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Research paper

Genetic variability and heritability of chlorophyll *a* fluorescence parameters in Scots pine (*Pinus sylvestris* L.)

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Current knowledge of the genetic mechanisms underlying the inheritance of photosynthetic activity in forest trees is generally limited, yet it is essential both for various practical forestry purposes and for better understanding of broader evolutionary mechanisms. In this study, we investigated genetic variation underlying selected chlorophyll *a* fluorescence (ChlF) parameters in structured populations of Scots pine (*Pinus sylvestris* L.) grown on two sites under non-stress conditions. These parameters were derived from the OJIP part of the ChlF kinetics curve and characterize individual parts of primary photosynthetic processes associated, for example, with the exciton trapping by light-harvesting antennae, energy utilization in photosystem II (PSII) reaction centers (RCs) and its transfer further down the photosynthetic electron-transport chain. An additive relationship matrix was estimated based on pedigree reconstruction, utilizing a set of highly polymorphic single sequence repeat markers. Variance decomposition was conducted using the animal genetic evaluation mixed-linear model. The majority of ChlF parameters in the analyzed pine populations showed significant additive genetic variation. Statistically significant heritability estimates were obtained for most ChlF indices, with the exception of DI_o/RC, φ_{Do} and φ_{Po} (F_v/F_m) parameters. Estimated heritabilities varied around the value of 0.15 with the maximal value of 0.23 in the ET_o/RC parameter, which indicates electron-transport flux from Q_A to Q_B per PSII RC. No significant correlation was found between these indices and selected growth traits. Moreover, no genotype × environment interaction (G × E) was detected, i.e., no differences in genotypes' performance between sites. The absence of significant G × E in our study is interesting, given the relatively low heritability found for the majority of parameters analyzed. Therefore, we infer that polygenic variability of these indices is selectively neutral.

Keywords: OJIP transient, pedigree reconstruction, photosynthesis.

Introduction

Analysis of chlorophyll fluorescence (ChlF) has provided large amounts of information on conifer physiology (particularly regarding plant response to various environmental factors) almost from the beginning of its utilization in forestry (reviewed, for example, in Mohammed et al. 1995). This approach is based on the fact that light energy absorbed by molecules of chlorophyll *a* in the photosystem II (PSII) antenna has three possible destinies—it is either utilized by the reaction center (RC) of PSII, dissipated as a heat or emitted as a light with shifted wavelength (ChlF). It is usually assumed that the rate of ChlF is inversely proportional to the rate of energy uptake by the RCs that is utilized in primary photosynthetic processes. This is correlated to plant health status as well as various other ecophysiological factors affecting plants (Maxwell and Johnson 2000, Kalaji et al. 2014). Chlorophyll fluorescence measurements are

usually very sensitive, and various instruments and analytical methods have been developed, which can be utilized both on individual and global scales (Kalaji et al. 2014).

In forestry, ChlF measurements have been recently used for various ecophysiological studies (Meroni et al. 2009, Pieruschka et al. 2014, Pollastrini et al. 2014, Damm et al. 2015, Masek et al. 2015) as well as in applied research, for example, for the monitoring of forest health status and productivity (Pontius and Hallett 2014). These applications suggest that measurements of ChlF may have value also in forest genetics and tree breeding. Breeders work with genetic variation in traits affecting productivity and stand health, and the ability to measure phenotypes associated with such traits efficiently, rapidly and accurately is an important asset to forest genetics and breeding. New methods for the detection of genetic variation, using molecular methods, can be coupled with efficient and cost-effective phenotypic measurements to enable more powerful studies of how genetic variation interacts with environmental variation to affect the health and productivity of forest stands (Neale and Kremer 2011). The measurement of ChlF allows sampling of a large number of individuals in a reasonable amount of time, which in turn enables powerful comparisons of multiple individual genotypes and is ideal for the purposes of quantitative genetics. In crop plants, this approach is considered to offer good prospects for breeding programs, particularly with regard to climate changes that we can expect to occur in the near future, as ChlF parameters are frequently well correlated to plant resistance to various stressors (Fiorani and Schurr 2013, Fahlgren et al. 2015). Resilience to climate change is likely to be an important trait for many forest tree species, and an easy and dependable method to evaluate potential adaptability to a changing environment will probably soon become a very desirable tool for forest genetics and tree breeding (Franklin et al. 2014, Harfouche et al. 2014). Estimating potential stress resistance of forest trees might, in principle, be based on ChlF analysis. However, studies utilizing ChlF measurements for such purposes in forest trees are still extremely rare (de Miguel et al. 2014).

Various approaches to ChlF analysis have been developed and some of them have also been applied to conifer trees. They include analysis of 77K ChlF spectra (Strand and Öquist 1985, Oquist and Malmberg 1989, Raskin and Marder 1997, Špunda et al. 1998, Ivanov et al. 2006), the measurements of delayed ChlF (Grigor'ev and Pakhar'kova 2001, Gyllenstrand et al. 2014) and solar-induced fluorescence (Louis et al. 2005). However, the approach most frequently encountered in studies dealing with ChlF in conifers utilizes a simple analysis of the kinetics of the induction of immediate ChlF. These kinetics can be separated into the fast and the slow phase (Kalaji et al. 2014). The majority of authors working with conifer trees use the slow phase analysis. This usually takes advantage of the phenomenon of so-called ChlF quenching, which is associated with the light activation of key Calvin cycle enzymes together with the regulation of stomata (photochemical quenching), but also with heat dissipation of excess excitation energy caused by the acidification of the thylakoid lumen, the xanthophyll cycle and changes in the PsbS subunit of PSII (non-photochemical quenching) (Murchie and Lawson 2013). Thus, it does not precisely dissect primary photosynthetic processes. Moreover, such analysis is somewhat disadvantageous for the utilization in quantitative genetics, where a very large number of samples (both individuals and technical replicates) need to be analyzed in the shortest time possible (to lessen the residual error). Providing one does not settle only for the calculation of a simple F_v/F_m index but wants to properly dissect various components of ChlF quenching, the measurement of one sample usually takes 10–20 min (depending on a particular instrument and methodical approach) (Kalaji et al. 2014).

