Drought Sensitivity of Norway Spruce at the Species’ Warmest Fringe: Quantitative and Molecular Analysis Reveals High Genetic Variation Among and Within Provenances

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ABSTRACT Norway spruce (Picea abies) is by far the most important timber species in Europe, but its outstanding role in future forests is jeopardized by its high sensitivity to drought. We analyzed drought response of Norway spruce at the warmest fringe of its natural range. Based on a 35-year old provenance experiment we tested for genetic variation among and within seed provenances across consecutively occurring strong drought events using dendroclimatic time series. Moreover, we tested for associations between $^{13}C_{25}$,700 variable SNPs and traits related to drought response, wood characteristics and climate-growth relationships. We found significant adaptive genetic variation among provenances originating from the species’ Alpine, Central and Southeastern European range. Genetic variation between individuals varied significantly among provenances explaining up to 44% of the phenotypic variation in drought response. Varying phenotypic correlations between drought response and wood traits confirmed differences in selection intensity among seed provenances. Significant associations were found between 29 SNPs and traits related to drought, climate-growth relationships and wood properties which explained between 11 and 43% of trait variation, though 12 of them were due to single individuals having extreme phenotypes of the respective trait. The majority of these SNPs are located within exons of genes and the most important ones are preferentially expressed in cambium and xylem expansion layers. Phenotype-genotype associations were stronger if only provenances with significant quantitative genetic variation in drought response were considered. The present study confirms the high adaptive variation of Norway spruce in Central and Southeastern Europe and demonstrates how quantitative genetic, dendroclimatic and genomic data can be linked to understand the genetic basis of adaptation to climate extremes in trees.

Norway spruce (Picea abies (L.) Karst.) is by far the most important timber species in Europe (Spieker 2000) and an important key species in mountainous, sub-alpine and boreal forest ecosystems. Quantitative genetic studies in Norway spruce so far mainly addressed local adaptation and trait variation at the cold end of the species’ distribution (Savolainen and Hurme 1997). Here, at its northern and altitudinal limit, temperature and photoperiodic constraints were found to result in natural selection on manifold quantitative traits (Howe et al. 2003), most notably bud burst, bud set, frost hardiness and growth (Skreppa 1991; Pulkkinen 1993; Hannerz et al. 1999). In contrast, genetic variation and local adaptation at the warm edge of the Norway spruce range have rarely been addressed (but see Mátyás et al. 2009; Kapeller et al. 2016), although global warming is expected to reduce the distribution and plantation area of Norway spruce significantly (Hanewinkel et al. 2013). In particular, the species’ sensitivity to drought periods (e.g., van der Maaten-Theunissen et al. 2013; Levesque et al. 2013) combined with increasing bark beetle damage (Seidl et al. 2008) are considered the main agents of ongoing and predicted Norway spruce decline.

Present Norway spruce populations in Central and Southeastern Europe share a complex history and originated from three to five refugial
Drought stress in trees may result in a variety of physiological and morphological changes: it can reduce the annual increment, can affect the xylem element architecture, may lead to alterations in the hydraulic properties, or may alter the chemical composition of the wood body (Grabner et al. 2006; Rosner et al. 2014; De Micco et al. 2016). Drought sensitivity is functionally related to various wood characteristics, because the vulnerability of the xylem conduits to hydraulic failure depends on conduit lumen and length as well as on cell wall thickness (Cruiziat et al. 2002). Therefore, wood characteristics have been suggested as screening traits for drought sensitivity to identify drought-tolerant individuals (Dalla-Salda et al. 2011; Rosner et al. 2014). However, it remains unclear whether observed trait correlations between drought sensitivity and wood characteristics (Cruiziat et al. 2002) are valid only within single populations or across populations and even species (George et al. 2015) and whether these correlations are under common genetic control. Generally, wood formation and the development of the secondary xylem constitutes a complex process that involves a combination of exogenous and endogenous factors during the different stages of cell differentiation (Fromm 2013), all of which are proposed to be coordinated by transcriptional networks (Carvalho et al. 2013; Zhong and Ye 2013; Liu et al. 2014).

Intraspecific genetic variation in drought response has been quantified in several tree species (e.g., Martinez-Meier et al. 2008; Eilmann et al. 2013; George et al. 2015), but meaningful estimates of genetic parameters which are required for breeding activities or to assess the evolvability of populations are still rare (but see George et al. 2016). The predominant reason for this is that genetic testing requires controlled trials in drought-prone environments and observations over several decades in order to assess drought response on adult trees in vivo. Another drawback of genetic testing in field experiments is that results on small sets of genotypes and seed provenances (i.e., seed material harvested from a single population) might not be transferable to other genetic backgrounds. To overcome time constraints in field tests, genome-wide associations between markers and environmental conditions and/or physiological and morphological traits were proposed and applied for several tree species (Neale and Kresmer 2011). Many association studies have identified SNPs and genes linked to wood and growth traits (e.g., poplars [Porth et al. 2013], pines [Dillon et al. 2011; Lamara et al. 2016]) or to environmental variables such as aridity, drought or cold tolerance (e.g., Eckert et al. 2010; Roschanski et al. 2016; Jaramillo-Correa et al. 2015). However, the amount of variance in quantitative traits explained by individual SNP markers is generally low and rarely exceeds 5% (Dillon et al. 2010; Guerra et al. 2013). Also, associations found in one study might not be comparable to other studies, even if they share similar sampling areas, environmental variables, and genetic loci (Gašić et al. 2016). This might be explained by missing heritability of traits and insignificant local adaptation to the tested environmental gradient.

