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Seasonal color shifts and genetic diversity in Scots pine: a generalizable RGB imaging and CNN-based framework for conifer seedlings

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Abstract

Key message Leveraging affordable red-green-blue (RGB) imaging and neural network algorithms, this study delivers a high-throughput method to quantify seasonal color shifts and genetic variation in Scots pine seedlings. Hue-saturation-brightness (HSB) color analysis, and RGB values can be used for population and seasonal differentiation and hold potential for advancing breeding programs in forestry under changing climatic conditions.

Context Scots pine (*Pinus sylvestris* L.) displays remarkable genetic and phenotypic diversity, with seasonal color changes such as autumn reddening, reflecting population-level responses to local environmental conditions. Advances in imaging and deep learning now enable precise quantification of such phenotypic variation, providing new insights into population-level variation.

Aims This study assesses seasonal color variation within and among Scots pine seedling populations, compares the effectiveness of RGB and HSB systems for population and seasonal differentiation, and investigates phenological patterns across progenies of three seed orchards from ecologically distinct populations.

Methods One-year-old seedlings from lowland (Plasy, Trebon) and upland (Decin) populations were imaged in a common garden trial in September, October, and January using a handheld camera. Needle-level segmentation was performed via a convolutional neural network. Genetic variability and population differences were analyzed using linear mixed models.

Results Population differentiation reached the highest values in the RGB blue channel ($Q_{ST-blue} = 0.64$ in September and $Q_{ST-blue} = 0.94$ in October) and in HSB ($Q_{ST-hue} = 0.61$, $Q_{ST-saturation} = 0.62$ in September and $Q_{ST-saturation} = 0.64$ in October). Color wheel visualizations revealed converging hue and saturation trajectories, indicating gradual phenological changes in the post-growing season. September values exhibited the highest heritability ($h^2_{RGB} = 0.12-0.25$; $h^2_{HSB} = 0.12-0.29$) among measured optical traits.

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Conclusion This study demonstrates that RGB and HSB color parameters, extracted from high-throughput image analysis using CNN-based needle segmentation, capture both seasonal and genetic variation in Scots pine seedlings. The highest genetic differentiation and heritability occurred during early autumn, particularly in the blue and saturation parameters. These findings suggest that autumn color traits, quantifiable using simple digital imaging, can serve as cost-effective indicators in tree breeding programs.

Keywords Scots pine seedlings, Phenology, Autumn reddening, Heritability, Forest nursery, Affordable phenotyping, RGB imaging, HSB color, Deep learning, Convolutional neural network

1 Introduction

Climate change represents an increasing threat to forests worldwide, weakening ecosystems' resilience through rising temperatures, altered rainfall patterns, and intensified droughts, pests, and wildfires (Anderegg et al. 2020; Dale et al. 2001). Abiotic and biotic stress factors negatively affect biodiversity, forest health, and stability, highlighting the need for adaptive forest management strategies (Senf and Seidl 2021). Selecting resilient tree populations by assessing and evaluating their genetic and phenotypic traits is a key approach for breeding programs aimed at enhancing climate adaptability (Aitken and Whitlock 2013; Isabel et al. 2020). Genetic variability is a critical factor in selecting resilient trees, as it provides the basis for both natural and artificial selection (Agashe et al. 2023).

Scots pine (*Pinus sylvestris* L.) is one of the most widely distributed conifer species, covering nearly the entire Eurasian continent. As a pioneer species, it thrives in a diverse range of environments—from sandy soils to bogs, and from sea level to the timberline (Durrant et al. 2016). This remarkable adaptability is driven by a combination of phenotypic plasticity (Beaton et al. 2022) and adaptive genetic variation (Savolainen et al. 2004). The existence of genetic variability in Scots pine is crucial for adaptation to climate change, particularly in phenological traits such as frost hardiness, growth cessation in autumn, bud set, and juvenile reddening of seedlings (Kaur et al. 2023; Savolainen et al. 2004). Notably, genetic variability in juvenile reddening of Scots pine has been discussed in relation to frost hardiness (Pulkkinen et al. 2011; Toivonen et al. 1991).

In the Czech Republic, Scots pine is the second most common tree species and is thought to gain more silvicultural importance as climate change progresses (Brichta et al. 2023). Established breeding programs leverage the species' genetic diversity with the aim of supplying resilient, high-quality timber and supporting reforestation efforts under changing climate conditions. These programs are based on clonal seed orchards, which produce consistent, abundant, and easily harvestable yields of genetically superior seeds (Funda and El-Kassaby, 2012). From this perspective, superiority encompasses traits such as height, diameter, growth increment,

stem straightness, phenological timing (e.g., bud set and flushing), and stress resilience.

To optimize adaptability, it is essential to integrate genetic data with phenotypic traits when evaluating the performance of individual genotypes and populations (Stejskal et al. 2023). This can be achieved through a comprehensive examination of genetic variation across multiple traits in pedigreed progeny trials, plantations where mother trees are known and their progeny are replicated and tested for several production traits (Cappa et al. 2022).

High-throughput phenotyping (HTP) technologies, such as the most common and affordable RGB imaging, allow researchers to observe phenotypic traits in natural environmental conditions, directly linking genotypic potential with practical adaptability. Recent advances in imaging technology, particularly RGB phenotyping, provide a more accessible and cost-effective means of assessing plant traits (Bian et al. 2022). RGB imaging is increasingly important for large-scale trait assessment in forestry and offers valuable insights for forest management and breeding programs (Mazis et al. 2020; Schiefer et al. 2020). To evaluate RGB-based phenological dynamics, imaging systems like the worldwide platform PhenoCam are used to monitor vegetation processes through RGB images with remarkable spatial and temporal resolution (Cao et al. 2021).

While HTP and RGB imaging facilitate large-scale data collection on plant traits, deep learning algorithms like convolutional neural networks (CNN) enable their precise image analysis, detailed segmentation, and trait extraction (Jiang and Li 2020). Furthermore, CNN can also be used to improve the information obtained by RGB monitoring methods (Cao et al. 2021), which increases the ability to extract accurate phenotypic data (Grondin et al. 2023; Jiang and Li 2020). There are several common areas of application of CNNs in agriculture, such as plant and leaf disease detection and classification (Kang et al. 2024), fruit counting (Kang et al. 2024), weed identification (Jiang et al. 2020), or plant phenotyping in the sense of obtaining phenological and morphological traits (Jiang and Li 2020). In forestry, CNNs have been used for similar purposes, such as species classification (Schiefer et al. 2020), disease and disturbance monitoring (Hu et al.

2022), inventory data acquisition (Grondin et al. 2023; Schiefer et al. 2020), or phenological monitoring (Cloutier et al. 2024). For color detection, the challenge lies in the segmentation, especially for conifers at the needle-level precision (Huang and Huang 2019). A significant knowledge gap persists in effectively utilizing advanced phenotyping methods to assess genetic variation in forest trees. Specifically, color models such as RGB and HSB for accurate phenotyping of conifer seedlings with their inherent challenges remain underexplored.

In this study, we address a knowledge gap in conifer phenotyping by quantifying foliage coloration in one-year-old Scots pine seedlings originating from three genetically distinct populations. These color traits were then used to assess genetic variation both within and among populations. To capture this seasonal variation in needle color, we used a handheld digital camera, followed by precise needle-level segmentation using a CNN YOLOv7 (You Only Look Once version 7; Wang et al. 2022), after which the RGB and HSB parameters were extracted.

To guide our investigation, we formulated the following research questions. How does genetic diversity influence the seasonal phenology of needle coloration across populations and families of Scots pine? To what extent do advanced image-based phenotyping techniques enable the detection of fine-scale genetic variation in needle coloration?

2 Material and methods

2.1 Plant material and experimental design

Workflow of an experiment is displayed in Fig. 1. To assess genetic and phenotypic variability, we evaluated one-year-old Scots pine seedlings in their second growing season, sourced from three Czech 1st generation seed orchards: Plasy (P), Trebon (T), and Decin (D), representing plus trees (superior individual trees selected from population based on their growth and vitality) from three populations with distinct ecological and geographical origins. The grafts from plus trees were collected in approximately a 50 km perimeter around seed orchards. The seedlings were measured in three different periods (September, October, January). In total, 1788 seedlings were analyzed: 746 from Plasy (385 m a. s. l.; 49.9087617°N 13.4427394°E), 750 from Trebon (430 m a. s. l.; 49.0166106°N 14.8247583°E) = both lowland regions, and 292 from Decin (465 m a. s. l.; 50.8191364°N 14.1172131°E) = upland region. The altitude of the Decin seed orchard, however, does not correspond to the original altitude of the plus trees. The Decin population is characterized by higher altitudes (up to 723 m a. s. l.). The experiment was conducted as a common garden experiment with a nested (population → family → individual) design. The seedlings were