An analysis of the fast phase of ChlF kinetics, called also the OJIP transient, would be much more advantageous for quantitative genetic analyses, as it is very fast and easily performed with many samples. Moreover, it offers a detailed dissection of the electron-transport chain in thylakoid membranes, i.e., the primary photosynthetic processes per se. Based on the theory of energy fluxes occurring in thylakoid membranes, developed by Strasser and Strasser (1995) and further expanded by the same as well as other scientists (Strasser et al. 2000, 2004, Stirbet and Govindjee 2011), various parameters characterizing 'individual' processes associated with the exciton trapping by light-harvesting antennae, energy utilization in PSII RCs and its transfer further down the photosynthetic electron-transport chain can be derived from ChlF along selected time points of the OJIP curve. Normalization of individual phases of OJIP transient kinetics can also yield additional information, for example, on the functionality of the oxygen-evolving complex, the size of the pool of the electron acceptors after PSI, the excitonic connectivity between PSII units, etc. (Kalaji et al. 2014).

In case of crop plants, ChlF parameters derived from the OJIP analysis have been used to assess heritable genetic variation, for example, in cotton (*Gossypium hirsutum* L.; Pettigrew and Turley 1998), maize (*Zea mays* L.; Šimić et al. 2014), rice (*Oryza sativa* L.; Gu et al. 2012), wheat (*Triticum aestivum* L.; Czyczyło-Mysza et al. 2011) or soybean (*Glycine max* L. Merr.), where some of the studied parameters were coassociated with yield (Yin et al. 2010, Hao et al. 2012). In conifers, the OJIP analysis has been used much less frequently. Several studies analyzed OJIP parameters as markers of plant response to various stress factors, for example, in Scots pine (*Pinus sylvestris* L.; Meinander et al. 1996), Aleppo pine (*Pinus halepensis* Miller; Manes et al. 2001), Swiss stone pine (*Pinus cembra* L.) and Mugo pine (*Pinus mugo* Turra; Lehner and Lütz 2003), or in Norway spruce (*Picea abies* L. Karst; Katanić et al. 2012, Pollastrini et al. 2014).

Genetically determined variation in ChlF parameters has been described for pines only in a few studies, linking this variation

mostly to variable adaptation of photosynthesis performance to seasonal changes (Lindgren and Hällgren 1993, Marshall et al. 2001, Corcuera et al. 2011, Salmela et al. 2011). Other studies dealt with genetically determined variation in ChlF parameters in case of different nutritional regimes (Bown et al. 2009), different water availability (Lüttge et al. 2011) or different adaptation to growth on serpentine soil (Koehn et al. 2003, Xue et al. 2013). However, these studies mostly did not deal with the parents–progeny relationship. Heritabilities in ChlF parameters were estimated very rarely and only for F_v/F_m parameter and for quenching indices (Corcuera et al. 2011, Xue et al. 2013); none of these papers dealt with the inheritance of OJIP parameters.

The first task of any quantitative genetics analysis aimed at dissection of the inheritance of some trait/parameter is to ascertain whether the examined parameter shows significant genetically determined variation and whether it is heritable. Dissection of various genetic mechanisms underlying this inheritance then follows. Currently employed methods are based almost exclusively on mathematical modeling. The statistical method that has come to be known as the 'animal model' has a long history of development and use within the animal breeding and statistical genetics literature (Henderson 1953, 1976, Meyer 1985, Thompson 2008). There was a significant time lag before its potential was fully recognized in other fields (but see Shaw 1987). However, over the last decade, this method has been utilized in estimating quantitative genetic parameters in natural populations (Kruuk 2004, Kruuk et al. 2008), and in plant breeding (Piepho et al. 2008, Cowling et al. 2015). Perhaps the primary reason for this is that the animal model makes use of information from all types of relationship within the complex, unbalanced pedigrees we expect to find in natural populations (Wilson et al. 2010). Its flexibility lies in the ability to cope with variable but nontrivial amounts of missing data (e.g., unknown paternities, unmeasured phenotypes), although missing data will obviously reduce estimate precision and in some circumstances can cause bias.

The aim of our study was to test the suitability of ChlF parameters calculated from the OJIP curve as a method for measuring variation in photosynthesis parameters in a structured population of Scots pine growing on two sites, with the objective of evaluating the amount and nature of genetic variability in ChlF using methods of quantitative genetics. We used pedigree reconstruction to enable use of the animal model, leading to more precise prediction of random effects, and then estimated the heritability of ChlF parameters. Subsequently, estimated heritable variability was related to selected growth traits. The long-term goal of this study was to assess the suitability of analysis of the fast phase of ChlF kinetics as a means of evaluating genetic differences among trees that may be associated with resistance to abiotic stresses, including climate change.

Materials and methods

Site description

Measurements were carried out on two half-sib progeny test plots planted in randomized incomplete block designs on two geographically distinct sites (Skelná Huť and Nepomuk) located in the western part of the Czech Republic (Western Bohemia; for specification, see Table 1). These test plots were grown from seed originating from two seed orchards, each composed of selected plus-trees of Scots pine, all originating from the Western Bohemia provenance (Kaňák et al. 2009).

Meteorological data

Air temperature and relative humidity were measured using an HMP45C (Vaisala, Finland) sensor, daily precipitation was measured using a precipitation gauge SR03 (Fiedler, České Budějovice, Czech Republic) and global radiation using a pyranometer CM11 (Kipp&Zonen, Delft, The Netherlands). We used one meteorological sensor per site—one sensor for relative humidity and air temperature, one for precipitation and one for global radiation. We also used the meteorological data from the nearest station with permanent technical support. Vapor pressure deficit (VPD) was calculated according to the Monteith equation (Goudriaan and Monteith 1990):

$$\mathrm{VPD} = \left(1 - \left(\frac{\mathrm{RH}}{100}\right)\right) \times \mathrm{SVP} \quad (1)$$

Table 1. Description of research sites.

Attribute	Skelná Huť	Nepomuk
Geographic coordinates	49°55′53.489″N, 13°6′43.268″E	49°29′40.735″N, 13°33′5.702″E
Progeny size	85 families	38 families
Total area (ha)	1.23	0.81
Year of establishment	1994	1991
Age of trees in the time of measurement (years)	20	23
Time of measurements	4–6 August 2014	14–18 July 2014
Altitude (m)	610	490
Leaf area index—hemisurface	5.2 (SE 0.1)	6.9 (SE 0.1)
Number of measured trees	207	318
Soil type	Planosol	Stagnosol, Dystric Cambisol

$$SVP = 610.7 \times 10^{7.5 \times T/(237.3+T)} \quad (2)$$

where RH is relative humidity, SVP is saturated vapor pressure and T is temperature.

For detailed meteorological data, see Table S1 available as Supplementary Data at *Tree Physiology* Online and Figure S1 available as Supplementary Data at *Tree Physiology* Online.