The objective of the present study was to analyze the drought response of Norway spruce in a drought-prone environment at the fringe of its natural range. Based on a 35-year-old provenance experiment we tested for genetic variation among seed provenances and estimated the degree of genetic determination of the drought reaction across consecutively occurring strong drought events. Moreover, we report here the first analysis in Norway spruce which tested for genomic associations to drought reaction, wood characteristics and climate-growth relationships. The joint interpretation of the quantitative genetic analysis together with association analysis on polymorphic SNPs should enable us to identify important genes or genomic regions with a general impact on drought performance and correlated wood characteristics.

MATERIALS AND METHODS

Trial site, plant material and sampling

We exploited data from a Norway spruce provenance trial that was established in 1978 as part of a larger test series of 44 trials in the eastern Alpine region (Nather and Holzer 1979). The trial site Porrau (48.548°N, 16.171°E) is located outside of the natural distribution area of Norway spruce at an elevation of 250 meters above sea level. Porrau is situated in the Northeast of Austria, a region characterized by high temperatures (Mean annual temperature: 9.1°C), low precipitation (Annual precipitation sum: 524 mm) and frequently occurring drought periods (e.g., Auer et al. 2005; Ethfymiadis et al. 2006; Nobilis and Godina 2006). Seed material for the trial Porrau and the complete trial series originates from commercial seed harvests in 1971 that comprised presumably autochthonous stands and included a large number of representative seed trees (Nather and Holzer 1979; Kapellet et al. 2012). In addition, seed provenances from outside Austria were contributed from seed material of the IUFRO trial 1964/68 (Kruttsch 1973). The trial was established with 5-year old seedlings in a randomized block-design and plant spacing of 1.8 × 1.5 m. For the present analysis, 11 provenances (Table 1) were selected representing the distribution of the species as wide as possible and given the availability of material in the trial (Figure S1). To reveal climate-growth relationships two cores per tree were taken from 22 to 55 individuals per provenance (323 trees in total) at breast height. Due to the advanced age of the trial and unequal tree numbers within blocks, sampling and consecutive statistical analysis could not consider the original block design.

Sample processing

Using a double-blade circular saw, tree cores were cut into cross sections of approximately 1.4 mm for X-ray densitometric analysis. These cross sections were placed on microfilms and exposed to a 10 kV (24 mA) X-ray source for 25 min. The films were analyzed with the software WinDENDRO 2009 (Regent Instrument, Quebec, CAN) and provided measurements of the following growth parameters: ring width (RW), earlywood width (EW, i.e., wood formed in the early phase of the growing season), latewood width (LW, i.e., wood formed late in the season consisting of narrow vessels and stronger cell walls) and latewood proportion (LWP), as well as the wood density characteristics;
mean ring density \((RD)\), earlywood density \((ED)\), latewood density \((LD)\), minimum density \((MIND)\), and maximum density \((MAXD)\) for each year of the cores. Ring width parameters were measured to the nearest 0.001 mm, while density parameters were measured in kg/m\(^3\). \(LWP\) is given in percentages as it expresses the relative proportion of latewood compared to total ring width. In order to reduce unspecific growth signals that were not caused by climatic and genetic factors \(e.g.,\) eccentric tree-ring patterns caused by compression wood, values from the two cores per tree were averaged. Data were converted into single-tree time series and mean chronologies using the dplR package in R \(\text{(Bunn 2010; R Development Core Team 2008)}\).

