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Visible wave spectrometric features of scots pine seeds: the basis for designing a rapid analyzer

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Abstract. The study is intended for engineers and small-scale forestry farmers because it offers a simple solution for forest seeds quality improvement by selection before sowing (especially aerial sowing). For coniferous trees seeds VIS spectroscopy is a very important tool that determines the energy of radiation, indicating viability and origin. However, despite many suggestions of potential for practical use, there are no specific recommendations given. Visible diffuse reflectance spectra of single seeds were scanned with spectrometer USB 4000 (Ocean Optics Inc., USA) from 450 to 1000 nm at 1 nm intervals. It is found that in the range from 660 to 715 nm the spectral allocate of seed coat color classes has three non-intersecting characteristic groups differing in the magnitude of the diffuse reflection and forming a possible grading zone. The results of this study are limited to this specific sample and cannot be interpreted for seeds of all coniferous species. There is a need for design of a device for rapid analysis of seeds by spectrometric parameters, which will have a practical application and which will allow a comprehensive sequential studies following the "spectrogram - germination - growth in the nursery-growth in the field" path.

1. Introduction

Non-destructive seed quality control [1] plays an important role in the purchase of seeds for use in small-scale forestry in contemporary reforestation. Also an important methodical factor specific to forestry is the portable design of devices which can used on field and low costs for the implementation. Table 1 illustrates the possibility of using different methods to analysis of forest seeds [2-15].

For example, the TTC-test has a high destructive effect on the seed coat and is now *«can be used to successfully estimate viability for very dormant species or for other hard-to-germinate species»* [6]. IDS-method includes the process of moisture absorption by seeds, where it is difficult to control the involuntary germination, drying and separation. It has a relatively high cost of operation due to the use of additional devices, so *«mainly used as a presowing separation to eliminate seeds that have lost viability during storage»* [16]. The NMR-method allows to determine, in addition to viability, in the composition of metabolites of seeds, while there is no *«the need for metabolite extraction from plant tissues, a process that can result in alteration of metabolites»* [17].

For forest seeds VIS and NIR spectroscopy is a very important tool that determines the energy of radiation not only their viability [18,19], but also the provenance [10]. Studies of seed coat in the infrared range and the absorption spectra have proved the possibility of optical separation of seeds [20]. Moreover, it is confirmed that the seeds of pine have several layers of coat, as well as hydroxyl



groups, which can be used as an indicator of viability. However, all researchers suggest the possibility of its use in practice, but do not give specific recommendations.

Table 1. Comparison of methods reported for the forest seeds analysis.

Methods	Analysis	Non-destructive	Use of reagents	Portability	Cost
Topographical tetrazolium chloride (TTC) test [2]	Quantitative	No	Yes	Yes	Medium
Flotation [3]; Incubation-Drying-Separation (IDS) [4]	Quantitative	No	No	No	High
Air Grading; modified specific gravity (MSG) separation [5]	Quantitative	No	No	No	High
Sieve [6] and non-sieve grading [7]	Quantitative	No	No	No	High
PREVAC [8]	Qualitative	No	No	No	High
Machine Vision [9]	Qualitative	Yes	No	No	Medium
NIR [10]	Qualitative	Yes	No	No	Medium
FT-IR [11]	Qualitative	Yes	No	No	Medium
Hyperspectral imaging [12]	Qualitative	Yes	No	No	Medium
Thermal imaging [13]	Qualitative	Yes	No	No	High
X-Ray [14]	Qualitative	Yes or No ¹	No	No	High
Nuclear magnetic resonance (NMR) [15] spectroscopy	Qualitative	Yes or No ¹	No	No	High

Notes: 1. High energies of the above radiation can generate valent or deformation changes in the internal structure of the samples, therefore, we consider the non-destructive method rather conditionally.

The variability of the seed coat color may be associated with genetic diversity, as well as with the signs of the disease, which, in turn, in different interpretations depend on the geographical location, soil conditions and other specific criteria. The possibility of precision automatic separation of seeds by the coat color without injuring would lead to the creation of new mobile devices working on a completely different principle than existing separators and sorters.

The design of mobile devices for quality control and grading of seeds based on the photonics principles requires big data for the implementation of the identification algorithm. The seed coat color of the coniferous trees is a qualitative indicator that is individual, hereditary stable [20–22] and due to the number and localization of phenolic pigments [23]. Separate direct seeding [24], especially aerial sowing [25], having different seed coat color, can provide cut costs due to the different seeds germination and growth intensity of seedlings [26,27]. To accumulate data on the spectrometric properties of seeds, it is necessary to design an analyzer that automates the process of seed supply, seed measurement and information processing.