Sample collection and measurements setup

Sampling and measurements of ChlF and parameters of tree water status took place during the summer of 2014 (Table 1). All trees included in our analysis were dominant or codominant within the forest stand, thus having sun-exposed crowns. Only fully sun-exposed branches were cut using telescopic pole-scissors. Cutting was performed on both locations within three to four consecutive days from 10:00 am to 4:00 pm, Summer Central European Time. At the end of each day, branches were transported to the laboratory (located in Prague, Czech Republic), where ChlF was measured the next day. The length of cut branches was at least 100 cm in all cases to minimize the desiccation of measured needles, and all samples were wrapped in moistened towels, transported (and stored overnight) under a controlled temperature below 20 °C.

Previous-year needles were selected for the ChlF analysis, because the needles from the current season were not fully developed at the time of our measurements. This meant that ChlF measurements took place at the ends of our branches, with ample amount of wood tissue between the cut end and the site of measurement, ensuring sufficient water supply to the needles. Moreover, according to Richardson and Berlyn (2002), measurements of ChlF in conifers need not be made in situ to be accurate and reliable—these authors showed that even 2 or 3 days after branch cutting, conifers display only small changes in fluorescence indices (in contrast to deciduous trees). Their conclusions were verified in a separate experiment made by us with our experimental pine trees and the results indeed indicated a negligible difference between ChlF indices measured at the time of cutting and after 24 h (see Figure S6 available as Supplementary Data at *Tree Physiology* Online). Moreover, this setup enabled us to minimize the variance associated with possible diurnal changes of solar radiation, as is recommended for such purposes (Richardson and Berlyn 2002, Kalaji et al. 2014). Prior to measurements, branches were kept in a darkened room at a constant temperature and the time interval during which the measurement of ChlF took place (which was similar to that of branch cutting and its sequence was the same) thus did not bring any bias into our experiments—the values of ChlF did not significantly change during this interval.

Chlorophyll *a* fluorescence

The measurements of the polyphasic rise of ChlF transient (OJIP) were made on dark-adapted (30 min) needles with a portable fluorometer FluorPen FP100max (Photon System Instruments, Brno, Czech Republic). The intensity of the saturating pulse (blue light, 455 nm) was 3000 μmol m^{-2} s^{-1}. All ChlF transients were recorded with a time scan from 10 μs to 2 ms with the data acquisition rate of one reading per 10 μs for the first 600 μs, one reading per 100 μs until $t = 14$ ms, one reading per 1 ms until $t = 90$ ms and one reading per 10 ms for the rest of the recording period. Fluorescence values recorded at 40 μs (F_0, initial fluorescence intensity), 300 μs (F_K, fluorescence intensity at K-step), 2 ms (F_J, fluorescence intensity at J-step) and 30 ms (F_I, fluorescence intensity at I-step), and $F_m \approx F_P$ (maximum fluorescence intensity), were used for the calculations of various ChlF parameters shown in Table 2, based on the theory of energy flow in photosynthetic electron-transport chain according to Strasser et al. (2000, 2004) and Stirbet and Govindjee (2011). Needles from each tree (branch) were measured in four technical replicates; these values were then averaged to obtain a value representing the respective tree and used in all subsequent statistical analyses.

Plant water status

Sap flow was measured in one position in different azimuthal directions on the stems of eight randomly chosen trees. A measurement point was chosen on the most suitable spot—from 80 to 130 cm above the ground. For transpiration stream measurement, the Trunk Sector Heat Balance (Čermák and Nadezhdina 2011) method was used. The amount of released water was estimated by two approaches. The average rate of transpiration per tree was calculated on an hourly or daily basis, or an average rate across trees was calculated with respect to different morphological parameters of the trees, which enables comparison of trees with diameter as a covariate. As the transpiration stream is a function of the diameter at breast height (DBH) (Wullschleger and King 2000), we used thickness class to be more precise in estimation of the transpiration stream per the whole forest unit. Thickness classes are circumferences of the stems in a centimeter at breast height, i.e., 30–40 stands for stems with a circumference between 30 and 40 cm. As well as this thickness class, we distinguish 40–50 and 50–60 thickness classes.

Leaf water potential (ψ, MPa) was determined using a pressure chamber (Model Pump-Up; PMS Instruments Co., Albany, OR, USA). Needles for estimation of water potential were cut from the trees at 4:00 am (predawn) and during various times of the day.

Plant morphology

Leaf area index was measured using an LAI-2200 Plant Canopy Analyzer (LI-COR, Inc., Lincoln, NE, USA). Tree height was assessed with a telescopic measuring rod (Sokkia, Atsugi, Japan) with an accuracy of 1 cm on the Skelná Huť site and a hypsometer Vertex III (Haglöf, Langsele, Sweden) with an accuracy of 10 cm at the Nepomuk site in autumn 2007. Stem DBH

Table 2. Selected photosynthetic parameters derived from the measurements of the polyphasic rise of chlorophyll *a* fluorescence transient. F_O, the initial fluorescence intensity (at 40 μs); F_K, the fluorescence intensity at the *K*-step (300 μs); F_J, the fluorescence intensity at the *J*-step (at 2 ms); F_I, the fluorescence intensity at the *I*-step (at 30 ms); $F_m \approx F_P$, the maximum fluorescence intensity; PSI, photosystem I; PSII, photosystem II; RC, reaction center.

Parameter	Definition	Formula
V_J	Relative variable fluorescence at the *J*-step	$(F_J - F_O)/(F_m - F_O)$
V_I	Relative variable fluorescence at the *I*-step	$(F_I - F_O)/(F_m - F_O)$
M_O	Approximated initial slope of the fluorescence transient	$4(F_K - F_O)/(F_m - F_O)$
φ_{PO}	Maximum quantum yield of primary PSII photochemistry	$(F_m - F_O)/F_m$
φ_{EO}	Quantum yield of electron-transport flux from Q_A to Q_B	$1 - (F_J/F_m)$
φ_{REO1}	Quantum yield of electron-transport flux until the PSI electron acceptors	$1 - (F_I/F_m)$
φ_{DO}	Quantum yield of energy dissipation	F_O/F_m
ψ_{EO}	Efficiency/probability with which a PSII-trapped electron is transferred from Q_A to Q_B	$1 - V_J$
ψ_{REO1}	Efficiency/probability with which a PSII-trapped electron is transferred until PSI acceptors	$1 - V_I$
δ_{REO1}	Efficiency/probability with which an electron from Q_B is transferred until PSI acceptors	$(1 - V_I)/(1 - V_J)$
γ_{RC}	Probability that a PSII chlorophyll functions as RC	$1/(\text{ABS/RC} + 1)$
ABS/RC	Average absorbed photon flux per PSII RC (apparent antenna size of an active PSII)	$(M_O/V_J)(1/\varphi_{PO})$
TR_O/RC	Maximum trapped exciton flux per PSII RC	M_O/V_J
ET_O/RC	Electron-transport flux from Q_A to Q_B per PSII RC	$(M_O/V_J)\psi_{EO}$
RE_{O1}/RC	Electron-transport flux until PSI acceptors per PSII RC	$(M_O/V_J)\psi_{REO1}$
DI_O/RC	Dissipated energy flux per PSII RC	$(\text{ABS/RC}) - (TR_O/\text{RC})$
PI_{ABS}	Performance index for energy conservation from photons absorbed by PSII antenna to the reduction of Q_B	$[1/(\text{ABS/RC})][\varphi_{PO}/(1 - \varphi_{PO})][\psi_{EO}/(1 - \psi_{EO})]$
PI_{TOTAL}	Performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors	$PI_{ABS}[\delta_{REO1}/(1 - \delta_{REO1})]$