### Identification of drought periods

For the identification of drought events, which occurred during the observation period of the trial, we used the standardized precipitation index SPI \(\text{(McKee et al. 1993)}\). Based on a long-term precipitation record \(\text{(in our study the period 1961-2011)}\), the SPI provides an estimate of deficit or surplus of precipitation for each point of the series. Drought events can be classified from ‘mild’ to ‘extreme’ drought events following the classification of McKee et al. \(\text{(1993)}\). We calculated the SPI for timescales of one and three months as these periods allowed identifying drought periods. Higher recovery stands for faster revitalization. Resilience \(\text{(George et al. 2015)}\) was calculated as the ratio between ring-width during drought \((Rt)\) and the ring-width during drought, with \(Rt = 1\) for trees which are not affected by drought, \(Rs = 1\) for full restoration and \(Rs < 1\) indicating lasting growth reductions. \(Rt\) is calculated as the ratio between ring-width during drought \((Dr)\) and mean annual increment of the two years before the drought event \((\text{preDr})\): \(Rt = Dr / \text{preDr}\). Recovery \(\text{(RC)}\) is calculated as the ratio \((Rc = \text{postDr} / Dr)\) of the mean annual increment across the two years following a drought year \((\text{postDr})\) and the ring-width during drought and describes how good a tree is able to recreate after a certain drought period. Higher recovery stands for faster revitalization. Resilience \(\text{(Rs)}\) describes the capacity of a tree to reach pre-drought increment after a drought event with \(Rt = 1\) for full restoration and \(Rs < 1\) indicating lasting growth reductions. \(Rs\) is given by the ratio of the increment of the two years after drought \((\text{postDr})\) and the pre-drought growth \((\text{preDr})\): \(Rs = \text{postDr} / \text{preDr}\). By accounting for the experienced growth reduction during drought, \(Rs\) can be extended to the relative resilience \(\text{(rRs)}\) as given by \(rRs = (\text{postDr} - Dr) / \text{preDr}\). Since a biologically caused negative long-term trend in ring width can be neglected within the relatively short time frame of each drought event and due to the equal age of the analyzed trees \(\text{(Pretzsch et al. 2013)}\), all drought response indicators were calculated from raw, untransformed ring width series. Also, transformations into the widely used basal area increment were unsuitable as any change of dimensionality of the measured traits affect the trait variances and thus the estimates of variance components and genetic parameters \(\text{(Houle 1992)}\).

### Climate-growth correlations

Growth dynamics of Norway spruce were found to depend on climate conditions of the local environment and the genetic origin of the seed material \(\text{(Suvanto et al. 2016)}\) and can be quantified by correlations between monthly climate variables and the annual increment as obtained from tree cores. For the present analysis of phenotypic trait correlations and associations to molecular markers, we calculated correlations between annual increment and monthly precipitation and temperature data for April of the preceding year to September of the current year using the entire ring chronologies. In a first step, the most important monthly climate variables were selected by testing for correlations between the mean increment across all trees as dependent variable and the monthly climate variables as independent parameter. In the second step, we removed all non-significant climate variables and calculated Pearson’s correlation coefficient between the significant climate variables and the annual increment of each individual tree and of provenance means. The obtained correlation coefficients for individual trees were then transformed to normality by using Fisher’s z-transformation.

### Variation among provenances

To test whether there is any genetic variation \(i)\) in drought sensitivity \(\text{(Rt, Rc, Rs, rRs)}\) within single drought periods, \(ii)\) in growth traits \(\text{(RW, EW, LW, LWP)}\) and \(iii)\) in wood characteristics \(\text{(RD, ED, LD, MIND, MAXD)}\), we tested for significant differences among provenances applying one-way analysis of variance \(\text{(ANOVA)}\). Here, provenances were treated as fixed effect and the respective drought, growth and wood traits as dependent variables. To confirm differences among provenances across consecutive drought periods, a repeated measure ANOVA was performed. The growth reactions of a single tree to consecutive drought events were considered as a repeated measure and treated as random effect, while provenances were treated as fixed effects. Duncan’s post hoc tests were used to analyze pairwise differences between provenances.
Degree of genetic determination

To estimate the degree of genetic determination usually requires parent-offspring correlations or estimates of variance components of full- or half-sib families (Falconer and Mackay 1996). As long-term trials with such experimental designs are rarely available, we made use of the repeated occurrence of drought events at our trial site and calculated the proportion of total variation of drought response that is due to differences between individuals. This proportion is defined as repeatability (r) and gives the upper limit of heritability of the given trait (Boake 1989, Falconer and Mackay 1996). Repeatability is calculated as the ratio of the between-group variance $\sigma^2_a$ and total phenotypic variance $\sigma^2_y$, where $\sigma^2_a$ is the sum of the between-group $\sigma^2_g$ and residual (within-group) variances $\sigma^2_e$ (Sokal and Rohlf 1995, Nakagawa and Schielzeth 2010): 

$$r = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_e}.$$ 

The variances $\sigma^2_e$ and $\sigma^2_a$ can be estimated via traditional ANOVA (Lessells and Boag 1987) or by using restricted maximum likelihood estimation (REML) in linear mixed-effect models, often referred to as the animal model (Nakagawa by using restricted maximum likelihood estimation (REML) in linear mixed-effect models, often referred to as the animal model (Nakagawa and Schielzeth 2010): 

For phenotype-genotype associations, provenances with significant repeatabilities for drought resistance (Rt) were grouped into a separate subset of trees referred to as SubsetQD (Subset with significant Quantitative variation in Drought resistance). We chose drought resistance as selection parameter for the subset as resistance shows the most direct reaction of a tree to drought, while other parameters might be affected by climate conditions after drought and the trees carbon storage capacity and thus reflect more complex trait architecture.

Phenotypic traits correlations

Drought sensitivity of trees functionally depends on xylem architecture and is related to wood characteristics. Such functional correlations indicate that wood properties, as for example wood density, might be suitable selection criteria for large-scale screening programs (Rosner et al. 2014) and may guide the selection of drought resistant individuals without the immediate occurrence of drought events. Thus, they are particularly beneficial in long-lived trees and field breeding experiments, where the appearance and intensity of drought events cannot be experimentally realized.

