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Correlation between the Spectrometric Parameters of Coniferous Seeds and the Molecular Indicators of Seedlings: Is It Possible to Apply It in Practice? †

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Abstract: Coniferous seeds as an integral part of Forest Reproductive Material (FRM) are a fairly valuable product that is transported by trade operations over long distances. Seed quality determines the rate of reforestation. Improving quality indicators and increasing the competitiveness of forest seeds is one of the promising directions of the many country's forestry development strategy and an opportunity to integrate into global reforestation initiatives. Based on the direct interaction of biophysical studies of seed spectrometric parameters, implementation of their genotype in different environmental conditions, biometric studies of seedling growth and development and genetic conditioning of these components, it is possible to develop and test a comprehensive concept for obtaining FRM with high quality indicators. What practical application can be expected? First, it is possible to study genetic variability among the seeds of the studied species using molecular DNA markers to obtain a comprehensive and categorical classification of samples that illustrates the genetic similarity and relationship structure relative to the desired characteristics of seedlings and seeds with high viability rates. Genetic and spectrometric data can be further combined to build a consensus tree of genetic similarity. Second, algorithms can be developed for integrating these parameters in the FRM-Library database to synchronize the quality indicators of forest reproduction material with information processing devices of optoelectronic graders and phenoseeders. Third, methods and algorithms can be developed for optimal selection of technology for coniferous seeds grading of different breeding values will be developed based on data extracted from the FRM-Library database for the production of forest reproductive material, considering various goals and frontiers methods of contemporary forest landscape restoration.

Keywords: coniferous seeds; seed spectrogram; seedling; genetic diversity; amplification curve; correlation; DNA markers; FRM-Library; forest seeds optoelectronic grader, forest seeds rapid analyzer

1. Introduction

When implementing contemporary forest landscape restoration [1–5], one of the main tasks is to obtain seedlings from seeds with improved properties. The first step in this direction is selection for the speed of growth and productivity of mother trees. This requires the development of new

methods for diagnostics of Forest Reproductive (seed and planting) Material (FRM) of newly created or restored stands. In addition, when breeding high-quality breeds and improved forest tree crops, success primarily depends on reliable reproduction at an early age [6]. Forest woody plants are characterized by significant intraspecific variability that increases the level of biodiversity of forest ecosystems [7], which is formed on the basis of seed quality [8] in conditions of extensive ecologically heterogeneous areas of forest-forming species.

Seed quality is defined as "*a measure of the attributes or attributes that will determine seed performance during sowing or storage*" [9]. This is a multiple concept that covers the physical, physiological, genotypic, pathological, and entomological factors that affect the seedlot productivity [10,11].

Many trees produce significant amounts of empty and fossilized seeds due to inbreeding, mismatch of phenological phases, or post-zygotic degenerations [12]. This is a major problem that reduces the quality of seed lots [13].

These seeds lack the miniature plant – embryo, and do not produce seedlings when sown. In addition, insect infestation [14–16] and environmental conditions during seed development [17] and the genetic constitution [18] of the seed lots influence their performance when sown or stored. Seeds partially consumed by larvae are usually less vigorous, while seeds that are severely attacked are completely empty of their contents, and hence failed to germinate. Despite several attempts to improve seed yield of several species, a substantial amount of empty, insect-infested and dead-filled seeds is still encountered in seed bulks, as there is no way of avoiding these seeds during collection. It is, therefore, imperative that such non-germinable seeds should be removed from the seed bulk prior to sowing in order to enhance the performance of seed lots when sown in the nursery.

A preliminary analysis of biophysical methods [19] for seed research has shown that there is a significant difference between the coniferous seed's spectrometric parameters of different geographical and species origin. Theoretical studies of the optical radiation formation [20] for detecting such seeds in the mobile rapid analyzer's optoelectronic system (patent RU 2,675,056 [21]), developed under the guidance of one of the authors, show a fairly high accuracy and the possibility of their analysis and grading.

Interdisciplinary interaction is designed to achieve a comprehensive solution to the problem of obtaining improved FRM in the future by studying the coniferous seeds spectrometric properties of different breeding potential and predicting the rate and quality of ontogenetic development of seedlings based on them.

What practical application can be expected from possible correlation between the spectrometric parameters of coniferous seeds and the molecular indicators of seedlings?

This requires interdisciplinary research that brings together specialists in the fields of molecular genetics, quantitative trait genetics, ontogeny genetics, fundamental optics (spectrometry), development of professionally oriented information systems (databases), and automation of forestry processes.

2. First application

First, the molecular variation among the forest seeds may be examined with the DNA markers exploring the genetic similarity/distance using the plant genetic materials. Utilizing molecular characteristics will illustrate the seed population structure and relationship regarding the tested quantitative variables, and will help to differentiate seedlings and seeds with high rates of viability. The genetic and spectrometric data may further be integrated for constructing a consensus genetic similarity tree.