(130 cm) was calculated from the circumference measured at the height of 1.30 m in autumn 2008.

Pedigree reconstruction

Parentage analysis was carried out using a battery of 10 microsatellite markers published previously: SPAC 11.4, SPAC 11.6, SPAC 12.5 (Soranzo et al. 1998), LOP 1 (Liewlaksaneeyanawin et al. 2004), PtTX 2146, PtTX 3025, PtTX 3107, PtTX 4001, PtTX 4011 (Auckland et al. 2002) and SsrPt_ctg64 (Chagné et al. 2004). Markers were selected according to their performance for this sample set (level of polymorphism, absence of nonspecific amplification and low probability of null allele occurrence). The polymorphic information content (Botstein et al. 1980) varied among loci between 0.355 and 0.951 with a mean of 0.701 (standard error (SE): 0.056). Two loci (SPAC 11.6 and PtTX 3107) showed relatively high level of potential null allele occurrence, 0.395 and 0.486, respectively. The remaining eight loci indicated a mean level of null alleles of 0.023 (SE: 0.008). It is important to point out that the reconstructed pedigree assigned the same parents regardless of whether we included these two questionable loci in the analysis or not.

Allele frequency analysis and subsequent parentage assignment were carried out under a maximum likelihood framework implemented in CERVUS 3.0 (Kalinowski et al. 2007), which also accommodates genotyping errors and takes into account the possibility of selfing. Genotype data were first used to simulate the critical logarithm of odds (it is obtained taking natural log (log base to e) of the overall likelihood ratio) score values and Δ statistics, and then parentage assignments were made.

The first scenario tested was paternal assignment with known maternal parent (half-sib progeny test), then an alternate scenario with both parents assumed unknown (combined nonexclusion probability 3×10^{-8}). The mutual comparison of pedigree reconstruction under those two scenarios helped us to reveal the mismatches in evidence. To further enhance the reliability of the reconstructed pedigree (for the purposes of G-matrix construction), we excluded from the final set of offspring those individuals where no match was found between the recorded mother based on seedlot identity, and either of the parents assigned by parentage reconstruction with both parents assumed unknown. This resulted in exclusion of 51 individuals in the Skelná Huť test and 90 individuals in the Nepomuk test.

Statistical analyses and genetic parameter estimates

The following statistical model was employed in the decomposition of variance in all studied traits:

$$y_{ijk} = \mu + S_i + \text{row}(S_i) + \text{column}(S_i) + P_j(\text{row}) \cdot S_i + P_j(\text{column}) \cdot S_i + a_{ijk} + e_{ijk} \quad (3)$$

where y_{ijk} is the respective phenotypic record, μ is the overall mean, S_i is the fixed effect of the *i*th site, row(S_i) and column(S_i) are the respective fixed effects of a row (or column) within the *i*th site, $P_j(\text{row}) \cdot S_i$ and $P_j(\text{column}) \cdot S_i$ are the respective *j*th orthogonal polynomial effects of a row (or column) where the mean is excluded within the *i*th site, a_{ijk} are the additive genetic values of the individual trees and e_{ijk} is the random error

assumed to be normally and independently distributed, with mean zero and variance σ_e^2; NID (0, σ_e^2).

Variance components estimates from the above model were used to estimate heritabilities (h^2) of the measured traits (ChlF indices):

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2} \quad (4)$$

where σ_a^2 is the additive genetic variance and σ_p^2 is the total (phenotypic) variance.

This analysis is based on a univariate animal model. We took advantage of the reconstructed pedigree based on microsatellite marker analysis to construct the relationship matrix, an integral part of the analysis. The model was developed and analyzed using ASReml software (Butler et al. 2007). First, both sites were evaluated independently by a simple animal model. Heritability for each trait of interest was estimated using the ASReml 'pin' function, based on Eqs (3) and (4). Subsequently, both sites were evaluated jointly, thus decreasing standard error of heritability and lowering possible bias at the same time. Site was included in our model as a fixed effect, which took account of different site conditions and different age of both progeny tests. However, site was not significant as a fixed factor in all cases as shown in Tables 4 and 5. Furthermore, the model for each trait was optimized by adding fixed effects such as row or column on each site separately or orthogonal polynomials to rows or columns. These special fixed effects were included to smooth up possible micro-site spatial variability, which is traditionally explained by spatial structures specified in ASReml syntax. As the original replicated design was not clearly visible in our dataset, the typical method for modeling spatial variation could not be applied. The random effect of additive genetic value versus site interaction was also inspected, but did not show any significance.

Model inference was based on traditional parameters such as Comp/SE ratio for random effects and *F* statistics for fixed effects. The Comp/SE statistic is the restricted maximum likelihood (REML) estimate of the variance component divided by the square root of the diagonal element (for each component) of the inverse of the average information matrix. The diagonal elements of the expected (not the average) information matrix are the asymptotic variances of the REML estimates of the variance parameters.

Log likelihoods were inspected for each individual model. The methods of model optimization and subsequent inference were based on Burgueño et al. (2000). For small data sets such as this one, it is important to avoid overfitting by identifying a parsimonious model rather than a complex one (Burgueño et al. 2000). However, traditional model inference tools (Akaike information criterion etc.) could not be used, as they rely on an increased number of random effects.