Moreover, genetic correlations are a product of local adaptation to specific environments and thus provide estimates of trait response to indirect selection (Roff 1996). As our experimental design did not allow estimating genetic correlations, we calculate phenotypic correlations, which are adequate estimates for genetic correlations if morphological and life history traits are being analyzed (Cheverud 1988; Roff 1996). We computed correlation matrices (Pearson’s product-moment-correlation) displaying the relationships between wood properties, drought stress indicators, and climate-growth relationships. Matrices were calculated across all provenances for the entire dataset (assuming equal adaptive history) as well as separately for every single provenance. Differences among provenances were expressed as minimum-maximum range for every trait combination and included in a heatmap using the package ggplot in R (R Development Core Team 2008).

SNP microarray design and genotyping

A custom Illumina Infinium HD iSelect BeadChip comprising 3,257 SNPs (assays) was developed by merging SNPs from a number of different resequencing and genotyping projects. The SNP assay comprised SNPs that were identified and applied in previous studies in Norway spruce (Heuertz et al. 2006; Chen et al. 2010; Chen et al. 2012a, 2012b; Pavy et al. 2013; Källman et al. 2014) and related conifers (Guillet-Claude et al. 2004); these were also found to be useful for applications in genetic structure analysis and association studies (Chen et al. 2016; Heer et al. 2016; Ganthaler et al. 2017). Further details on chip design are given by Ganthaler et al. (2017) and a list of the compiled assays is provided in Table S1. Infinium BeadChips were manufactured by Illumina in a 24×1 format. The association analysis was done by considering 147 trees from 11 provenances (Table 1). This selection of individual trees from the total number of 323 trees was based on the phenotypic variation of drought resistance and covered extreme phenotypes with approximately 25% of trees with the highest and the 25% of tree with the lowest drought resistance. The SubsetQD further reduced the number of trees for association analysis to 72 trees by removing provenances without significant variation in drought resistance. For each sample, genomic DNA was extracted from lyophilized needle tissue using a CTAB protocol (van der Beek et al. 1992) with minor modifications made for the processing of 96-well plates. DNA concentrations were quantified on a 0.8% agarose gel. SNP genotyping was conducted according to the manufacturer’s recommendations and the microarray signals were detected on Illumina’s iScan System by the IMGM Laboratories GmbH, Germany. All SNP data were analyzed with GenomeStudio v. 2011.1 (Illumina).

Population structure and relatedness

260 polymorphic neutral SNPs were used to investigate population stratification and relatedness among individuals as they can lead to false positive detection during association analysis. The selection of neutral SNPs followed a two-step procedure: first, only those SNPs located outside of genes or within gene introns were selected; second, these SNPs were filtered for a 100% call rate and a minor allele frequency (MAF) above 0.15. Population stratification was first investigated with the Bayesian model-based software STRUCTURE (Falush et al. 2003) which is used to infer distinct populations and to assign individuals to the identified populations. The model allows admixture and correlated allele frequencies and was run with a burn-in period of 105 and Markov chain Monte Carlo (MCMC) replicates after burn-in (run length) of 106. Three independent runs (iterations) were conducted for each putative number of clusters (K). Sampling location information was considered by applying the prior model parameter (LOCPRIOR) to the population model (Hubisz et al. 2009) and possible numbers of K tested ranged from one to nine. For each scenario, Structure Harvester (Earl and von Holdt 2012) was used to estimate the most probable number of K using Evanno’s method (Evanno et al. 2005). Based on the first results (see Population structure), a second structure analysis was performed excluding individuals that were not related or admixed with the majority of the respective provenances, assuming that such trees may result from identification failures during seedling production or outplanting. This exclusion of trees from the total dataset increased the number of polymorphic neutral SNPs using the selection procedure above
to 264 SNPs. Kinship, i.e., relatedness among individuals was estimated with TASSEL’s centered IBS method using the same neutral SNPs.

**Association analysis**

Associations between the different quantitative traits including drought response, wood traits, climate-growth correlations and the various SNPs was performed using TASSEL (Bradbury et al. 2007). SNPs with MAF less than 5% and missing genotypes higher than 10% were excluded for the association test. Overall, this resulted into a final set of 1,714 SNPs, while for the SubsetQD (i.e., provenances with significant degree of genetic determination) 1,707 SNPs were tested in 72 trees. Marker-trait associations (MTAs) were calculated for all provenances and SubsetQD individually.

