome pre-sequencing to develop primers,
possible primers universality for different species, and relatively low cost. At the same time, ISSR technology is considered more reliable due to the longest primers [13].

2. Methods and Materials
The study objects were plus trees of Scots pine growing in the Bashkir Urals' forest conditions in the territories of the Diuriulinsky and Tuymazinskii forest districts. The research covers forest-seed plantations of this tree species. The presence of objects of a single selection complex of the main forest-forming tree species in the region was specified and analyzed according to the national forestry register's reporting documents. The sample plots were established, taking into account the requirements of All-Union Standard [14]. The scientific methods and methodologies used in forest taxing, forest-cultural and selection-genetic studies are applied. At the experimental facilities, 9 plus trees of Scots pine (Pinus sylvestris L.) were counted and measured according to the main morphometric criteria. A polymerase chain reaction using 5 ISSR primers was carried out to study the genetic variability of Scots pine grown on forest-seed plantations in the Bashkir Urals.

DNA was isolated using the standard STAV method [15]. Fresh pine needles were used as a raw material for the experiment. Forty Scots pine trees on four forest-seed plantations were analyzed in total.

3. Results and Discussion
The total forest area on the territory of the Republic of Bashkortostan is 5.7 million hectares. The republic's forest cover is 39.9% (on average, in Russia, 46.6% and in the Volga Federal District, 36.5%). The annual maximum allowable cut volume is 9.6 million cubic meters, including 1.1 million cubic meters for coniferous farming [16]. The area of artificial forest stands is 6.8 thousand hectares. In general, the current silvicultural production system does not provide a balance between the reforestation areas and forest disposal.

According to the forecasts of the Republic of Bashkortostan's forestry development for 2019-2024, the ratio of felled forest stands should correspond to the area of reforestation (figure 1).

Figure 1. The ratio of the area of reforestation and clear filling is a thousand hectares.

The federal project "Forest Conservation" was developed to increase the reforestation volume. The project aims "to ensure the balance between forest disposal and reforestation in the ratio of 100 % by 2024". To increase forest plants' volume harvesting seeds with improved hereditary properties and obtain high-quality planting material from them, it is necessary to radically change the approaches to forest seed production management and the nursery base's development.

The basic requirements for improving the productivity, quality and sustainability of forests are the scientifically-based exploitation of existing breeding facilities of the main forest-forming tree species and the creation of new ones, which will ensure the production of seeds and planting material with improved hereditary properties. Currently, the Republic of Bashkortostan has an inadequate forest
seed production system, allowing reforestation and afforestation activities using high-quality planting material. As of 01.01.2018, the area structure of the objects of the unified genetic breeding pool (UGBP) of the Republic of Bashkortostan is presented in figure 2.

![Figure 2. The area structure of the objects of the unified genetic breeding pool.](image)

The main object is the forest genetic reserve of Sukachev's larch, allocated on an area of 4577.8 hectares, which is 75.3 % of the total area of the objects of the UGBP. Plus plantings are allocated on the area of 939.3 hectares (15.5 %). Forest-seed plots (PFSP) occupy 384.8 ha (6.3 %). Seed plantations and other objects are located in small areas, varying from 0.1 % to 1.8 %. The number of plus trees is 952. For various reasons, their number has decreased by 27 % over the past 10 years; the number of plus plantings has decreased by 20 %. This indicates the need to resume breeding work on selecting plus trees and plantings of the main forest-forming tree species growing in the region.

The volume of harvesting of seeds of the improved breeding category is deficient. Over the past 2 years, their share was on average 3.4 % of the total volume of seed harvesting, including 2.4% of Scots pine. In 2020, 76.5 kg of the improved breeding category seeds were harvested from forest seed plantations, which is 3.5 % of the total volume of forest seed harvesting. These volumes do not meet the needs of the forestry of the republic for high-quality seeds. It is necessary to increase their share in the total volume of seed harvesting by increasing the seed productivity of Scotspine growing on forest seed plantations and collecting seeds from all trees. As it was noted, in some cases, the seeds from Scots pine trees growing on forest seed plantations are not fully and properly collected. The work on the cultivation of high-quality planting material with a root-balled tree system has been started to improve forest stands. The technology of creating forest crops using seedlings with a root-balled tree system allows increasing the work efficiency and optimize the reforestation cost. It is necessary to increase the volume of harvesting of improved seeds to solve this problem successfully. For the cultivation of planting material, the republic has 105 forest nurseries with a total area of 790 hectares. More than 70 million seedlings and plantlets are there, while 90 % of them are coniferous species. The annual demand for planting material for reforestation measures is 30 million pieces. A comparative analysis of the trunk diameter and height was made to distinguish the best individuals from Scots pine plus trees. The Scots pine plus trees are characterized by good vital condition, rapid growth and development.
The excess in trunk diameter was from 114 to 138 %; tree height excess was from 101 to 109 %, compared to the passport data. Plus, trees with passport numbers 108/33, 573/70, 575/72, 577/74 and 581/78 had the best indicators for the trunks' diameter and height.