Results

Pedigree reconstruction

The assigned paternal and maternal gametic contributions based on results given by Cervus were used to construct a bubble chart depicting the mating pattern across the seed orchard's parents (Figure 1a for site Skelná Huť and Figure 1b for site Nepomuk). The number of seedlots planted at Skelná Huť site as given in Table 1 does not match the number of female parents identified as shown in the chart. This difference is due to discarding some individual trees because of a lack of agreement between the pedigree records of female ancestry and the results from Cervus assuming a parentage-unknown model.

Water potential and transpiration rates

The mean value for water potential ψ was –0.18 (SE: 0.11) MPa, which signifies plants without any water stress. We thus concluded that water was not the limiting factor for tree growth on our experimental sites due to the good availability of ground water (for details, see Figure S2 available as Supplementary Data at *Tree Physiology* Online). Very irregular precipitation distribution during spring and the beginning of summer (see Figure S1 available as Supplementary Data at *Tree Physiology* Online)

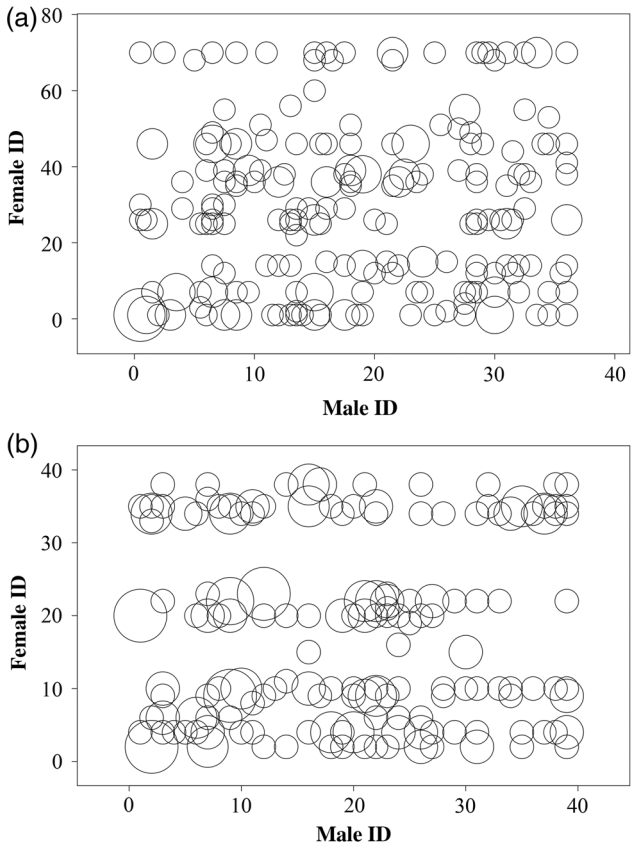

Figure 1. Pedigree reconstruction on Skelná Huť (a) and Nepomuk (b) site. The radius of a bubble depicts the relative size of each progeny ranging from one to six.

had no effect on tree water status. There was no clear trend during the day in plant water potential (see Table S2 available as Supplementary Data at *Tree Physiology* Online), and differences in plant water potential between days were also not significant as the meteorological conditions (precipitation, air temperature, global radiation and relative humidity) were very similar (see Figures S1, S3 and S4 available as Supplementary Data at *Tree Physiology* Online). This enabled us to reasonably compare both sites and in some cases to make a joint analysis of the data. Daily rates of transpiration of dominant pines were correlated with global radiation (see Figure S5 available as Supplementary Data at *Tree Physiology* Online). The overview of the total water released during the day was dependent on the thickness class, for example, on the position of the tree within a canopy and ranged from 4 kg per tree up to 27 kg per tree (see Table S5 available as Supplementary Data at *Tree Physiology* Online).

Chlorophyll fluorescence parameters

Differences between experimental sites The majority of ChlF parameters calculated from the OJIP curve (Table 3; for mean OJIP curves of both sites with indicated variance, see Figure 2) displayed statistically significant differences between the Skelná Huť and Nepomuk sites. Indices M_0, φ_{DO}, ψ_{EO}, ABS/RC, TR_0/RC, ET_0/RC, RE_{01}/RC and DI_0/RC had higher values at the Nepomuk site; indices V_J, φ_{PO}, γ_{RC}, PI_{ABS} and PI_{TOTAL} were higher at Skelná Huť. The most significant differences between sites were found for indices ABS/RC, DI_0/RC and PI_{ABS}. Index ABS/RC estimates the apparent light-harvesting antenna size of PSII expressed as absorbance per RC. Its higher value in Nepomuk indicates a possible higher efficiency of energy capture by PSII. Index DI_0/RC, representing the amount of excess excitation energy that is dissipated per active RC, was also higher in Nepomuk, indicating that the captured irradiation was not particularly effectively utilized for the photosynthetic electron transport. The value of the performance index PI_{ABS} (which is an indicator of an overall PSII performance and includes in its formula both excitation capture and electron transfer up to Q_B electron acceptor) was higher at the Skelná Huť site, which suggests that trees at Nepomuk might actually be less photosynthetically effective.

Table 3. Mean fluorescence parameters for both analyzed sites. Site effect describes the statistical differences between both sites; asterisks denote statistical significance (***$P \leq 0.001$, *$P \leq 0.05$, ns = not significant). Number of trees analyzed: $N_{Skelná\ Huť} = 207$; $N_{Nepomuk} = 318$.

Index	Skelná Huť		Nepomuk		Site effect
	Mean	SE	Mean	SE	
V_J	0.467	0.002	0.455	0.002	***
V_I	0.808	0.002	0.803	0.002	ns
M_0	0.981	0.008	1.012	0.007	***
φ_{PO}	0.822	0.001	0.809	0.001	***
φ_{EO}	0.439	0.002	0.441	0.002	ns
φ_{REO1}	0.158	0.002	0.160	0.002	ns
φ_{DO}	0.178	0.001	0.191	0.001	***
ψ_{EO}	0.533	0.002	0.545	0.002	***
ψ_{REO1}	0.192	0.002	0.197	0.002	ns
δ_{REO1}	0.359	0.003	0.361	0.003	ns
γ_{RC}	0.283	0.001	0.268	0.001	***
ABS/RC	2.551	0.014	2.749	0.014	***
TR_0/RC	2.096	0.010	2.220	0.009	***
ET_0/RC	1.115	0.004	1.208	0.004	***
RE_{01}/RC	0.400	0.003	0.436	0.004	***
DI_0/RC	0.455	0.004	0.530	0.005	***
PI_{ABS}	2.150	0.037	1.930	0.028	***
PI_{TOTAL}	1.235	0.031	1.139	0.029	*