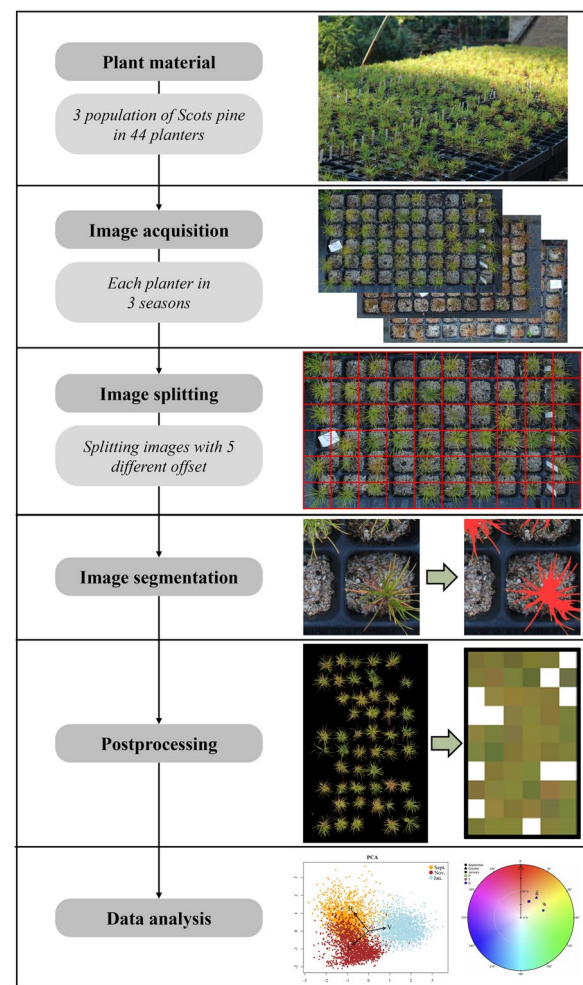


Fig. 1 Overview of the high-throughput phenotyping assay. Three populations of Scots pine from different seed orchards in the Czech Republic were grown in 44 planters. Images were captured of each planter across three periods (September, October, and January) to observe seasonal phenotypic changes. Each image was split into parts with five different offsets to maximize detail capture. Image segmentation was performed using a YOLOv7 CNN model to identify and isolate needle structures precisely. Post-processing steps were applied to remove the background, resulting in clean, segmented images of individual seedlings. Finally, data analysis was conducted on HSB and RGB color parameters, examining seasonal and genetic variability through statistical analysis

cultivated in the arboretum Sofronka (331 m a. s. l.; 49.789113°N 13.386193°E) in planters containing 60 individuals of the same origin. In a single planter, there were always only seedlings from one of the populations, and single half-sib families shared the same line of cells. The study used progeny from 289 mother trees (P–96, T–122, D–71). The planters were arranged in an area of 1.6 m × 4.4 m (7.04 m²) close together. This area was in an open space without shade and with conditions as

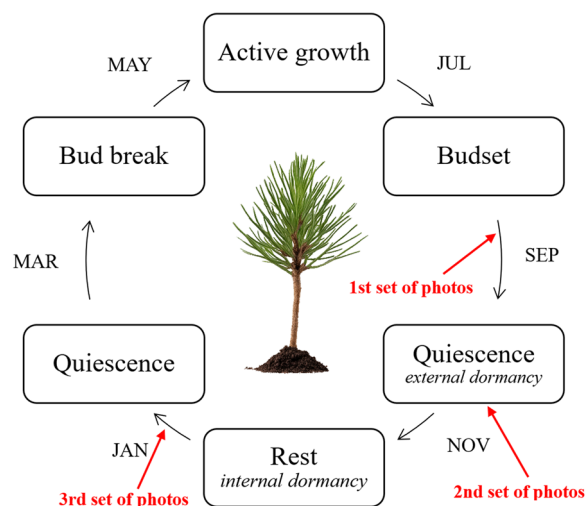


Fig. 2 Image acquisition in relation to the phenological cycle. The red arrows indicate the specific periods within the phenological cycle when the images were captured

homogeneous as possible. The figure of the mean and minimum temperature is included in the Appendix Fig. 9. For more details, see Stejskal et al. (2023).

2.2 Image and hyperspectral data acquisition

Photographs were taken during three different periods (Fig. 2). The first observation day was September 10th, 2021. The second observation day occurred on October 23rd, 2021, and the third on January 22nd, 2022. Each time, the images were taken between 2:30 PM and 3:30 PM without interruption to minimize changes in ambient conditions. The photographs were taken with a digital single-reflex camera Canon EOS 1200D, handheld and saved in the DNG format (5184×3456 pixels), which does not compress the images. The camera's auto mode settings were utilized for each observation day to handle the adjustments.

2.3 Color information acquisition from seedlings

A series of sequential steps was required to obtain average RGB or HSB values for each seedling, which will be described below. The codes for the individual steps and other algorithms described in this section were developed in various programming languages (ImageJ Macro Language, R-software, Python). The entire segmentation and color acquisition pipeline is available at Figshare (Chuchlík 2025) or in Github: https://github.com/jirkachuchl/pine-seedling_segmentation.

2.3.1 Image standardization using a white reference

The first step involved color standardization using a white reference to minimize external variability, such

as changes in lighting caused by cloud cover or variations in sunlight angle at different times of day, ensuring the images were comparable. The standardization process was conducted using a custom macro in FIJI software (Schindelin et al. 2012). Each image of the planter was split into its RGB color channels, allowing for separate standardization of each channel. A white reference area was selected in each channel, representing the full intensity value (255) for that color channel. The average intensity value of the white reference area was measured for each channel. To achieve standardization, the maximum intensity value (255) was divided by the measured average intensity of the white reference for each channel. This ratio was then applied to each pixel in the respective channel. After standardizing all three channels, they were recombined into a single RGB image for further analysis.

2.3.2 Image transformation

The next step involved compensating for the irregular shape of the planter in an image caused by handheld photography. We applied a perspective transformation (from a pre-built OpenCV package in Python) to rectify the planter into a rectangular shape. First, it was necessary to save the pixel coordinates of the corners of each seed tray. The pixel coordinates of each planter's corners were used to transform the image. During the transformation, the image was also cropped. The edges of each image were trimmed to leave only 400 pixels (approximately 5 cm) from the line connecting the seed tray's marked corners, forming the planter's edge. The transformation was implemented in Python, version 3.12 (Van Rossum and Drake 2009).

2.3.3 Image splitting

After the transformation, the images were split into parts of 600×600 pixels due to the limited size of the input images to the YOLOv7 models. Using larger images would first require resizing, which would significantly reduce quality and could result in unsuccessful or significantly worse segmentation (Wang et al. 2022). Each image was cut five times with different offsets: the first cut started at the pixel coordinates (0,0), and subsequent cuts were shifted twice (200 and 400 pixels offset), both horizontally and vertically. This multiple cutting was used for two reasons. The first reason is that segmentation on the shifted part may be more precise. The second reason is that more overlapping parts create smoother transitions when combined into a whole image. Each part name included the planter number, offset details, and row and column information. Cutting the images into parts was implemented using a custom function in R software, version 4.2.2 (R Core Team, 2023).

Table 1 Hyperparameters used during training. Image size (resolution in pixels); epochs (training iterations); batch size (images processed before model parameters update); learning rate (step size controlling the parameters shift); momentum (inertia term that smooths parameter updates); weight decay (penalty to prevent overfitting)

Hyperparameters	Values
Image size	640 × 640
Epochs	200
Batch size	16
Learning rate	0.01
Momentum	0.937
Weight decay	0.0005

2.3.4 Segmentation model

The custom YOLOv7 model for instance segmentation was created using training data, with 417 parts of the images annotated using the online application Roboflow ("Roboflow," (n.d)). The annotated dataset was split into training and validation sets in an 80:20 ratio. The training dataset was extended using data augmentation to enhance model robustness and generalization while reducing the risk of overfitting. In our study, data augmentation was performed in Roboflow using several augmentation steps: horizontal flip, 90° rotations, random crop between 0 and 31% zoom, rotation and shear between $\pm 15^\circ$, hue adjustment between $\pm 31^\circ$, saturation, brightness, and exposure adjustment between 25%, blur—up to 2 pixels, noise—up to 5% of pixels, cutout—11 boxes with 8% size each, and bounding box 90° rotation. As a result, the model was trained on 1081 images, with 996 images in the training dataset and 85 images in the validation dataset.

Training the segmentation model and the segmentation itself was performed in the Google Colab environment ("Google Colab," (n.d)). Google Colab is an environment that allows writing and running scripts in Python and provides free access to the NVIDIA Tesla T4 graphics processing unit (GPU). This study used a transfer learning approach due to the relatively small dataset. Instead of training the YOLOv7 model from scratch, the training process was initialized using pre-trained weights from the COCO dataset (Lin et al. 2014). The hyperparameters used during model training are provided in Table 1. The total training time was 180 min.

As evaluation metrics were used: "precision," "recall," and "mAP@0.50" (mean Average Precision at an intersection over the union threshold of 0.50). "Precision" measures the ratio of correctly identified positive instances to the total predicted positive instances. "Recall" assesses the ability of the model to identify all relevant instances,

Table 2 Values of the evaluation metrics. The box values indicate bounding-box detection parameters; Seq values indicate instance segmentation parameters

Metrics	Box	Seq
Precision	0.933	0.933
Recall	0.910	0.910
mAP@0.50	0.950	0.942

showing the proportion of true positives out of all actual positives. The "mAP@0.50" evaluates overall object detection accuracy, reflecting the model's performance in correctly identifying and localizing objects. The values of the evaluation metrics are shown in Table 2. Class names and bounding boxes were removed from the segmentation output. The segmentation result can be seen in Fig. 3B.