Woody species naturally exist in populations or communities. Population as a level of organization of individuals is characterized by the following processes: migration, mutation, gene drift, and inbreeding. During the ontogenesis of each tree, both the interaction of genes and their expression occur. For example, gene expression was studied in detail in coniferous tree species that have a high economic value, in particular, in frankincense pine [19]. In this species, genes were identified whose variability is associated with adaptive traits, as well as affecting the expression of genetic information and the level of metabolites. For this purpose, a genome-wide approach was

applied, i.e., a description of the sequence of nucleotides of all exons of the body, i.e., sections of DNA encoding proteins. Single — nucleotide polymorphisms were used as markers- differences between two sequences per nucleotide that occur as a result of point mutations and are steadily transmitted from generation to generation. This change, as it turned out, can dramatically affect the body. A correspondence was found between the variability of the studied single-nucleotide polymorphisms and the expression of a number of genes, as well as the corresponding plant traits. As a result, gene networks were obtained, the analysis of which revealed the key genes responsible for adaptation to various environmental factors, including drought. There are also studies showing that evolutionary and transgenerational processes affect the expression of the offspring trait, the parent environment can pre-adapt the offspring to similar environmental conditions, the degree of influence of maternal traits on the offspring depends on the context, changes in the seedling growth environment have a profound effect on the productivity of the offspring [20].

Genomic techniques are very valuable and useful tool for the genetic study of many organisms from all very simple prokaryotic cells to eukaryotes, e.g. tree plants. These methods enable scientists to investigate the genetic mechanism behind different plant characteristics, perform evolutionary evaluation, and enable biologists to conduct fundamental tasks such as inferring recent and historical demographic trends of populations in response to environmental changes.

However, due to some limitation in trees, i.e. the large size and the complexity of many tree genomes, application and development of species-specific genomic techniques might be challenging. Many tree plants like gymnosperms are polyploid with a large genome with a high proportion of repetitive content, which makes the genetic studies more complicated. In addition, the wide geographical distribution of forest trees along with a large ecological range determine the formation of various life forms (from prostrate shrubs to trees). Challenges in taxonomic classification of subspecies are also related to high variability of morphological traits in trees. Many of the species in forest trees are polymorphic species with intricate and often controversial taxonomy [21]. In addition, a strong geographic bias towards temperate tree species constrains our ability to decipher the long-lasting evolutionary impact of climate change across forested lands worldwide, a task of utmost importance to forecast the response of forests to emerging mega-disturbances [22–24]. Even with the advent of reliable and fast modern techniques to study the genomes (e.g., genome-wide sequencing and association studies), genomic research on forest trees still remains partly unsolved regarding the genetic factors and their interaction with environmental factors.

A comprehensive study of the relationship between the spectrometric parameters of seeds of promising woody plants obtained by different breeding techniques, their sowing qualities, quantitative characteristics of gene expression, and biometric parameters of seedlings will allow us to obtain reliable correlation models of growth and development of coniferous trees.

3. Second application

Second, algorithms can be developed for integrating spectrometric (spectral absorption bands etc.) genetic (DNA markers, amplification curve etc.) and biometric (growth rate etc.) parameters into the FRM-Library (FRMLib) database [25]. This will allow us to synchronize FRM quality indicators with optoelectronic graders [26] and phenoSeeders [27] based on fuzzy logic algorithms [28,29], moving to the implementation of a closed-loop control system that is most effective in terms of accuracy of analysis and grading.

The solution to this problem requires the following conditions to be met:

- there is a universal criterion for selecting viable seeds with certain improved genetic characteristics;
- availability of an optimal method for conducting such testing;
- availability of technical means for such testing that meet the requirements of energy conservation and environmental safety.

To date, the implementation of all these conditions in the practice is carried out mainly in two directions: quantitative and qualitative.

The quantitative approach assumes the use of geometric parameters of seeds and their mass as a selection criterion. In its pure form, seed size separation can change the genetic diversity of the seedlot [30]. It is a rather invasive method and does not fully take into account hereditary features.

The qualitative approach assumes the use of spectrometric parameters of seeds obtained in the visible, infrared, or submillimeter wavelength ranges as a selection criterion. At the same time, there is currently no consensus on which division range best predicts the subsequent growth and development of seedlings [31]. At the same time, the optical radiation plays an important role in the evaluation and preparation of forest seeds for sowing and storage. The different ability of radiation absorption by seeds has a certain significance in genetic diversity [32] due to the high heritability of the seed coloration gene [33–36]. For example, the offspring of Scots pine obtained from seeds with a light skin color sharply "*changes the ranks of germination time and fluctuations in the frequency of the best families from 0 to 400% of the norm*" [37]. Many scientists have studied the mechanisms of using optical radiation for diagnostics, analysis, and prediction of further development of seeds [37–46]. However, there is no holistic approach to conducting spectrometric studies of forest seeds and mobile equipment for rapid analysis, and data on the existence (or not) of correlation interactions between the spectrometric characteristics of seeds and the biometric indicators of seedlings obtained from them are poorly presented in the scientific forestry literature [5].