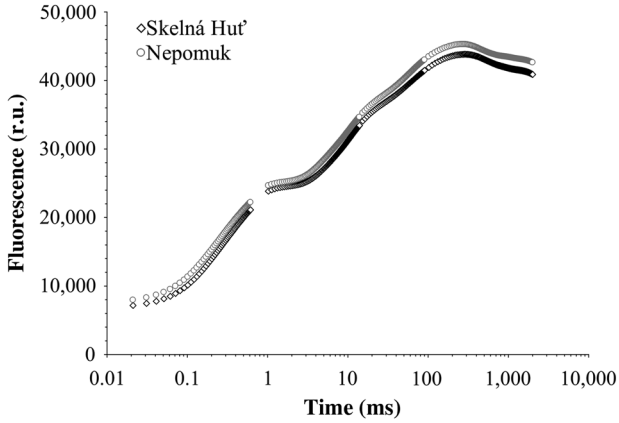

Figure 2. Mean ChlF transient 'OJIP' curves for both analyzed sites; standard errors are within the thickness of the curves; $N_{Skelná\ Huť} = 207$, $N_{Nepomuk} = 318$.

Heritability of the JIP test parameters The majority of ChlF parameters in the analyzed pine populations showed significant additive genetic variation. Mixed models analysis with the inclusion of random effect (relationship matrix) resulted in significant estimates of additive genetic variance, also reflected in the Comp/SE ratio. A simple formula (Eq. 4) enabled us to compute heritability estimates of ChlF indices (Table 4). With the exception of DI_0/RC, φ_{DO} and φ_{PO}, all of the measured ChlF parameters showed significant heritability in our study. These heritabilities varied around the value of 0.15. The maximal value of heritability (0.231; SE: 0.104) was estimated for the ET_0/RC parameter, which indicates electron-transport flux from Q_A to Q_B per PSII RC.

Heritability of V_t at various time points along the OJIP curve Significant heritability of parameters V_J and V_I led us to explore heritability of V_t at several other time points along the OJIP transient. Values of V_t were calculated as $(F_t - F_0)/(F_m - F_0)$ for all time points and plotted in the graph (Figure 3; for absolute values of V_t for selected time points, see Table S6 available as Supplementary Data at *Tree Physiology* Online). The heritabilities were calculated for V_t at several time points and are presented in Table 5 and Figure 3, where they are indicated by dots with SE indicated.

Table 4. Heritabilities (h^2) of selected ChlF parameters. Asterisks denote statistical significance of a given model term included in the header (***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$, ns = not significant as a model term); Pol-1 indicates linear orthogonal polynomial and Pol-2 indicates quadratic orthogonal polynomial; $N = 525$.

Index	Site	Skelná Huť site		Nepomuk site		Comp/SE	h^2	SE
		Row	Column	Row	Column			
V_J	***	ns	ns	ns	ns	2.28	0.169	0.071
V_I	ns	ns	ns	ns	ns	2.52	0.197	0.074
M_0	***	Pol-1***	ns	ns	ns	2.30	0.182	0.075
φ_{P0}	***	Pol-2***	ns	***	***	0.64	0.043	0.068
φ_{E0}	ns	Pol-1***	ns	ns	ns	2.06	0.144	0.067
φ_{RE01}	ns	Pol-1*	Pol-1*	ns	ns	2.25	0.158	0.068
φ_{D0}	***	Pol-1*	ns	***	***	0.31	0.019	0.062
ψ_{E0}	***	Pol-1***	Pol-1**	ns	ns	2.21	0.152	0.066
ψ_{RE01}	ns	Pol-1**	ns	ns	ns	2.29	0.166	0.069
δ_{RE01}	ns	ns	ns	ns	ns	2.41	0.186	0.074
γ_{RC}	***	Pol-1**	Pol-1*	Pol-1*	Pol-1*	2.06	0.161	0.075
ABS/RC	***	Pol-1*	ns	Pol-1*	Pol-1*	1.98	0.151	0.073
TR_0/RC	***	Pol-1*	ns	Pol-1*	ns	2.26	0.189	0.080
ET_0/RC	***	ns	ns	***	*	2.10	0.231	0.104
RE_{01}/RC	***	Pol-1*	ns	ns	ns	2.25	0.166	0.071
DI_0/RC	***	Pol-1*	ns	**	ns	1.36	0.093	0.067
PI_{ABS}	***	**	Pol-1***	ns	ns	2.18	0.191	0.083
PI_{TOTAL}	*	Pol-1***	Pol-1*	ns	ns	1.91	0.115	0.059

Table 5. Heritabilities (h^2) of V_t at various time points (t). Asterisks denote statistical significance of a given model term included in the header (***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$, ns = not significant as a model term); Pol-1 indicates linear orthogonal polynomial and Pol-2 indicates quadratic orthogonal polynomial; $N = 525$.

t (μs)	Site	Skelná Huť site		Nepomuk site		Comp/SE	h^2	SE
		Row	Column	Row	Column			
101	***	***	***	ns	ns	0.21	0.013	0.061
151	***	Pol-1***	Pol-1*	Pol-1*	Pol-1*	2.14	0.158	0.071
221	***	**	Pol-1***	ns	*	2.21	0.272	0.114
461	ns	**	Pol-1***	ns	ns	2.52	0.256	0.094
1021	*	*	Pol-1***	ns	ns	2.42	0.224	0.087
4621	ns	Pol-1**	Pol-1**	ns	ns	2.20	0.157	0.069
10,021	ns	Pol-1*	Pol-1**	ns	ns	2.27	0.163	0.069
21,321	ns	Pol-1**	Pol-1*	ns	ns	2.39	0.177	0.071
46,321	ns	Pol-1*	ns	Pol-1*	Pol-1***	2.10	0.138	0.064
101,621	*	ns	ns	Pol-1**	ns	2.02	0.129	0.062
211,621	*	ns	ns	Pol-1***	ns	1.89	0.116	0.060
461,621	***	Pol-2***	ns	***	***	1.99	0.236	0.112
1,001,621	***	Pol-1*	ns	***	***	0.96	0.085	0.088
1,441,621	***	ns	ns	***	***	1.61	0.194	0.115
2,001,621	***	ns	ns	***	***	2.10	0.338	0.146

Relationship among ChlF parameters and growth traits Calculation of coefficients of correlation between selected ChlF indices commonly used in the OJIP analysis or V_t parameters at chosen time points and tree morphological characteristics (DBH and height) jointly for both sites revealed no statistically significant correlation of any ChlF parameter with either height or DBH (Figure 4). For correlations between ChlF indices and growth parameters for both sites separately, see Tables S7–S10 or Figures S7 and S8 available as Supplementary Data at *Tree Physiology* Online.