2.3.5 Connecting parts to a whole picture and unification of offsets

After removing the background, individual parts were combined into complete images. Five outputs were obtained from each planter. The first output was aligned with no offset, while subsequent outputs used horizontal or vertical offsets of 200 px or 400 px. An example of a single uncombined output is shown in Fig. 3D. These images were combined into the final output to ensure smooth, seamless transitions. If a pixel had a color in any image, it was preserved during the merging process. An example of such a unified final output can be seen in Fig. 3E. The unification process was implemented in Python.

2.3.6 Identification of individual trees and acquisition of RGB and HSB values

Each seedling was assigned to the correct planter cell by defining the perimeter of the planter through two selected diagonal corners on an image. Each planter had ten columns and six rows, with cell dimensions determined by dividing the distance between corners by the number of rows and columns. Using a Python script, the coordinates of each pine seedling's terminal bud were recorded interactively by clicking on each bud. These coordinates were matched to planter cells, and each tree was assigned an identification number.

The average RGB and HSB values were calculated for each seedling based on a radius around the terminal bud, defined by the shorter side of the cell. Although the selected diameter primarily encompassed the needles of the target seedling, it occasionally included parts of neighboring seedlings' needles. However, due to the optimized radius, such cases were infrequent and

had a negligible effect on the average color. All black pixels within this area were excluded, focusing only on colored pixels representing needles or buds. This data was assigned to each identified seedling. The average color detection scheme is shown in Fig. 3F–G. The entire color table from different periods is included in Fig. 4. The acquisition of RGB and HSB was implemented in Python.

2.3.7 HSB and RGB color systems

To better evaluate the changes in RGB channels, the differences of these channels across seasons were calculated. The HSB color system is a cylindrical representation that organizes colors based on three main dimensions: hue—the color type, measured in degrees from 0° to 360°, saturation—the intensity or purity of the color, from 0% for grayscale to 100% for fully saturated, and brightness—value from 0% for black to 100% for maximum brightness (Hanbury and Wien 2003). Because the hue value in the HSB color model is circular, we used circular statistics for basic analysis (the *circular* package in R-software). For modeling, however, we used the untransformed data since our measured hues ranged only from 2 to 109° and did not cross the 0°/360° boundary. To avoid confusion, we are clarifying here the abbreviations of individual RGB channels and HSB parameters: R—red channel, G—green channel, B—blue channel, HUE—hue parameter, SAT—saturation, and BRI—brightness. If a color is mentioned in the text by word and not by abbreviation, it is not related to RGB but a description of HUE or some elements.

2.4 Statistical analysis

For statistical analysis, R-software, version 4.2.2 (R Core Team, 2023) and the ASReml-R package, version 4 (Butler et al. 2023) were used. A univariate linear mixed model was fitted to evaluate individual color traits with the following terms:

$$y = X_1\beta + Z_1f + Z_2u + e \quad (1)$$

where y corresponds to the vector of phenotypic traits, β represents the fixed effects associated with the origin of the population (P, T, or D), u is the random effect of the planter, and f is the random effect of the mother. X_1 , Z_1 , and Z_2 are the respective incidence matrices associated with fixed and random effects, and e is the random error term with $e \sim MVN(0, R_{mat})$, where R_{mat} is the variance–covariance matrix of errors for each field position.

Two forms of the R_{mat} were evaluated: one considering independent errors, i.e., $R_{mat} = \sigma^2 I_n$, where I_n is an identity matrix of order n and another based on a separable first-order autoregressive process (AR_1) in rows and columns, where the R_{mat} is:

$$R_{mat} = \sigma^2 [AR_1(p_{col}) \otimes AR_1(p_{row})] \quad (2)$$

where σ^2 represents the residual variance, and $AR_1(p_{col})$ and $AR_1(p_{row})$ represent first-order autoregressive correlation matrices. This approach reduces the influence of spatially correlated residuals by down-weighting information from neighboring positions. Additionally, the box was included as a random effect to capture planter-related variability and correct for systematic differences introduced during planting.

Several models were fitted, considering independent or autoregressive errors and with or without polynomial terms. The best model was selected based on likelihood ratio tests (LRT) (Isik et al. 2017). Wald tests (approximated F -test; Isik et al. 2017) were applied to assess differences among populations fitted as fixed effects. Population-level means were extracted with all three pairwise contrasts (D – P, D – T, P – T); standardized effect sizes (Cohen's d) for the same contrasts were calculated with the *effsize* package. Due to the complexity of our dataset, including a 2D autoregressive structure, several missing values, and multiple random effects, freely available packages could not accommodate our modeling needs. ASReml-R was the only tool capable of handling this combination. Full ASReml-R code is available on Figshare (Chuchlík et al. 2025) to support reproducibility: <https://doi.org/10.6084/m9.figshare.28239326.v2>.

Narrow-sense heritability (h^2) was estimated as:

$$h^2 = \frac{4 \times \sigma_f^2}{\sigma_f^2 + \sigma_u^2 + \sigma_e^2} \quad (3)$$

where σ_f^2 is the random effect of mother (1/4 of additive genetic variance $-\sigma_a^2$); σ_u^2 denotes the variance attributable to planter effects, and σ_e^2 is the residual variance. We derived the approximate standard errors (SE) of h^2 using the Delta method (Butler et al. 2023). The significant values of h^2 in the results are also based on the likelihood ratio test (LRT). The LRT compares the goodness of fit between the null model (without the genetic component) and the model based on Eq. 1.

Variance at population level was evaluated using the Q_{ST} metric (Spitze 1993). We used a similar model as in Eq. 1, except that the population level was treated as a random effect to extract the respective variance component σ_p^2 – among populations genetic variance:

$$Q_{ST} = \frac{\sigma_p^2}{\sigma_p^2 + 2 \times \sigma_a^2} \quad (4)$$

Q_{ST} is defined as the proportion of variation in a given trait that is attributable to genetically based differences among populations, as scaled to the total genetic

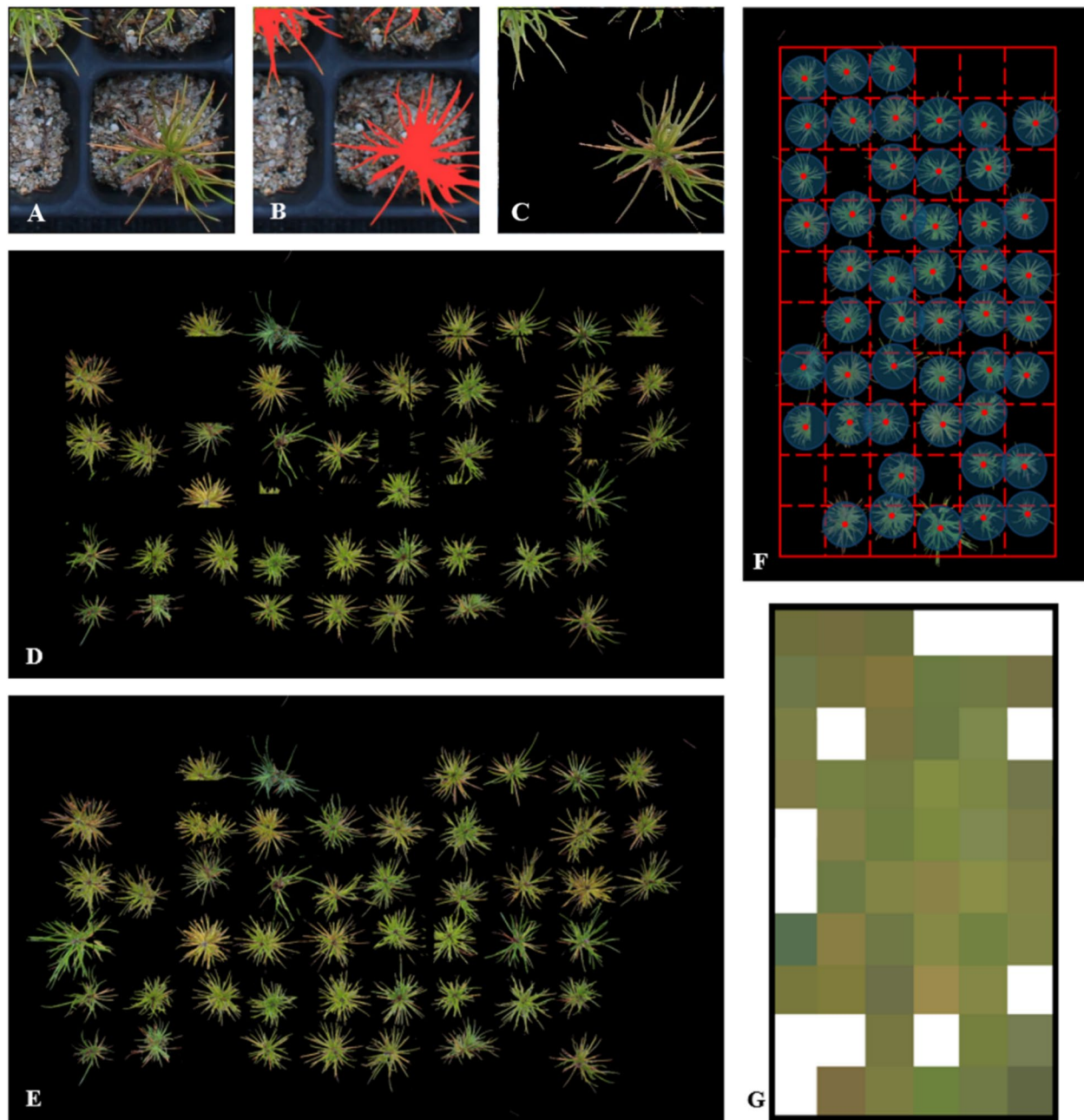


Fig. 3 Framework of pine seedling segmentation. **A** Part of original RGB image, **B** segmented areas highlighted by red, **C** the object of interest without background, **D** output after joining the individual segmented parts, **E** the unification of individual outputs into the final image, **F** identification and detection of average RGB values—the solid red line marks the planter's edge, the red dashed lines mark the edges of individual cells, the red dots mark the marked terminal buds, and the blue circles mark the area from which the average RGB/HSB values were obtained for each seedling, **G** map of average colors obtained from the corresponding seedling; white squares represent empty cells

variation in the trait. LRT and SE were calculated similarly as mentioned before, with a null model with the random population term omitted.