Association analyses were performed by using a mixed linear model taking into account population structure and kinship (MLM+Q+K) (Yu et al. 2006). No compression (reduction of the dimensionality of the kinship matrix to reduce computational time) was applied and variance component was re-estimated for each marker. The $p$-value threshold was corrected with the standard Bonferroni procedure resulting into the following corrected $p$-values for a given significance level $\alpha$ ($\alpha = 0.0001: P < 5.83 \cdot 10^{-7}; \alpha = 0.01: P < 5.83 \cdot 10^{-6}; \alpha = 0.05: P < 2.92 \cdot 10^{-5}; \alpha = 0.1: P < 5.83 \cdot 10^{-5}$) for all provenances and to ($\alpha = 0.0001: P < 5.85 \cdot 10^{-7}; \alpha = 0.01: P < 5.85 \cdot 10^{-6}; \alpha = 0.05: P < 2.93 \cdot 10^{-5}; \alpha = 0.1: P < 5.86 \cdot 10^{-5}$) for SubsetQD. The amount of variation explained by a SNP was obtained for each significant association as Rsq_MARKER value ($R^2$). Q-Q plots were used to assess the number and magnitude of observed associations between SNPs and traits under study, compared to the association statistics expected under the null hypothesis of no association. This procedure resulted in $-\log_{10}$ ($p$-values) that were ranked in the order from lowest to highest on the $y$-axis and plotted against the distribution that would be expected under the null hypothesis of no association on the $x$-axis (expected values). Deviations from the identity line suggest either that the assumed population stratification or cryptic relatedness is incorrect or that the sample contains values arising from other reasons, as most likely by true associations (Wellcome Trust Case Control Consortium 2007; Pearson and Manolio 2008).

In addition to associating raw trait means to the candidate SNPs, we transformed wood property traits into binomial and categorical data (three categories). For the association study, these traits were denoted by adding b (binomial) or t (categorical) to the original trait name (ex: RW, RWb, RWt). Drought resistance values of the drought events (1993 and 2000) were combined into a single binomial or categorical measure by considering different scenarios as cut-off between categories (Figure S2). Drought resistance in 2003 was excluded from these combinations as individuals could be expected to show a carry-over effect from the previous drought event in the year 2000.

**Exploration of significant associations**

To understand the molecular function of associated markers, we examined the Pfam domains (http://pfam.xfam.org) of the respective genes. Because of the limited quality of the available Norway spruce preliminary annotation at congenie.org, sequence similarity was assessed by BLASTP 2.6.1 (Altschul et al. 1997) against the GenBank NR database. SNPs located within a coding region can have a direct impact changing the amino acid sequence of a protein. Nevertheless, markers located in non-coding regions (introns, downstream regions or gene-empty regions) should also be considered due to their possible impact on gene expression through different regulatory elements as some of them could be directly involved in phenotypic variation (Webb et al. 2009).

All genes linked to the significantly associated SNPs were examined on the recently developed gene expression database NorWood (Jokipii-Lukkari et al. 2017). This resource facilitates exploration of the associated gene expression profiles and co-expression networks during wood formation and is a powerful community tool for understanding the regulation of wood development in *P. abies*.

**Data availability**

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. File S1 contains phenotypes for each individual. File S2 contains genotypes for each individual. File S3 contains the structure (Q) matrix used for the association study. File S4 contains the kinship (K) matrix used for the association study. File S5 contains GWAS output for all provenances. File S6 contains GWAS output for SubsetQD.

**RESULTS**

**Identification of drought periods**

Using the SPI we identified three years (1993, 2000 and 2003) with extreme deficit of rainfall compared to the long-term record (Figure 1). These droughts were SPI = -2.41 (1993), SPI = -2.37 (2000), and SPI = -2.00 (2003) given in standard deviations according to McKee et al. (1993). These drought events significantly affected tree growth across all provenances. For example, the range of provenances’ growth performance (expressed as the drought stress indicator $R_s$) to the drought event in 1993 was 0.82 to 0.63, whereas it was 0.32 to 0.21 in 2000. Although the drought event in 2003 was an extreme event across Europe (Ciais et al. 2005), it was slightly weaker at our trial site and had smaller effects on annual increment than the two previous periods. However, the moderate growth reduction of 2003 compared to the previous two years was probably also due to the lasting effect of the previous drought in 2000. Beside these three drought events, which occurred at the beginning or in the middle of the growing season (May or June), there were also few years with SPI values of similar magnitude, but without any notable effect on tree growth (e.g., 1983, 1988, and 1993, Figure 1). These further drought events were not considered for the analysis, since they occurred toward the end of the growing season (end of July, August, September; Table S2) when earlywood formation in Norway spruce was probably already completed or had at least slowed down (e.g., Bouriaud et al. 2005).

**Climate-growth correlations**

Climate-growth analysis across the complete ring chronologies of all provenances confirmed the negative effects of high temperatures on incremental growth of Norway spruce at our trial site, as we obtained significant negative correlations between annual growth and the monthly temperatures of January (CorJanT), March (CorMarT), May (CorMayT), June (CorJunT) and August (CorAugT) of the current year (Table S3). In contrast, high precipitation resulted in higher incremental growth as revealed by significant positive correlations to precipitation in February (CorFebP), May (CorMayP) and June (CorJunP). Similar correlations for individual provenances to these eight monthly climate variables confirm their importance for growth and demonstrate among-provenance variation in climate-growth response (Table S4) as correlation coefficients varied among provenances. For example, increment of provenances R6 and X5 was not correlated to precipitation in June (Table S4). Correlation coefficients between these eight monthly climate variables and ring width were calculated for each individual tree and used as traits for genetic correlation and association analysis.
Genetic variation among provenances

Significant differences in drought response were observed among provenances for the drought events in 1993 and 2000, significant but to a lesser extent in 2003 (Figure 2, Table S5). Generally, the highest variation among provenances was observed in 2000, while also the strongest growth decline across all provenances was observed. Repeated measure ANOVA confirmed the significant differences among provenances across all three drought periods (Table S6). The best performing provenance across all drought events was R06, followed by provenances S10, Q14 and I19 (Figure 2). Significant genetic variation among provenances was also found for incremental growth and wood density parameters (Figure 3, Table S5).