Discussion

In the present study, we analyzed ChlF parameters derived from the fast ChlF (OJIP curve) analysis in structured populations of Scots pine grown at two sites in Western Bohemia. Our objectives were to examine genetic variation in ChlF indices under optimal regime (no apparent stress), to estimate the heritabilities of these indices and to investigate possible correlations of such indices with selected growth traits.

Studies of genetically determined variability in ChlF parameters in conifers are rare, and to our knowledge, this is the first study estimating heritability of OJIP ChlF parameters that describes individual parts of primary photosynthetic processes. Previously, heritabilities of ChlF parameters were estimated in maritime pine (*Pinus pinaster* Aiton) only for F_v/F_m index ($h^2 = 0.06$), Φ_{PSII} index ($h^2 = 0.03$) and for quenching processes expressed by indices qP ($h^2 = 0.41$) and non-photochemical quenching ($h^2 = 0.23$ in warm winter and $h^2 = 0.39$ in cold winter) in response to winter stress (Corcuera et al. 2011). Broad sense clonal heritability was estimated for F_v/F_m index also in slash pine (*Pinus elliottii* Engelm.) trees growing on serpentine soil with $H^2 = 0.2$ (Xue et al. 2013). These studies associated variation in ChlF parameters with some environmental stress. However, ChlF analysis can reveal variability that is not connected with any visible stress (as pointed out in our study) and can be utilized in a more general quantitative genetic analysis (e.g., Fracheboud et al. 2002, Yang et al. 2007). Based on our results, it seems that OJIP ChlF parameters (and thus the efficiency of primary photosynthetic processes) are influenced by a large number of genes with relatively small effects (i.e., the infinitesimal model). More detailed segregation analysis might reveal few genes with relatively larger effects (quantitative trait loci, QTL), even though the combined effects of polygenes would likely explain most of the trait's variability. de Miguel et al. (2014) identified QTL for F_v/F_m measured in light-adapted state for maritime pine needles, which explained 44% of phenotypic variation in this parameter; however, this was analyzed under conditions of water insufficiency.

Comparison of the absolute values of other OJIP parameters available in the literature as measured in several pine species

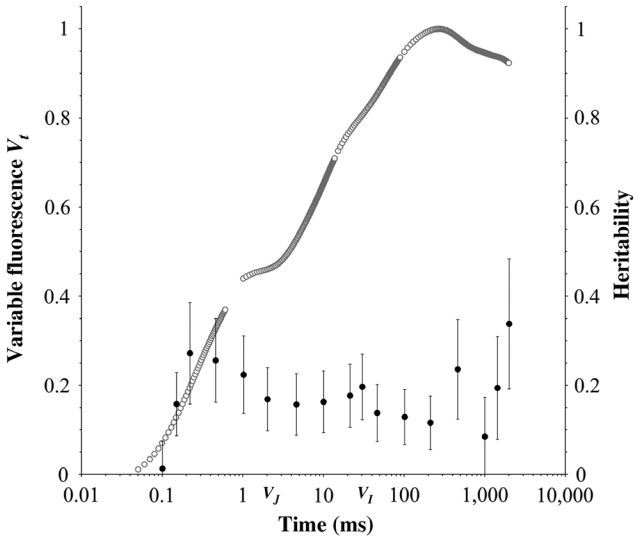

Figure 3. Mean variable ChlF (left *y*-axis) from both experimental sites with heritabilities (indicated by dots, right *y*-axis) for relative variable fluorescence at selected time points along with their respective SEs; $N = 525$.

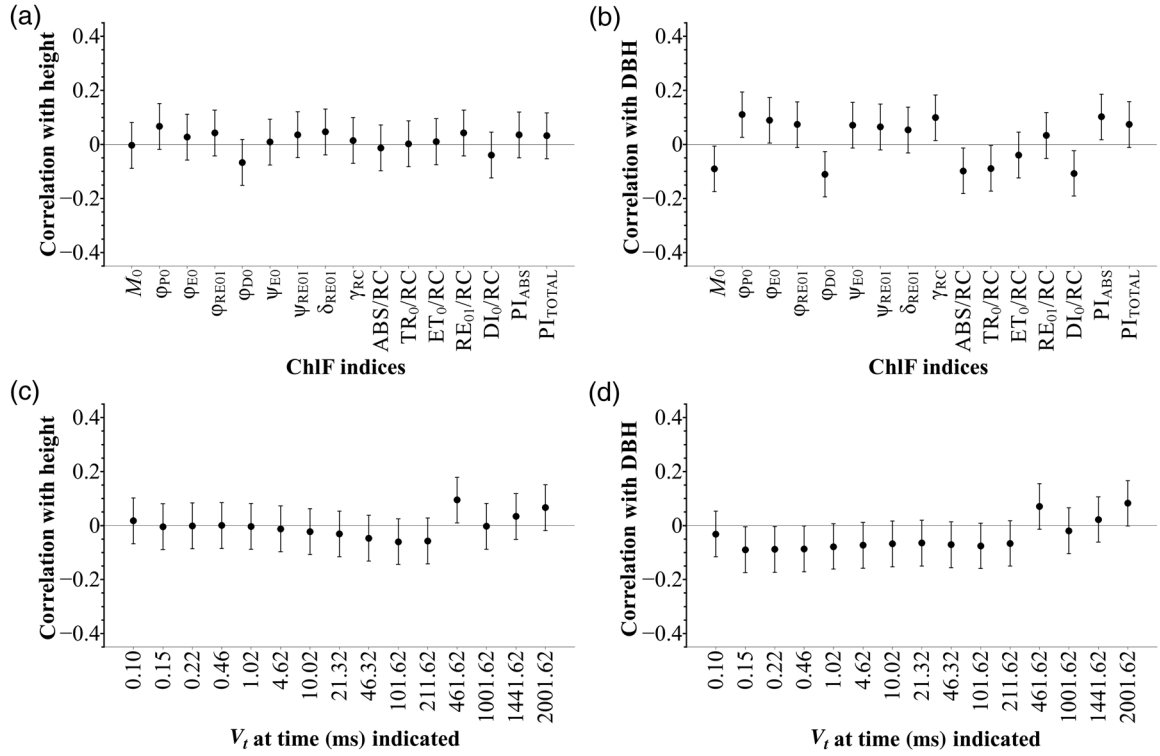

Figure 4. Combined correlations of tree growth parameters with ChlF parameters, jointly calculated for Skelná Huť and Nepomuk sites; SEs indicated; $N = 525$. (a) Correlation of height with standard ChlF indices, (b) correlation of DBH with standard ChlF indices, (c) correlation of height with V_t at chosen time points and (d) correlation of DBH with V_t at chosen time points.