For season differences, a repeated-measure model was fitted as:

$$y = X_1\beta_1 + X_2\beta_2 + X_3\beta_{1 \times 2} + Z_1f + Z_2u + e \quad (5)$$

where y corresponds to the vector of phenotypic traits, β_1 represents the fixed effects associated with the origin of the population, β_2 the season, and $\beta_{1 \times 2}$ their interaction, u represents the random effect of the planter and f the

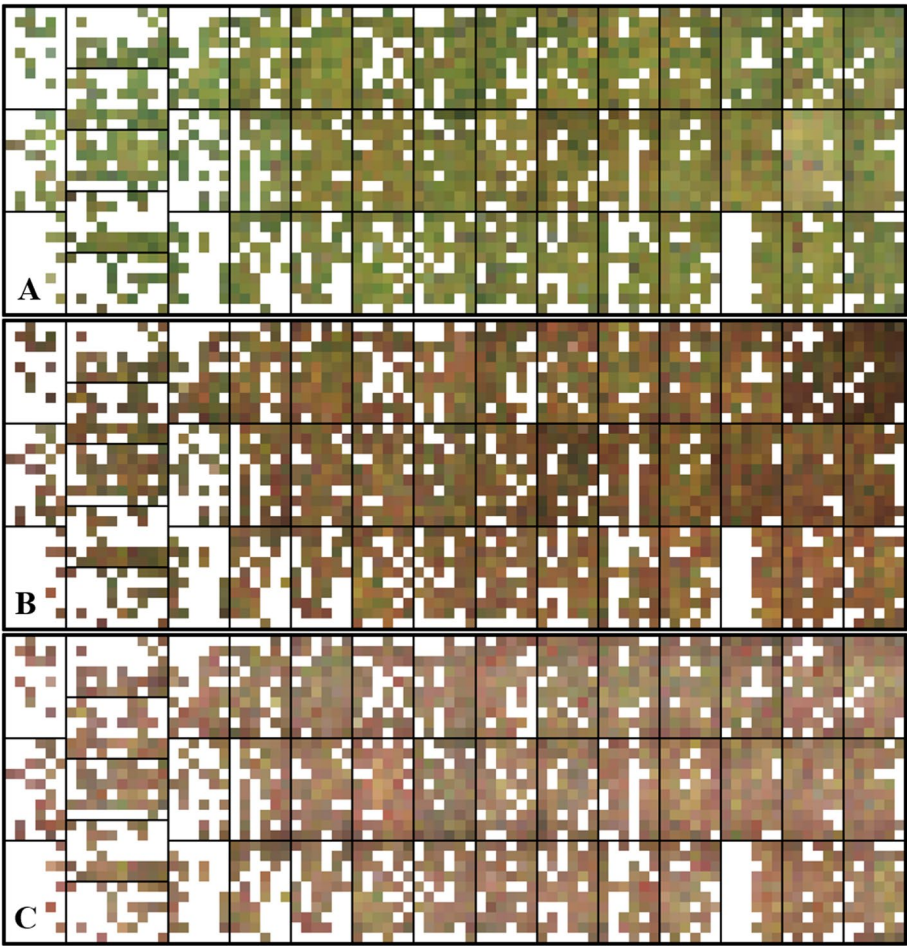


Fig. 4 Average colors obtained from 3 periods. Black lines separate individual planters. Each cell represents the average color of an individual seedling. Blank cells represent empty cells or dead seedlings. **A** Average colors in September, **B** average colors in October, **C** average colors in January

Table 3 Pairwise comparison of seasons in RGB and HSB. Differences in each parameter between seasons are shown along with the corresponding *p*-values. Significant differences (*p*-value < 0.05) are highlighted in bold

	R		G		B		HUE		SAT		BRI	
	diff.	<i>p</i> -value	diff.	<i>p</i> -value	diff.	<i>p</i> -value	diff.	<i>p</i> -value	diff.	<i>p</i> -value	diff.	<i>p</i> -value
Sept-Oct	11.64	<0.0001	36.00	<0.0001	3.66	<0.0001	0.08	<0.0001	−0.06	<0.0001	0.04	<0.0001
Oct-Jan	−45.55	<0.0001	−32.06	<0.0001	−32.96	<0.0001	0.02	<0.0001	0.19	<0.0001	−0.13	<0.0001
Sept-Jan	33.91	<0.0001	−3.94	<0.0001	29.29	<0.0001	−0.10	<0.0001	−0.15	<0.0001	0.09	<0.0001

random effect of the mother. X_n and Z_n are the respective incidence matrices associated with fixed and random effects, and e is the random error term with $e \sim MVN(0, R_{seasons})$, with $R_{seasons}$ being a block-diagonal unstructured 3×3 variance–covariance matrix for the repeated seasonal records on the same seedling ID. Significance of seasonal contrasts was calculated using the *predictPlus* function from the *asremlPlus* package.

3 Results

3.1 Differences in RGB channels due to phenology

RGB channels of pine seedling needles observed across seasons exhibited differences as demonstrated by pairwise comparison from Eq. 5 (Table 3). In all RGB channels, seasons significantly differ. While all seasons significantly differ, the smallest differences were in September–October in B and in September–January in G.

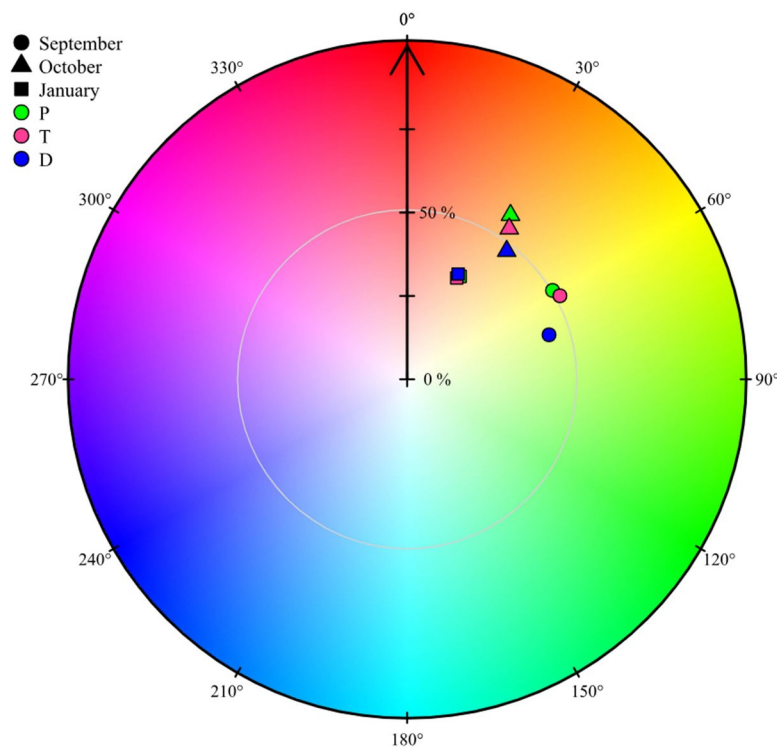


Fig. 5 Color wheel with hue and saturation means of individual populations. The angle 0–360° indicates the hue of the color, and the distance from the center of the circle indicates the saturation of the color. The colors in the center of the wheel are achromatic, and the colors on the circle's edge are saturated. The auxiliary grey circle indicates a 50% saturation. Individual shapes correspond to seasons, and their colors correspond to populations

3.2 Differences in HSB parameters due to phenology

Similar to RGB, HSB color parameters showed distinct seasonal variations in the needle color of Scots pine seedlings (Table 3). For HUE, the smallest but still very significant differences were between October and January. The smallest difference in SAT was between September and October. For BRI, the most similar seasons were September and October.