Degree of genetic determination

Treating Norway spruce as a homogenous species, the repeatability for four drought response indicators across provenances varied between nil for resilience up to 0.18 for resistance. Significant repeatabilities across all provenances were found for resistance, recovery and relative resilience (Table 2). When calculated for individual provenances, repeatabilities varied significantly between individual provenances ranging from nil to 0.44 (Table 2). The Bulgarian provenance d14 revealed significant repeatabilities for all four response measures, while the other provenances revealed significant repeatabilities only for one to three response measures. Provenances b03, d04, X05, Y18 and ST did not show significant degree of genetic determination for drought resistance Rs and thus were excluded from the SubsetQD (Table 2). Repeatabilities closely resemble mean pairwise correlation coefficients between drought response indicators of the individual drought periods (Table S7).

Phenotypic trait correlations

Genetic and phenotypic trait correlations are important indicators for indirect selection on specific traits and could guide the development of screening tools for drought-resistant genotypes in breeding programs. When we tested for provenance-specific differences in the complex relationships between wood properties, drought stress indicators, and climate-growth correlations, the correlation matrices revealed ample differences among provenances for numerous trait combinations (Figure 4). Generally, variation in trait correlations among wood properties, among drought indicators and among climate-growth correlations were small, but correlations between drought indicators and wood properties or between drought indicators and climate-growth correlations were high and ranged from positive correlations in one provenance to negative correlations within another. For example, the correlation between resilience during the drought in 1993 (Rs93) and growth response to May precipitation (CorMayP) was 0.62 for provenance B3 whereas it was −0.55 for provenance Q14 (both significant at $P < 0.001$ and $<0.01$, respectively; Figure 5). When we tested for correlations across all provenances (i.e., for the entire dataset), the same correlations were rather weak or even non-significant, justifying provenance specific trait correlations (Figure S3).

Population structure

To avoid spurious false positive associations between SNPs and quantitative traits, we tested for population structure and relatedness with 260 polymorphic neutral SNPs. When considering 155 trees from eleven provenances in population structure analysis, the likelihood (LnP) of K increased reaching a continuous plateau with large variances (Figure S4A). Using Evanno’s method, the Delta K plot showed low values in all Ks that were tested for, but two peaks for K = 4 and K = 7 with the latter one being the highest (Figure S4A). Bar plots with 155 individuals illustrated the uncertain assignment of individuals to the seven groups. In particular, six individuals from provenance S10 and one individual each of d04 and b03 showed genotypic information that was very distinct from all analyzed provenances (Figure S4A). After these eight genotypes were removed and the structure analysis recalculated with 147 individuals from eleven provenances the LnP of K increased reaching a continuous plateau with reasonably small variances (Figure S4B). Evanno’s method resulted into a highest Delta K for K = 2 as illustrated by bar plots of the 147 individuals (Figure S4B). This structure result (Q) was subsequently used for all association analyses. Kinship estimated for these 147 individuals resulted in a matrix with values ranging mainly from 0.1 to −0.1 and a slightly negative mean kinship value of −0.007 (Figure S5).

Association analysis

147 trees from 11 provenances (Table 1) were considered for the association analysis (Figure S6). First, all provenances were included in the association study. Based on the quantitative genetic analysis a second analysis was performed with a subset of provenances that revealed significant quantitative genetic variation within provenances, i.e., significant degree of genetic determination (SubsetQD with provenances R06, d14, I19, Q14, R13 and S10). With the MLM+Q+K model, 1,714 SNPs (all provenances) and 1,707 SNPs (SubsetQD) were tested against 51 traits: four drought scenario traits (Scen1, Scen2, Scen3, Scen4), 12 drought stress parameters ($Rt93$, $Rc93$, $Rs93$, $Rt00$, $Rc00$, $Rs00$, $Rt03$, $Rc03$, $Rs03$, $Rt06$, $Rc06$, $Rs06$), eight climate-growth correlations

![Figure 1](https://example.com/image1.png)

**Figure 1** Drought occurrence and resulting increment declines of Norway spruce at a trial site located in the northeastern Austria. The bars show the standardized precipitation index SPI (given in standard deviations) on time scales of 1 (SPI-1) and 3 (SPI-3) months. The line plots illustrate the course of annual increment, as dimensionless ring width index (RW index). Arrows mark the three most distinct drought events which effects were analyzed in the present study.
Among 87,414 marker-trait pairs 17 significant associations were found for all provenances. For the SubsetQD, we found 20 significant associations (Table 3, Figure 6, Figure S7) among the 87,057 marker-trait pairs. As some SNPs were associated with several correlated traits and some SNPs were significant in both sets, a total of 29 SNPs—physically located within or in close neighborhood of 24 genes—were found to be associated with the studied traits (Table 4). Significant SNPs found in both sets (all provenances and SubsetQD) revealed genes and genotypes that are linked to drought response and wood quality across the complete Central European range of Norway spruce, while those markers found to be significant within the SubsetQD might indicate local adaptations to drought prone environments. Among all SNPs detected, 12 were mainly detected in single individuals having extreme phenotypes of the respective trait (Table 3).