Table 6. Comparison of the mean values of selected OJIP ChlF indices from our study with the values of the same indices reported for unstressed populations of various pine species. na, not assessed.

	Meinander et al. (1996)	Manes et al. (2001)	Lehner and Lütz (2003)		Pollastrini et al. (2014)	Current study	
Index	*Pinus sylvestris*[1]	*Pinus halepensis*[2]	*Pinus mugo*[3]	*Pinus cembra*[3]	*Pinus sylvestris*[4]	*Pinus sylvestris*	
						Skelná Huť	Nepomuk
F_v/F_m (φ_{P0})	0.81–0.82	na	0.68	0.78	0.83	0.82	0.81
ABS/RC	1.30–1.40	2.50	3.50	2.60	na	2.55	2.75
TR_0/RC	1.10–1.20	na	2.40	2.00	na	2.10	2.22
ET_0/RC	1.10–1.20	1.60–1.80	1.20	1.00	na	1.12	1.20
ψ_{E0}	0.98	na	0.49	0.49	0.63	0.53	0.55

[1]Two-m-high saplings grown in fumigation chamber.
[2]Four-year-old trees grown in pots under controlled growth conditions.
[3]Trees naturally growing in high altitudes, measured in May.
[4]Fourteen-year-old trees from experimental plantation.

(F_v/F_m, ABS/RC, TR_0/RC, ET_0/RC and ψ_{E0}) with our data indicated no apparent stress in our population. Our data were similar to values characterizing unstressed (usually control) trees from other studies (Meinander et al. 1996, Manes et al. 2001, Lehner and Lütz 2003, Pollastrini et al. 2014; see Table 6).

The lowest heritabilities of conventional OJIP ChlF indices were found for DI_0/RC, φ_{D0} and φ_{P0} (F_v/F_m) indices in our case. The low heritability of index DI_0/RC that we detected in our study on Scots pine differs from the data reported for maize, where this index showed significant heritability with an average value comparable to other fluorescence indices (Šimić et al. 2014). This could simply express interspecific differences between conifer trees and annual crop plants. Absence of significant heritability for the F_v/F_m index in our study is in accordance with Lüttge et al. (2011), who tested different provenances of Scots pine in different water regimes and found no apparent variation for this parameter. Bown et al. (2009) also found no influence of genotype on F_v/F_m as tested in seedlings of radiata pine (*Pinus radiata* D. Don) growing in pots with various nitrogen and phosphorus nutrition.

The structure of heritable variability presented in our study is probably unique to our particular scenario of pine population, location and climatic conditions. As noted by Wilson (2008), heritability should be interpreted as being conditioned on any fixed effects included in the model. This work suggests major model dependency of h^2 estimates. We were aware of such pitfalls prior to modeling our data, especially when dealing with fixed effects. Any upward bias caused by reckless inclusion of a fixed effect was carefully avoided.

Genetic variation in ChlF indices is usually considered to reflect local adaptation of pine populations embodied by differences in response to environment of pines originating from different provenances (Ma et al. 2010, Corcuera et al. 2011, Reinhardt et al. 2011). In our case, the measured trees are all descendants of parents originating from the Western Bohemia provenance (Kaňák et al. 2009), so the genetic variation we detect is within-provenance. We did not detect any genotype × environment interaction (G × E), i.e., there were no differences in performance of the same families at different sites. The absence of significant G × E in our study is interesting, given the relatively low heritabilities found for the majority of parameters analyzed, because low heritability is usually assumed to mean that the phenotype is heavily influenced by environmental variation. Missing G × E interaction and relatively low differences in ChlF indices may be attributed to the homogeneity of our populations. As all parental trees originate from the local Western Bohemian provenance, the analyzed populations might be uniformly adapted to conditions of this region.

We have not detected any statistically significant relationship of measured ChlF indices with selected growth parameters (height, DBH) of measured trees. This result suggests the hypothesis that residual variation in photosynthesis is selectively neutral. Photosynthesis is central to plant growth, and has no doubt been subject to selection pressure in the past, but it may be that factors other than photosynthetic efficiency are rate limiting for height and diameter growth in the two stands we measured. The presence of heritable variability of a trait within a population is essential for that trait to evolve. Evolution of a trait is directed by natural selection if the trait value is correlated with fitness (at present time, in present niche), or the trait is neutral if there is no link to fitness. Traits connected with fitness often show low heritability, because natural selection on those traits reduces genetic variation, whereas traits that are less intimately tied to fitness may display greater genetic variability and so have higher heritability (Falconer et al. 1996). It is possible that the heritable variation we discovered for ChlF indices has no statistically significant association with fitness in the experimental stands at the time the measurements were made. Although some examples of association of ChlF indices with fitness in the natural population do exist (Arntz et al. 2000*a*, 2000*b*, Molina-Montenegro et al. 2013), direct correlations between photosynthetic rates and fitness are not a rule (Ackerly et al. 2000). However, we attempted to link

ChlF parameters to only two growth traits (height and DBH) under growing conditions where the trees were not stressed, and the possibility to link ChlF parameters to other important traits for forestry (e.g., viability, fertility, germination or resilience to abiotic stress, as some studies indicate) remains. To answer this question, more detailed study would be necessary.

Conclusions

We have detected statistically significant genetic variation in various ChlF indices calculated from the fast ChlF transient (OJIP) analysis in structured population of Scots pine growing on two sites without any apparent stress. Processes enumerated by ChlF indices are considered to be polygenic, so tools of quantitative genetics must be used to dissect their genetic variation and mechanisms of inheritance. These were found to be of a simple additive character for all OJIP ChlF parameters examined. Estimated heritabilities for 18 ChlF indices derived from the OJIP transient were about $h^2 = 0.15$ in most cases, with the highest value of 0.23 for the ET_0/RC parameter. Only DI_0/RC, φ_{D0} and φ_{P0} (F_v/F_m) parameters showed no significant heritability. Normalized chosen points along the OJIP transient (V_t) displayed similar pattern of heritability, i.e., heritabilities around $h^2 = 0.15$. Possible further analysis of QTLs linked to such parameters might reveal loci involved in primary photosynthetic processes as they enable dissection of the heritable structure of primary photosynthetic processes on a fine scale.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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