HUE and SAT differences among populations are displayed in the color wheel (Fig. 5). Populations varied in HUE depending on the period. September HUE was light green to yellow, October HUE was brown-orange, and January HUE was orange-red. SAT in September and October measurements were relatively similar. In January, SAT dropped significantly (also visible in Fig. 4). During September and October, the Decin (D) population exhibited more pronounced differences in both HUE and SAT compared to the other two populations. SAT of the D population was lower, and HUE was slightly higher. On the other hand, in September and October, the Plasy

(P) and Trebon (T) populations had a similar HUE and relatively similar SAT. In January, SAT and HUE of all three populations hardly differed.

3.3 RGB heritability and population comparison

Differences among populations were calculated based on the parameters of the RGB model. Color channels were used as response variables, and narrow-sense heritability (h^2) was estimated from the random variance components. The h^2 and corresponding standard error for RGB in various seasons are detailed in Table 4. In September, h^2 was significant in all channels and reached the highest values, ranging from 0.11 to 0.25. In October and January, the only significant h^2 was shown in G.

The Wald test was used to test the significance of differences among populations and is determined by the p -value, reported in Table 5. Significant population differences emerged in B in September and in G and B in October. A similar pattern is visible in Fig. 6. Details on

Table 4 Narrow-sense heritability and Q_{ST} of color trait. The table shows the heritability estimates (h^2) and its standard error (SE_{h^2}), and Q_{ST} and its standard error ($SE_{Q_{ST}}$), indicating population differentiation for each color trait. Statistically significant values (LRT p -value < 0.05) are highlighted in bold

Color traits in seasons		h^2	SE_{h^2}	Q_{ST}	$SE_{Q_{ST}}$
September	R	0.25	0.074	0.07	0.099
	G	0.24	0.070	0.09	0.129
	B	0.12	0.039	0.64	0.264
	HUE	0.12	0.071	0.61	0.279
	SAT	0.15	0.056	0.62	0.268
	BRI	0.29	0.074	0.02	0.056
October – September shifts	R	0.28	0.065	0.13	0.167
	G	0.20	0.060	0.20	0.216
	B	0.16	0.041	0.44	0.299
October	R	0.05	0.045	0.32	0.392
	G	0.11	0.059	0.27	0.278
	B	0.03	0.042	0.94	0.210
	HUE	0.03	0.073	0.31	0.418
	SAT	0.16	0.072	0.64	0.253
	BRI	0.05	0.045	0.30	0.373
January – October shifts	R	0.00	0.000	1.00	3.06×10^{-6}
	G	0.02	0.038	0.79	0.348
	B	0.00	0.000	1.00	1.17×10^{-6}
January	R	0.00	0.000	0.99	0.451
	G	0.12	0.068	0.05	0.089
	B	0.00	0.000	0.12	0.000
	HUE	0.24	0.087	0.01	0.036
	SAT	0.05	0.064	0.00	0.000
	BRI	0.00	0.000	1.00	0.033

population-specific differences, effect sizes, and post-hoc test p -values are provided in Table 5. In September, the lowland populations (P and T) showed no differences across any channels; however, the upland population D differed significantly from both lowland populations. By October, notable differences appeared between populations D-P and P-T in G and B.

To evaluate the relative contribution of population-versus family-level genetic variation, in RGB channels, Q_{ST} was calculated (Table 4). Higher Q_{ST} values indicate stronger population differentiation, while low values suggest most genetic variance occurs within populations. Q_{ST} for R and G in September is very low and insignificant, suggesting little genetic differentiation among populations for these channels this month. B, however, shows a moderate Q_{ST} , indicating more pronounced population-level variance for blue coloration. In October, strong population variation is shown in B. In G, the Q_{ST} indicates a moderate population differentiation. In January,

none of the RGB channels displayed a significant portion of population-level variation.

3.4 Heritability of RGB shifts and population comparison

To further elucidate the genetic basis of color change across seasons, h^2 was estimated for the differences between RGB channels across consecutive seasons. Estimates of h^2 and their SE are presented in Table 4. Notably, the transition from September to October exhibited significant h^2 in all RGB channels, with the highest h^2 observed for R. For the differences from October to January, h^2 dropped sharply.

RGB shifts in season show significant differentiation in all channels in both transitions except for R in the January–October period (Table 5). In the October–September period, Trebon differed strongly from both Decin and Plasy, especially in B, while Decin and Plasy did not differ significantly from each other. For the January–October difference, the most notable differentiation was consistently between Plasy and Trebon across all channels, with the strongest effects in G and B. Differences are visually demonstrated in the boxplot in Fig. 7.

Q_{ST} values were calculated to attribute the proportion of variance in color change that is due to population-level genetic differences (Table 4). For the October–September difference, the Q_{ST} value was moderate for G and higher for B. In contrast, the January–October difference showed an extremely high Q_{ST} value for B ($Q_{ST}=1.000$) and a high value for G (0.791), though these are accompanied by very low or zero heritability. This pattern indicates that, while almost all genetic variance in these color shifts is among populations.

3.5 HSB heritability and population comparison

Narrow-sense heritability and population differences for the HSB model were calculated similarly to the RGB model, with detailed h^2 estimates and the population differentiation (Q_{ST}) presented in Table 4. In September, all three HSB parameters showed significant h^2 , with BRI exhibiting the highest estimate. By contrast, in October, only SAT had a significant h^2 , while in January, only HUE remained significant.

Statistically significant population differences were observed in HUE and SAT in both September and October. Differences between the lowland (P and T) and upland (D) populations were significant in September and October. Notably, significant differences between the two lowland populations (P and T) appeared only in October for SAT. In January, populations did not differ significantly in any HSB parameter (Table 5). These differences are also shown in Fig. 8.

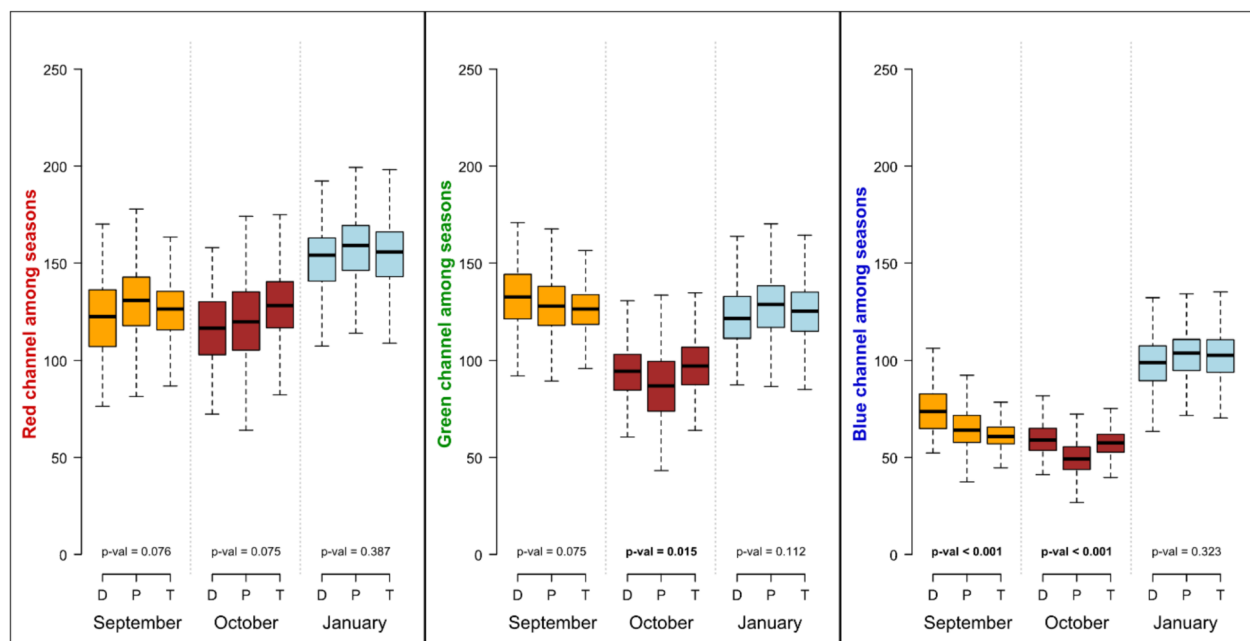


Fig. 6 Boxplot with differences among populations across seasons in RGB. Individual colors of boxplots represent seasons; in each season, there are 3 boxplots representing values from individual populations (P–Plasy, T–Trebon, D–Decin) in the given season. The significance of differences is demonstrated by the p-value under each triplet of boxplots. The significant values are bold

Table 5 Differences among populations based on color traits. The Wald test (modified F-test) tests for overall differences among populations. Pairwise differences between populations are assessed using the Least Significant Difference (LSD) test. For each comparison, differences (diff.), p-values, and Cohen's d (standardized effect sizes) are shown. Significant p-values ($p < 0.05$) are in bold