Research is being conducted with the support of universities' own funds under the project "Development of technology for the production of forest seed material with specified quality characteristics" (<https://www.researchgate.net/project/Development-of-forest-seeds-production-with-the-specified-characteristics>). The goal of the project is to increase the efficiency of forest seed production for direct automated seeding (ground or air seeding [47,48]) by developing technology and designing automated technical means for high-quality seed grading based on photon technologies.

The current level of technology development, the presence of positive international and domestic experience in the field of genetics, biophysics, biochemistry, optics, electronics and mechanics makes it possible for the research team to develop the design of devices [26,49–51] that can separate forest seeds by quality (spectrometric) characteristics.

At the same time, a prerequisite for improving the design is the availability of experimental data on the expression of seedling genes during ontogenesis, depending on the spectrometric characteristics of coniferous seeds, which are negligible at this time.

4. Third application

Third, based on data extracted from FRMLib, and taking into account frontier interdisciplinary (biophysical, biochemical, biological, engineering, and other) methods, optimal technological regulations can be developed for non-destructive quality control and sorting of coniferous seeds of various breeding values for the production of forest reproductive material, taking into account various purposes (reforestation, plantation forestry, etc.) and methods (for storage, for ground-based directive seeding, for aerial seeding, for planting, etc.).

In the future, the forest restoration method on hard-to-recover forest sites [52] can be upgraded based on updated technological regulations. Sites that are difficult to restore include:

- released as a result of felling, including burning felling, inefficient for the operational technology of ground-based seeding or planting;
- released as a result of forest fires that are not effective for the operational technology of ground-based seeding or planting;
- inaccessible to ground-based mechanization facilities for climatic and geomorphological reasons;
- inaccessible to people due to the complication of the radiation background and (or) after man-made disasters.

When implementing forest restoration method, including group of site preparation operations [53], group of FRM preparation operations, group of seeding [47,54,55] or planting [56,57] on site, additionally includes group of monitoring operations [58,59]. Alternative rapid analysis [16,31] and

encapsulation [60] operations carried out in field conditions using mobile equipment [26,49–51] are additionally introduced into a group of FRM preparation operations. In the group of seeding or planting operations the additional operation of spot sowing of seeds or planting of seedlings from air is carried out, which is carried out using unmanned aerial vehicles of helicopter, aircraft or hybrid types, equipped with taking into account their take-off mass navigation equipment based on closely-integrated inertial-satellite system [61–64], equipment [65] for simultaneous storage of sown seeds or planted seedlings, control of specified seeding rate of seeds or planting seedlings by quantitative or mass basis and accurate seeding or planting of seedlings depending on specified scheme of location of forest crops and speed of unmanned aerial vehicle, energy accumulating equipment.

Advantages of the proposed forest restoration method on hard-to-recover sites are the acceleration of the reforestation process and the higher environmental safety by reducing the number of operations which disturb the forest ecosystem, use of environmentally safe energy sources in technical means of monitoring, preparation of reproductive material, owing or planting, reduction of costs for performance of energy-intensive operations of site preparation, transportation of seed to the place of preliminary preparation and back.

The socio-economic effect of the use of new technological regulations is explained by the fact that coniferous seeds as an integral FRM-part are a fairly valuable product that is moved by trade operations over long distances [66]. The role of seeds is quite significant [8]. their quality determines the rate of reforestation and afforestation. Improving quality indicators and increasing the competitiveness of forest seeds is one of the most promising areas for the development of the forest complex in many countries. This direction involves active interaction between the triad "state forest owners – private forest owners – forest seed producers", which corresponds to the main provisions of the methodology for assessing recovery opportunities [67] for use in global reforestation initiatives [68–70]. According to Dominic DellaSala et al., "*we must commit to thoughtful, science-based restoration to ensure that future generations can experience and enjoy pristine, diverse forest landscapes with the highest ecological integrity* [71]."

4. Conclusions

Thus, based on the direct interaction of biophysical studies of the spectrometric parameters of seeds, the implementation of their genotype in different environmental conditions, biometric studies of the growth and development of seedlings and the genetic conditionality of these components, it is planned to develop and test a comprehensive concept for obtaining FRM with high quality indicators.

Integration of the claimed approaches will allow creating a fundamentally new information and analytical FRMLib-database [25], which provides a solution to the problem of synchronization of FRM quality indicators with the decision-making module when performing the technological process of reforestation [52], as well as with software complexes of automated optoelectronic devices [26,49–51,60,65] for rapid analysis, grading, encapsulation, seeding and monitoring of the reforestation results.

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The authors hope to establish and expand interdisciplinary relations with colleagues from other countries.

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