In general, the amount of variation explained by associated SNPs was higher for SubsetQD (R² = 0.23-0.43) compared to all provenances (R² = 0.11-0.31). For SubsetQD, markers GQ03709-C10.1.1133 (R² = 0.33) and GQ03204-K15.1.405 (R² = 0.36) are significantly associated to drought resistance scenario one and two which combine continuous drought resistance in 1993 and 2000 into a single binomial trait (Figure 6, Figure S2). For GQ03204-K15.1.405, homozygous TT genotypes had higher drought resistance than heterozygous CT. On the other hand, homozygous CC genotypes of GQ03709-C10.1.1133 were found to have higher drought resistance than TT or CT genotypes. From the continuous drought traits, associations were only found for response variables in the periods 2000 and 2003. Traits of the drought event 2000 (Rc00, Rs00 and rRs00) were associated to four SNPs with R² between 0.26 and 0.36, while from the drought 2003 only a single response variable (Rc03) was associated to a SNP (ss538944271) with R² = 0.26. Finally, three SNPs with R² between 0.23 and 0.25 were found to be associated to the correlation between June temperature and annual ring width (CorJunT). Significant genotype-trait associations for 7 SNPs were also found for wood property traits (RW, EW, LW, RD, ED, LD, MAXD, MIND, +b [binomial], +t [categorical]) showing the highest R² (between 0.28 and 0.43) within our study. Among them, SNP 08Pg07115c was significantly associated to both RW and EW (R² = 0.27-0.29). SNP GQ03709-C10.1.483 which is associated to LW (R² = 0.42) is located 799 nucleotides upstream of GQ03709-C10.1.1133, i.e., one of the SNPs associated with drought response. Also, a single
### Table 2: Repeatabilities within each single and across all provenances as given by $r \pm SD$

| Provenance | Measure | $\sigma^2_p$ | $\sigma^2_g$ | $\sigma^2_e$ | $r_p$ | $r_g$ | $r_e$ | $r$ |
|------------|---------|-------------|-------------|-------------|-------|-------|-------|-----|
| b03        | $\sigma^2_p$ | 0.011 ± 0.088 | 0.043 ± 0.114 | 0.000 ± 0.000 | 0.053 ± 0.082 |
| d04        | $\sigma^2_p$ | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| R06*       | $\sigma^2_p$ | 0.198 ± 0.133 | 0.035 ± 0.077 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| ST         | $\sigma^2_p$ | 0.233 ± 0.137 | 0.059 ± 0.129 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| X05        | $\sigma^2_p$ | 0.071 ± 0.120 | 0.013 ± 0.078 | 0.000 ± 0.000 | 0.041 ± 0.093 |
| d14*       | $\sigma^2_p$ | 0.071 ± 0.120 | 0.019 ± 0.112 | 0.019 ± 0.115 | 0.053 ± 0.118 |
| I19*       | $\sigma^2_p$ | 0.192 ± 0.140 | 0.253 ± 0.141 | n.e.         | n.e.   |
| Q14*       | $\sigma^2_p$ | 0.373 ± 0.148 | 0.149 ± 0.116 | 0.000 ± 0.000 | 0.022 ± 0.089 |
| R13*       | $\sigma^2_p$ | 0.575 ± 0.105 | 0.057 ± 0.105 | 0.057 ± 0.105 | 0.153 ± 0.089 |
| S10*       | $\sigma^2_p$ | 0.048 ± 0.129 | 0.324 ± 0.133 | n.e.         | n.e.   |
| Y18        | $\sigma^2_p$ | 0.061 ± 0.112 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Overall    | $\sigma^2_p$ | 0.022 ± 0.018 | 0.032 ± 0.021 | 0.016 ± 0.012 | 0.014 ± 0.012 |

* Provenances that were grouped into the SubsetQD for additional association analysis.

$\sigma^2_p$ - Variance among provenances; $\sigma^2_g$ - Variance within groups; $\sigma^2_e$ - variance among groups. Boldface indicates significant repeatabilities.

Association to wood density ($RD$, $RD_t$) was detected (WS-2.0-GQ0036. TB-K03.1-397, $R^2 = 0.25-0.26$).