Color traits in seasons		p-value (Wald)	Decin–Plasy			Decin–Trebon			Plasy–Trebon		
			diff.	p-value	Cohen's d	diff.	p-value	Cohen's d	diff.	p-value	Cohen's d
September	R	0.076	–8.794	0.025	–0.46	–3.022	0.429	–0.19	5.772	0.099	0.32
	G	0.075	5.956	0.104	0.33	8.292	0.020	0.57	2.336	0.479	0.17
	B	<0.001	10.750	0.001	0.73	14.244	<0.001	1.43	3.494	0.251	0.40
	HUE	<0.001	0.040	<0.001	1.13	0.031	<0.001	0.96	–0.008	0.087	–0.24
	SAT	<0.001	–0.074	<0.001	–0.98	–0.082	<0.001	–1.25	–0.008	0.563	–0.17
	BRI	0.280	0.002	0.883	–0.01	0.020	0.164	0.32	0.017	0.178	0.31
October - September	R	0.043	–6.164	0.287	–0.28	7.484	0.185	0.48	13.648	0.009	0.69
	G	0.025	–1.206	0.814	–0.11	10.831	0.030	0.66	12.037	0.010	0.69
	B	0.004	0.147	0.971	–0.08	11.307	0.004	1.21	11.160	0.002	1.01
October	R	0.075	–2.846	0.571	–0.13	–10.681	0.029	–0.65	–7.834	0.085	–0.43
	G	0.015	7.315	0.049	0.47	–2.648	0.465	–0.18	–9.963	0.003	–0.65
	B	<0.001	10.572	<0.001	1.15	2.666	0.228	0.25	–7.906	<0.001	–1.04
	HUE	0.005	0.014	0.001	0.48	0.009	0.025	0.31	–0.005	0.141	–0.17
	SAT	<0.001	–0.098	<0.001	–1.47	–0.062	<0.001	–0.93	0.036	<0.001	0.60
	BRI	0.078	–0.011	0.588	–0.12	–0.041	0.031	–0.65	–0.031	0.084	–0.43
January - October	R	0.067	0.744	0.897	0.15	–10.517	0.060	–0.55	–11.261	0.031	–0.62
	G	0.004	12.171	0.005	0.78	–0.572	0.893	0.05	–12.743	0.001	–0.80
	B	0.001	12.880	0.000	0.99	4.269	0.203	0.42	–8.611	0.006	–0.67
January	R	0.387	–3.716	0.205	–0.36	–0.864	0.761	–0.15	2.851	0.269	0.21
	G	0.112	–4.545	0.038	–0.37	–1.969	0.354	–0.19	2.576	0.167	0.19
	B	0.323	–2.952	0.143	–0.32	–2.376	0.223	–0.25	0.576	0.741	0.06
	HUE	0.431	–0.005	0.225	–0.13	–0.001	0.706	–0.02	0.003	0.323	0.11
	SAT	0.451	0.002	0.798	0.06	0.012	0.251	0.20	0.009	0.325	0.14
	BRI	0.388	–0.015	0.206	–0.36	–0.003	0.764	–0.15	0.011	0.269	0.21

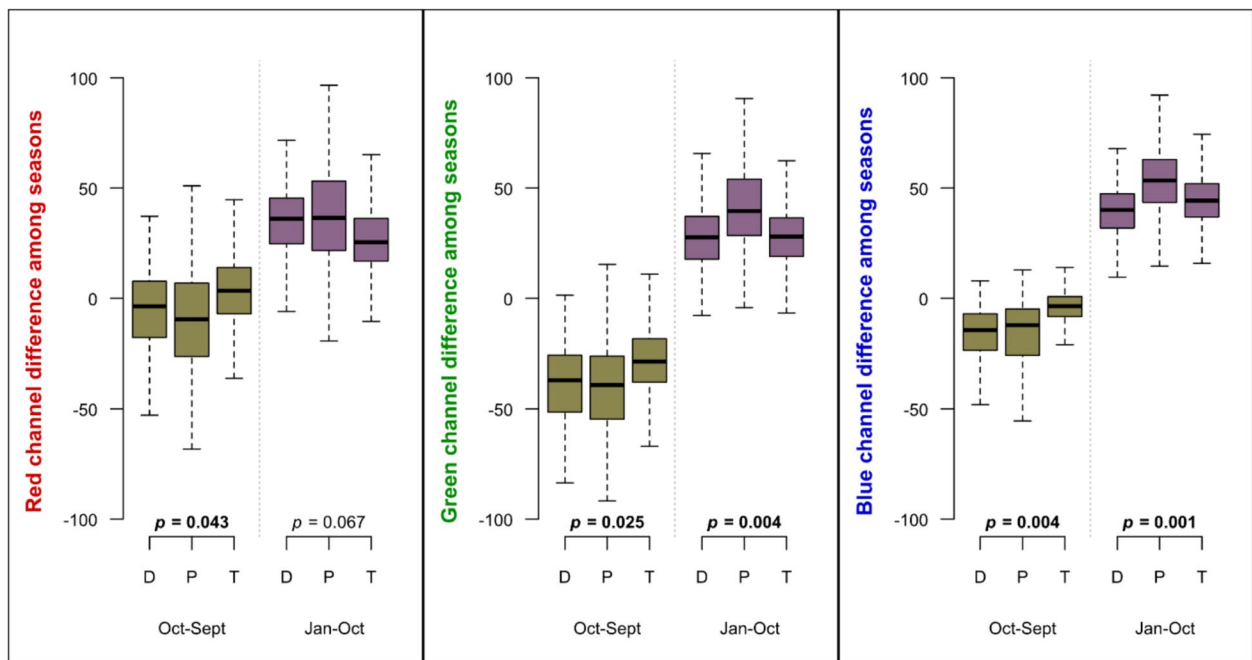


Fig. 7 Boxplot with differences among populations across seasons in RGB shifts. Individual colors of boxplots represent shifts in seasons; in each change, there are 3 boxplots representing values from individual populations (P–Plasy, T–Trebon, D–Decin). The significance of differences is demonstrated by the p -value under each triplet of boxplots. The significant values are bold

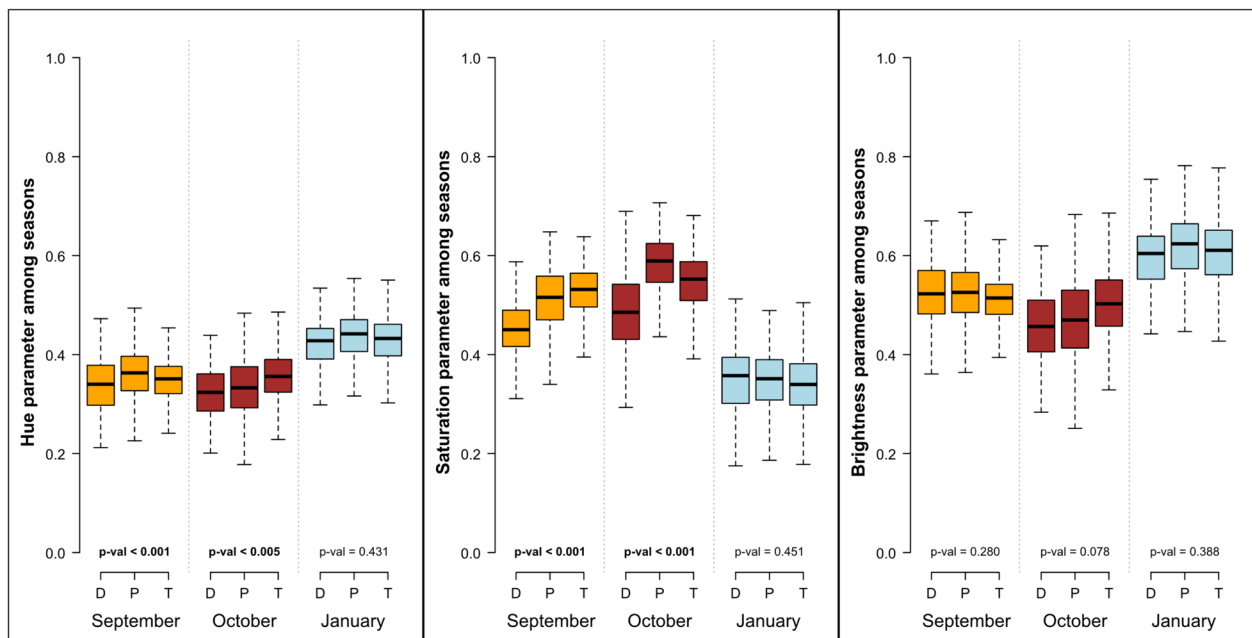


Fig. 8 Boxplot with differences among populations across seasons in HSB. Individual colors of boxplots represent seasons; in each season, there are 3 boxplots representing values from individual populations (P–Plasy, T–Trebon, D–Decin) in the given season. The significance of differences is demonstrated by the p -value under each triplet of boxplots. The significant values are bold

In September, both HUE and SAT show high Q_{ST} values, indicating substantial genetic differentiation among populations for these color traits. In October, Q_{ST} remains moderate to high for SAT, showing continued

strong differentiation among populations. HUE displays moderate Q_{ST} values, reflecting some population structure but less pronounced than in September. In January, Q_{ST} values for HUE and SAT drop to near zero, indicating

negligible population differentiation for these parameters in winter.

4 Discussion

This study demonstrated that seasonal color variation and the assessment of within and between population genetic variation in Scots pine seedlings using RGB and HSB imaging combined with CNN-based segmentation. In the following sections, we contextualize these findings within the biological significance of phenological changes like autumn reddening in conifer seedlings, exploring its potential implications for breeding.

4.1 Seasonal color variability observed in pine seedlings

Color differences between October 2021 and January 2022 are visible in shifts of R and B, resulting in needle reddening, which could be observed in seedlings of every population and every family. Nozzolillo et al. (1990) observed that coloration is most prominent in first-year seedlings.