In this study, we have tried to answer the following questions, related to design of mobile devices for practical application of visible spectroscopy in assessing the quality of *Pinus sylvestris* seeds. Is there a relationship between the number of seed coat color fractions and the seed engineer properties for separation? Is there a relationship between seed position and seed spectrometric properties? Is there a need to design a seed analyzer and create a database of spectrometric properties of seeds?

2. Materials and methods

Cones of *Pinus sylvestris* L. were collected from trees, in natural forest in autumn of 2017, located in the Pavlovsky district of the Voronezh region, Russian Federation (Latitude 50.462169; Longitude 40.096446, Altitude 83 m). Seeds were extracted from cones and further processed (pre-cleaning, extraction, de-winged) using the standard procedures and equipment (BCC AB, Landskrona, Sweden) at the Voronezh containerized forest nursery, Russian Federation (Latitude 51.567094; Longitude 39.243006, Altitude 105 m asl). Original seedlot was placed in a storage facility in a glass bottle at $+5\pm 2^{\circ}\text{C}$ and humidity of 60 %.

Seeds sampling for the study from storage was made as follows. From the original seedlot, three random samples of 0.5 kg were extracted in May of 2018. The samples were kept for 24 hours at $+20\pm 2^{\circ}\text{C}$ and humidity of 75%. From each samples we selected three seed coat color classes in using an organoleptic test: black, brown and white. Three representative samples (figure 1) were taken from each seed-color classes. Additionally, these seeds were classified in the Munsell [21] color system (table 2) using the digital camera Canon Digital IXUS 100 IS 12.1 MPix (Canon Inc., Tokyo, Japan) for obtaining images and for image processing Digital Color Guide android-software (DIC Corp., Tokyo, Japan).

Table 2. Color classes and sample coat color of *Pinus sylvestris* seed used in the study.

Seed color group	Seed color (Munsell system)
1 – Black (K)	N 0.8
2 – Brown (B)	3.5 YR 3.0/5.3
3 – White (W)	0.1 Y 8.3/3.0

Visible diffuse reflectance spectra of single seed samples were scanned with spectrometer USB 4000 (Ocean Optics Inc., USA) from 450 to 1000 nm at 1 nm intervals. Spectrometer has a high sensitivity in the medium-wave part of the visible spectrum (from 490 to 750 nm). Every seed was placed in a special holder, oriented about the axis in four different positions. Such positions simulate the seed orientation in the vertical pipeline of the rapid analyzer. The average spectrum from 32 scans (8 for each position) was obtained for further analysis.

The optical radiation reflected from the seed passes through the integrating sphere FOIS-1 (Ocean Optics Inc., USA), the fiber-optic cable P400-2-VIS-NIR (Ocean Optics Inc., USA) to the entrance slit of the spectrometer. At the output of the optical system, a voltage proportional to the light intensity was generated using a CCD line. The reflectance spectra as a dependence of the reflected radiation intensity R (in percentage) from wavelength λ (in nanometers) was obtained by analog-digital conversion. The results were processed on a personal computer using Spectra Suite application software.

3. Results and discussion

Figure 1 shows that used organoleptic test can be classified in three specific color groups of Scots pine seeds as black (figure 1a), brown (figure 1b) and white (figure 1c). Baldwin (1942) classified seeds into four colour classes (“dark, brown, black or gray with mottling” [21]), Tillman-Sutela and Kauppi (1995) into three colour classes (“dark, light and mixed” [23]) Mukassabi et al. (2012) into two colour classes (“dark and light, light seeds are divided into 4 sub ranks” [27]).



Figure 1. Representative selected samples for the organoleptic test in K (a), B (b) and W-color (c) groups.

The seed coat color is correlated with the seeds germination and seedlings growth, with the majority of research shows that “the darker the seed the better the germination” [21,27] and growth [27]. The author's own research [26] carried out with seeds from the same original seedlot established the highest growth in transplanted seedlings produced from light seeds. The fact of better seedling growth from seeds of opposite color may be associated with geographical variability [20], genetic diversity [28] and may depend on the abrasion [21] of the outer layer of the seed coat obtained in the process of extraction and purification. Brown seed resulted in the lowest survival rate [26], much lower than black and white seed. Frequent mention in studies of light, brown and dark seeds justifies the separation of seeds by color of the predominant pigment. However, the organoleptic method of determining color is quite subjective and depends on the perception and characteristics of the human eye. To confirm the technological possibility of seeds separation by color into three groups, we will carry out spectrometric measurements of samples from K, B and W-color groups (see table 2).