When selecting plus trees, it is also important to consider the crown's size and the length of the branch-free zone (table 1).

Table 1. Average statistical indicators of Scot pine plus trees.

| Number of the plus tree | The length of the branch-free zone, m of the trunk, x±mx | % of height | Crown length, m x±mx | % of height | Crown diameter, m x±mx | % of height |
|-------------------------|------------------------------------------------------|-------------|----------------------|-------------|------------------------|-------------|
| 108/33                  | 21.1±0.52                                            | 67.6        | 10.5±0.25            | 33.6        | 7.5±0.09               | 24.1        |
| 573/70                  | 21.8±0.63                                            | 66.5        | 11.2±0.18            | 33.9        | 8.2±0.08               | 25.1        |
| 575/72                  | 20.6±0.31                                            | 65.2        | 10.8±0.16            | 34.3        | 8.5±0.10               | 26.9        |
| 577/74                  | 25.3±0.86                                            | 82.1        | 7.8±0.09             | 25.3        | 7.2±0.12               | 22.3        |
| 578/75                  | 23.2±0.43                                            | 71.7        | 9.2±0.11             | 28.2        | 6.8±0.11               | 23.2        |
| 579/76                  | 24.2±0.54                                            | 76.3        | 7.3±0.10             | 23.0        | 5.6±0.06               | 22.8        |
| 580/77                  | 22.6±0.32                                            | 70.0        | 10.5±0.19            | 32.5        | 7.6±0.14               | 23.5        |
| 581/78                  | 24.8±0.26                                            | 80.2        | 8.4±0.14             | 27.2        | 6.3±0.12               | 20.3        |
| 582/78                  | 22.3±0.31                                            | 72.1        | 9.2±0.18             | 29.7        | 7.1±0.14               | 23.3        |

*indicators of plus trees depending on the average trunk height, m

The plus trees of Scots pine with numbers 577/74, 579/76 and 581/78 had the best indicators of the trunk branch-free zone length and crown size. Among the selected Scots pine plus trees, those with the passport numbers 577/74 and 581/78 had a high breeding value proven by their indicators. They are the most promising for improved harvesting seeds, cuttings and creating high-quality plantation-type forest crops, seed and clone plantations of Scots pine. The studies on the genetic variability of Scots pine trees growing on forest-seed plantations of the Bashkir Ural were conducted using ISSR markers. Based on the ISSR analysis results of Scots pine trees' DNA, the occurrence frequency of locus alleles was calculated for the analyzed population of Scots pine plus trees. The proportion of polymorphic loci was 78-87 %, and the average value for all identified loci was 82%. The length of the amplified DNA fragments in different primers varied from 1700 to 200 pairs of nucleotides. The obtained frequencies of ISSR fragments were used to estimate the main parameters of the genetic variability of plus trees of this species. The calculations showed that the effective number of alleles (Ne) in the studied forest – seed plantations was 1.385, and the total genetic diversity (H) was 0.239. The results obtained are consistent with the data of studies made by [2], in which the proportion of polymorphic loci was 85%, and the expected heterozygosity was 0.250. Such studies identify the existing gene pool of valuable tree species and confirm the origin of forest seed plantations. The DNA analysis method is currently successfully used in the examination of illegal wood trafficking [17].

4. Summary

One of the main problems in reproducing highly productive forests in the region today is the lack of an effective systematic organization and management of forest selective seed production, focused on increasing the volume of harvesting improved seeds of the main forest-forming tree species. This factor significantly slows down the production of high-quality planting material used to cultivate artificial, highly productive plantings. The areas of forest crop plantations, especially where coniferous species are grown, should be expanded using seedlings and plantlets with a root-balled tree system grown in forest nurseries from seeds of an improved breeding category. Molecular genetic analysis
using ISSR-markers allowed obtaining new data on the Scots pine's genetic variability growing on forest seed plantations of the Republic of Bashkortostan.

The results obtained prove a relatively high level of genetic variability of trees of this species. To preserve the high-quality gene pool of the Republic of Bashkortostan's main forest-forming tree species, it is necessary to actively introduce methods of genetic monitoring of objects of the unified genetic selection complex into the practice of forest breeding seed production. The existing plus trees of Scots pine have not lost their breeding status. They are the most promising for harvesting valuable reproductive seed material of an improved breeding category and vegetative cutting material for creating artificial, clonal and family forest plantations. Forestry specialists should pay special attention to the safety of the selected Scots pine plus trees. They must monitor their condition, collect cones in a timely and high-quality manner and prepare seeds of the improved breeding category without mixing them with other seeds.

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