Autumn reddening, or purpling, has been observed in seedlings of various pine species in response to shortening day lengths and colder temperatures (e.g., *Pinus banksiana* Lamb.—Nozzolillo et al. 1990; *Pinus sylvestris*—Toivonen et al. 1991). The pigments responsible for this purpling observed in *Pinus* species were described as anthocyanins, specifically cyanidin-3-glucoside and delphinidin-3-glucoside (Toivonen et al. 1991). Krol et al. (1995) described this reddening in *Pinus* seedlings as a protective mechanism for mesophyll cells against low temperature and photoinhibition.

Pulkkinen et al. (2011) observed higher long-term survival in individuals with pronounced autumn purpling, suggesting a potential adaptive advantage. While there is a link between population origin and the extent of reddening, a study by Toivonen et al. (1991) reported no direct correlation between purpling and frost hardiness, indicating that further research is needed to understand the adaptive significance of this phenomenon fully.

Aside from anthocyanins, the reddish coloration may result from seasonal shifts in pigment composition, particularly the increased ratio of carotenoids to chlorophyll, which is characteristic of leaf senescence (Neuwirthová et al. 2021). Consistent with this, previous studies have observed a decline in chlorophyll a + b and a relative increase in the carotenoid-to-chlorophyll ratio during late summer and autumn in pine needles (Wang et al. 2003). As chlorophyll content diminishes during cold acclimation, the presence of carotenoids becomes more visually apparent (Seyednasrollah et al. 2021). These seasonal pigment dynamics facilitate the acclimation of conifers to the low-light and low-temperature conditions typical for autumn (Gamon et al., 2016;

Miettinen et al. 2025). This agrees with our observations, where G effectively distinguished September from October and January, highlighting the potential of G as a marker for early-autumn phenology assessment. For instance, a G channel-based index has been shown to correlate strongly with chlorophyll content during seasonal transitions in conifers (Seyednasrollah et al. 2021).

Seasonal RGB shifts demonstrated pronounced population-level differentiation, with the highest heritability (h^2) observed for R during the autumn transition (September–October). These findings align well with studies on conifers, which have shown that seasonal shifts in canopy color captured by indices such as green and red chromatic coordinates are robust indicators of physiological transitions and are sensitive to environmental factors (Liu et al. 2020; Seyednasrollah et al. 2021).

Similar to RGB, we observed seasonal changes in the HSB parameters of pine seedling needles; specifically, the color changes from green to orange HUE from September to October. The green HUE of needles in September is primarily a result of chlorophyll's strong absorption of light at specific wavelengths. We suppose that in October, the chlorophyll content decreased, and the anthocyanin content increased.

4.2 Narrow-sense heritability in RGB and HSB parameters

We demonstrated the presence of additive genetic variability in all color parameters, with h^2 estimates reaching up to 0.29. Genetic variability in color traits could be attributed to variable pigment content. Several studies have confirmed significant h^2 of chlorophyll content (Čepl et al. 2018; Tao et al. 2021), likely manifested by significant h^2 of various color parameters. Comparable studies estimating h^2 for color parameters are limited, particularly in juvenile conifer seedlings. Notably, Tao et al. (2021) reported significant heritability estimates (site averages of 0.21–0.26) for all three RGB channels in a 7-year-old slash pine (*Pinus elliottii* Engelm.) plantation, based on replicated progeny trials and multispectral imaging conducted in September. In contrast to Tao et al. (2021), we also investigated h^2 in October and January, when h^2 was significant only in G, not reaching the September values in both months.

4.3 Among-population variability in RGB and HSB parameters

In our study, we find a significant variation among the studied populations in RGB channels. Based on Grubinger et al. (2025), changes observed in B early in the season could be partially explained by the presence of waxes, which, as shown by Holmes and Keiller (2002), increase reflectance in the blue spectral region (270–480 nm). The presence of waxes could be related to acclimation to high irradiance conditions typical of mountain environments (Grubinger et al. 2025). This would be in accordance with

the upland origin of D population, further supported by the high Q_{ST} values for B. The very high Q_{ST} value in October was caused by the almost zero heritability of such a parameter compared to the among-population variance.

In contrast to the September results, October revealed differentiation between the two populations from distinct altitudinal regions (D and P), as well as between the two lowland populations (P and T). No significant difference was detected between T and D, despite their contrasting geographic origins. This agrees with Stejskal et al. (2023), who measured hyperspectral reflectance with fiber optical cable and found significant differences in visible spectra in blue and green spectral regions only between D-P and P-T populations. This comparable result reveals the relative quality of RGB channels for population differentiation.

In September and October, variation in HUE and SAT is apparent between the upland population (D) and the lowland populations (P, T). This differentiation may stem from the D population's adaptation to harsher upland conditions. A study on Scots pine seedlings (Taulavuori et al. 2010) showed that populations growing at higher latitudes are more frost-resistant.

Contrary to findings by Toivonen et al. (1991), who observed earlier reddening in northern populations, in our study, the D population exhibits greener HUE; thus, presumably different pigment content in September and October than the lowland populations. At higher elevations, increased solar radiation reduces the need for high light-harvesting capacity, while simultaneously increasing the risk of photodamage due to excess light energy. Under such conditions, shifts in chlorophyll-to-carotenoid ratios may help prevent over-excitation of the photosystems (Tausz et al. 2001). This suggests that population variance observed in SAT may be caused by variance in chlorophyll and carotenoid levels. By January, before the start of the vegetative season, however, the average HUE and SAT values for each population almost met at the same position on the color wheel. This overlap may reflect a gradual phenological trajectory, where different populations of contrasting altitudinal regions ultimately reach a similar final state with variations only in the timing of this transition. This observation aligns with findings by Repo et al. (2000), who noted that while frost hardiness in Scots pine began at different times, fully hardened pines from different latitudes showed similar hardiness levels, indicating that all populations were sufficiently adapted to endure winter conditions.

In contrast to HUE and SAT, the populations do not exhibit differentiation based on BRI. This can be attributed to the standardization process applied to images to ensure their comparability. Through this standardization, factors such as varying ambient light conditions were eliminated. Consequently, the variance in BRI, which is

closely tied to the variability of ambient lighting (Vadivel et al. 2005), was also removed as part of this process.

5 Limitations

This study is limited by data collected on only three points, which restricts the full picture of temporal phenological trends and dynamics. Incorporating a long-term monitoring sensor for high-density temporal phenological monitoring (e.g., fixed camera above seedlings in a nursery setting) could provide a more comprehensive understanding of seasonal dynamics.

Due to the nature of our experiment, the images were taken under ambient light conditions, which may have introduced a bias. To minimize such bias, artificial illumination could eliminate variation caused by changing sunlight conditions.

Segmentation of individual seedlings was sometimes difficult due to overlapping needles from neighboring plants. To reduce this issue, the average color was calculated within a defined perimeter around the terminal bud. In some cases, small overlap remained, but the number of such pixels was low and likely did not affect the final color values in a significant way.

This study is limited by the absence of biochemical sampling, which means we do not have direct biochemical evidence (ground truth) to support our findings. To partially overcome this absence, we incorporated spectral vegetation indices, known to be correlated with vegetation pigments (see Appendix 2).

6 Conclusion

This study highlights the effectiveness of RGB and HSB imaging combined with CNN-based segmentation in distinguishing seasonal color variations and quantifying genetic variation in Scots pine seedlings. The findings reveal phenological changes, such as autumn reddening and the loss of green color towards winter. By confirming inter- and intra-population variability and heritability in color parameters such as HUE and SAT, HSB-based parameters proved to be informative metrics, capturing meaningful patterns of population-level color variation and offering an intuitive link to physiological and seasonal dynamics.

Notably, September exhibited the highest heritability in both RGB and HSB parameters, aligning with the peak in color variation. Seasonal shifts were evident in RGB and HSB metrics, particularly in the G channel, as well as in HUE and SAT. By January, the convergence of average HUE and SAT values across both altitudinal regions suggests a gradual phenological alignment, with different populations reaching a similar color status before the onset of the vegetative season.

These findings provide a foundation for integrating color-based phenotyping into genetic selection frameworks. Specifically, this research highlights the potential of early-autumn RGB and HSB traits, readily obtainable using simple digital cameras, as accessible and cost-effective markers that may be linked to adaptive traits. Future research should focus on exploring genetic correlations between color-based phenotyping and adaptive traits. The insights gained from this study advance our understanding of color variation in Scots pine seedlings and its relevance to within and among population-level variation.