Figure 2 illustrates the diffuse reflectance spectra of all seed color groups. In the spectral field under consideration, the reflectivity of the samples is allocated as it decreases in the order of the seed coat color: «white – brown – black». Let's divide the spectrogram into three areas: short-wave (from 450 to 500 nm), medium-wave (from 500 to 750 nm) and long-wave (from 750 to 1000 nm). The high level of spectral lines oscillation is observed in all color groups in the short-wave (from 450 to 500 nm) and long-wave (from 900 to 1000 nm) range, which is characterized by low sensitivity of the device in these areas.

In the field of average wavelengths from 500 to 750 nm, the bandwidths are as follows: for seeds with white peel a flat growing line, for seeds with brown peel a wave-like curve, and for seeds with black peel a straight line parallel to the abscissa axis, to a value of 700 nm, then a growing concave curve. Least variability fluctuations of spectral bands within color classes have a seed coat of white color (the blue line in figure 2) and seed coat of black color (the black line in figure 2), the highest – seed coat of brown color (the red line in figure 2). It should be noted that the spectral line of the brown seed coat in the range from 500 to 550 nm is superimposed on the spectral line of the black seed coat, and in the range from 725 nm and above – on the spectral line of the white seed coat.

The nature of the change in the intensity of the reflected radiation in the field from 660 to 715 nm indicates the possibility of a conditional grade of the spectrum of seed coats into three intervals: from 35 to 45 %, from 50 to 65 %, from 70 to 85 %. The separation of the seeds into three fractions by the organoleptic test is confirmed by the spectrogram on figure 1, where the dark seeds correspond to the

spectrum with the lowest reflectivity, light seeds with the highest reflectivity. On figure 1, three grouped spectrograms are distinguished, which confirms in one case the feasibility of dividing this original batch of seeds into three color groups in another case, there is no significant difference in the orientation of the seed in space when performing the analysis.

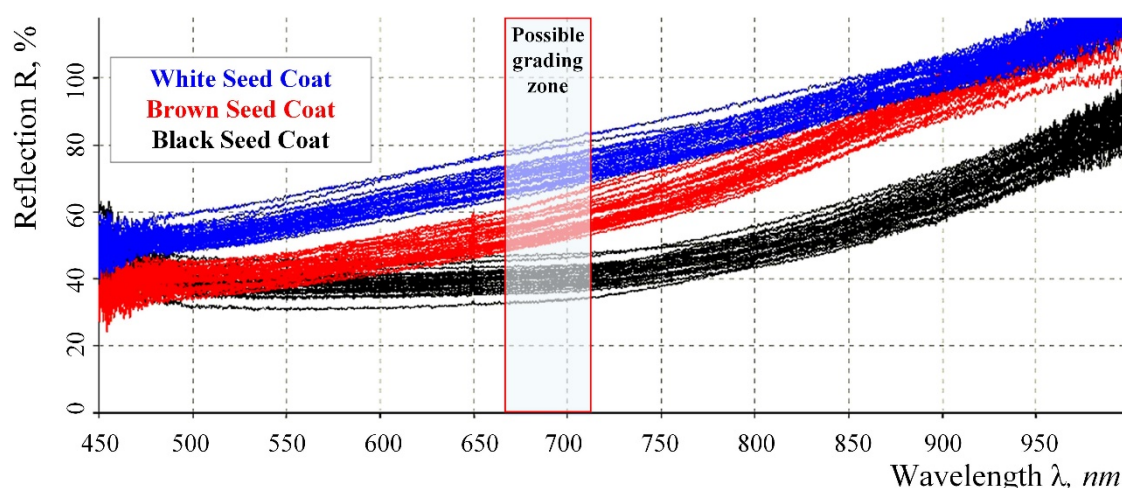


Figure 2. Diffuse reflection spectra samples of *Pinus sylvestris* seeds.

However, there is an evidence of bimodal color [29] in coniferous seeds, so the results of the study can only give specific data on specific seeds and they cannot be interpreted for all coniferous seeds. Therefore, it is necessary to systematize and collect data for seeds collected from different sources.

4. Conclusion

For Scots pine seeds collected in Pavlovsk district of Voronezh region, Russia can be divided by the color of the seed coat in the wavelength range from 660 to 715 nm. To design mobile devices for determining the seeds quality and their subsequent sorting, it is necessary to create a data Bank including characteristic spectrograms of seeds of different growing conditions, as well as information about the germination and growth of seedlings from the same seeds. To do this, it is necessary to design a device for rapid analysis of seeds by spectrometric parameters and to plan comprehensive sequential studies "spectrogram - germination - growth in the nursery-growth in the field".

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