Appendix 1
Temperature development in the common garden experiment

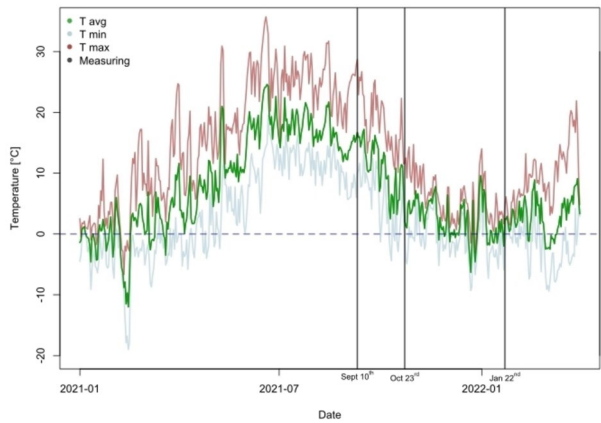


Fig. 9 Average, minimal, and maximal temperature (data from chmi.cz) in Arboretum Sofronka. Measurement dates are marked with vertical black lines, the average temperature is marked in green, the minimum in blue, and the maximum in red

Appendix 2
Correlation of color traits with reflectance indices
Our current study did not directly quantify pigment concentrations via biochemical assays. To overcome this shortage, we utilized spectral reflectance-derived indices, known from literature to reliably indicate respective pigment content (Appendix Table 6). These indices were subsequently correlated with image-based color parameters obtained from digital imaging, particularly RGB, HSB, and color coordinate metrics at the family level, using best linear unbiased predictions (BLUPs). Chromatic coordinates (R_{cc} , G_{cc} , and B_{cc} for red, green, and blue channels, respectively) were calculated as in Meyer and Neto (2008).

The hyperspectral reflectance was measured for each seedling between September 7th and 10th. The ASD FieldSpec 4 Wide-Res portable spectroradiometer (Malvern Panalytical Ltd., United Kingdom) was used to record the biconical reflectance factor in the span of 350 to 2500 nm. The measurements were done by fiber optical cable and were carried out under a clear sky with stable light. For more details, see Stejskal et al. (2023).

For correlation analysis, the Best Linear Unbiased Predictors (BLUPs) were obtained as random effects of individual mothers from Eq. 1. The Pearson correlation coefficient was calculated in R software. The observed correlations between reflectance indices and color parameters are presented in Appendix Fig. 9. BLUPs obtained from color traits show significant correlation with spectral vegetation indices. The correlation ranged from -0.628 to 0.631. The highest positive and negative correlation is shown in R_{cc} , highest with BLUP from spectral index Vog2, and lowest with spectral index MNDV18. The whole correlation matrix, including significances, is shown in Appendix Fig. 10.

The Wald test and pairwise comparison, h^2 , and Q_{ST} were also calculated from all 3 chromatic coordinates. Significant h^2 ranged from 0.12 to 0.16. The Wald test showed population differentiation in all chromatic coordinates in September and October. Q_{ST} was significant in coordinates among all seasons except B_{cc} in January. All results are shown in Appendix Table 7 and Appendix Table 8.

Table 6 Spectral vegetation indices

Index	Equation	Reference
RMCARI	$(X_{750} - X_{705}) - 0.2 \times (X_{750} - X_{550}) \times \frac{X_{750}}{X_{705}}$	Croft et al. (2014), Wu et al. (2008)
NDChI	$\frac{X_{925} - X_{710}}{X_{925} + X_{710}}$	Le Maire et al. (2008)
Vog2	$\frac{X_{734} - X_{747}}{X_{715} + X_{726}}$	Vogelmann et al. (1993), Dmitriev et al. (2024)
N705	$\frac{X_{705} - X_{675}}{X_{750} + X_{670}}$	Mišurec et al. (2016); Campbell et al. (2004)
MNDV18	$\frac{X_{755} - X_{730}}{X_{755} + X_{730}}$	Croft et al. (2014), Mutanga and Skidmore (2004)
SR1	$\frac{X_{750}}{X_{710}}$	Moorthy et al. (2003), Zarco-Tejada and Miller (1999)
GM_94	$\frac{X_{750}}{X_{700}}$	Croft et al. (2014), Gitelson and Merzlyak (1994)
ARI	$\frac{1}{X_{550}} - \frac{1}{X_{700}}$	Gitelson et al. (2001), Tao et al. (2021)
PRI	$\frac{X_{570} - X_{530}}{X_{570} + X_{530}}$	Gamon et al. (1992), Seyednasrollah et al. (2021), Wong and Gamon (2015)

Table 7 Narrow-sense heritability and Q_{ST} of RGB chromatic coordinates. The table shows the heritability estimates (h^2) and its standard error (SE_{h^2}), and Q_{ST} and its standard error ($SE_{Q_{ST}}$), indicating population differentiation for each color trait. Statistically significant values (LRT p -value < 0.05) are highlighted in bold

Chromatic coordinates in seasons		h^2	SE	Q_{ST}	SE
September	R _{cc}	0.16	0.066	0.627	0.260
	G _{cc}	0.08	0.069	0.264	0.285
	B _{cc}	0.12	0.055	0.701	0.241
October	R _{cc}	0.10	0.071	0.650	0.279
	G _{cc}	0.08	0.075	0.127	0.179
	B _{cc}	0.21	0.077	0.521	0.272
January	R _{cc}	0.07	0.060	6.13×10^{-7}	5.55×10^{-7}
	G _{cc}	0.28	0.088	1.57×10^{-7}	5.29×10^{-8}
	B _{cc}	0.18	0.078	0.001	0.026

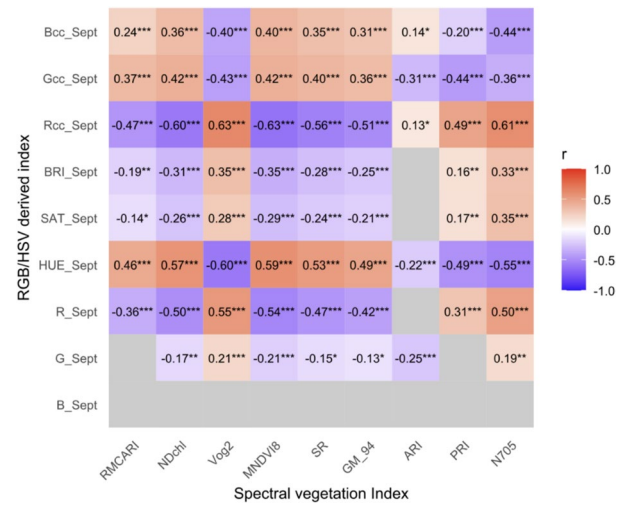


Fig. 10 Correlation matrix of BLUPs of spectral indices and color traits. Pearson correlation coefficients are used with asterisks denoting statistical significance as follows: for $p < 0.05$ *; for $p < 0.01$ **; and for $p < 0.001$ ***; statistically non-significant values are not displayed

Table 8 Differences among populations based on RGB chromatic coordinates. The Wald test (modified F -test) tests for overall differences among populations. Pairwise differences between populations are assessed using the Least Significant Difference (LSD) test. For each comparison, differences (diff), p -values, and Cohen's d (standardized effect sizes) are shown. Significant p -values ($p < 0.05$) are in bold

Chromatic coordinates in seasons		p -value (Wald)	Decin–Plasy			Decin–Trebon			Plasy–Trebon		
			diff	p -value	Cohen's d	diff	p -value	Cohen's d	diff	p -value	Cohen's d
September	R _{cc}	< 0.001	− 0.036	< 0.001	− 1.292	− 0.032	< 0.001	− 1.225	0.004	0.339	0.135
	G _{cc}	0.003	0.010	< 0.001	0.539	0.003	0.359	0.177	− 0.007	0.004	− 0.373
	B _{cc}	< 0.001	0.027	< 0.001	1.098	0.030	< 0.001	1.319	0.003	0.548	0.180
October	R _{cc}	< 0.001	− 0.038	< 0.001	− 1.205	− 0.022	< 0.001	− 0.757	0.015	< 0.001	0.510
	G _{cc}	0.021	0.010	0.004	0.381	0.005	0.126	0.215	− 0.005	0.106	− 0.172
	B _{cc}	< 0.001	0.027	< 0.001	1.270	0.017	< 0.001	0.785	− 0.010	< 0.001	− 0.517
January	R _{cc}	0.594	0.002	0.575	0.087	0.003	0.306	0.152	0.001	0.619	0.064
	G _{cc}	0.560	− 0.002	0.311	− 0.108	− 0.001	0.743	− 0.018	0.002	0.420	0.095
	B _{cc}	0.413	0.001	0.758	0.017	− 0.002	0.401	− 0.146	− 0.003	0.193	− 0.169

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Authors' contributions

Conceptualization: J.C., J.Č., E.N., and J.S. Methodology: J.C., J.Č., and J.S. Statistical Analysis: J.C., J.Č., and J.S. Formal Analysis: J.C., J.Č., J.S., and D.P. Investigation: J.C. Resources: J.C., J.Č., and J.S. Writing—Original Draft Preparation: J.C., J.Č., E.N., J.K., and J.S. Writing—Review and Editing: J.C., J.Č., E.N., J.K., and J.S. Visualization: J.C. and J.Č. Supervision: J.Č., J.S., and E.N. Project Administration: J.Č., J.K., and J.S. Funding Acquisition: J.Č., J.K., and J.S. All authors reviewed the manuscript.

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Data Availability

The datasets generated during and analyzed during the current study are available in the Figshare repository, <https://doi.org/10.6084/m9.figshare.28239326.v2> and referenced as Čuchlík (2025). The custom code generated during the current study are available in the Figshare repository, <https://doi.org/10.6084/m9.figshare.28805840.v2>.

Declarations

Competing interests

The authors declare no competing interests.

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