When all provenances were considered for the association study, no single SNP was found to be associated with a scenario of drought resistance. However, several significant associations were found for drought response indicators for the drought events in 2000 and 2003. Traits of the drought 2000 ($Rc<0$, $Rs<0$) were associated to five SNPs ($R^2 = 0.14-0.15$), with one of them (WS-2.0-GQ02827.B7-M03.1-1062) also detected within the SubsetQD. For 2003, ten SNPs were associated to three traits ($Ri03$, $Ri03$ and $Rs<0$). In few cases, SNPs were associated to different drought traits of the same drought event, but also across different drought events. Two SNPs were significantly associated with wood properties: 00930-O17-366 was associated to ED ($R^2 = 0.11$) and GQ00410.B3-M23.4-2998, also detected within the SubsetQD, to LW ($R^2 = 0.24$).

SNPs significantly associated were mainly located within exons (18 SNPs). Others (11 SNPs) belonged to untranslated regions (UTR) like introns, downstream regions or gene-empty regions (Table 4). Variations in five exon-located SNPs were found to affect the amino acid sequence (non-synonymous substitution; missense change). On the other hand, variations in the remaining 13 exon-located SNPs were found to have no effect on the amino acid sequence (synonymous substitution) as they are located mainly in the 3rd codon position.

**Exploration of associated genes**

Exploration of the 24 genes in which the 29 SNPs are located or to which they were closely linked indicates a highly diverse assortment of Pfam domains (Table 4). Additionally, BLASTP analysis (Table S8) allowed annotations of some genes (e.g., MA_39636g0010: Putative hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase involved in lignin biosynthesis pathway; MA_90007g0010: ARM repeat protein interacting with ABF2) but did not reveal similarity to any known genes for some others (e.g., MA_10434266g0010; MA_588952g0010).

Exploration of the expression profiles of the associated marker genes within the gene expression resource NorWood (Figure S8) resulted in expression data for 20 out of the 24 associated genes. Hierarchical clustering of these expression data revealed seven clusters (Figure S8A). Out of these, one cluster (cluster-c) showed a well defined expression profile across the stem section (Figure S8B) with maximum expression within cambium (T-03, T-04) and xylem expansion cells (T-05, T-06). Cluster-c comprised 5 genes including multicopper oxidase / L-ascorbate oxidase, Leucine Rich Repeat...
DISCUSSION

Under global warming, extreme climate events are expected to increase in frequency, duration and intensity and such events are considered the main causes for forest mortality and dieback (Allen et al. 2010). In this study, we analyzed the genetic variation of drought response of Norway spruce by means of quantitative and molecular genetic analysis of specimens growing for 35 years in a drought-prone environment. During this time, three strong drought events resulted in growth reductions of up to 80% revealing significant genetic variation among and within the tested provenances. On basis of the observed quantitative genetic variation, we found significant associations between drought response indicators and wood properties for a total of 29 SNPs, though 12 of them were due to single individuals having extreme phenotypes of the respective trait. These included SNPs closely linked to genes involved in lignin biosynthesis, cell differentiation or transcription. Our study is one of the first that links dendroclimatic analysis, quantitative genetic provenance and breeding research with genomic association studies. By making use of existing trials that experienced in situ stress events that were archived in tree rings, we were able to provide meaningful phenotypes for the association analysis. Moreover, restricting association analysis to provenances with significant quantitative genetic variation among individuals resulted in stronger and likely more meaningful phenotype-genotype associations.

Quantitative genetic variation

Among various European conifers, Norway spruce was found to show the highest vulnerability to extreme heat and drought events (Lévesque et al. 2013; Zang et al. 2014). Thus, it is remarkable that we observed significant variation in drought response among as well as within some of the tested provenances. However, this can be explained by the broad geographic amplitude of the tested plant material, which comprised provenances of the Eastern Alpine range, the western Carpathian Mountains, the Bohemian Massive and the Rhodope Mountains at the southern fringe of the species. This geographic area harbors high plastid diversity as a consequence of multiple refugial areas and post-glacial migration history as confirmed by previous genetic studies (Collignon et al. 2002; Tollefsrud et al. 2008; Mengl et al. 2009) and

(lrr) and ERBB-3 binding protein 1; Table 4). Other gene clusters have maximum expression within the zone of secondary wall formation (cluster-a), or within mature xylem (cluster-c).
as expected at the rear edge of the species’ range (Hampe and Petit 2005). However, neutral genetic diversity in nuclear genes within the Alpine and Central European domain was found to be relatively low in Norway spruce (Heuertz et al. 2006) as we could confirm in our analysis with 264 neutral SNPs. These markers show only a low differentiation with a global $F_{st}$ of only 0.015, probably resulting from extensive gene flow via pollen which is able to mediate effective gene transfer even between refugial lineages (Liepelt et al. 2002) On the other hand, analysis of the 29 SNPs which are associated to drought and wood traits revealed a higher global $F_{st}$ of 0.045 and thus indicates slightly higher differentiation at markers which are likely to be under selection.

Generally, studies on the genetic variation of drought response in Norway spruce are rare. Burczyk and Giertych (1991) analyzed growth reaction of 17 polish provenances within drought years in the 1980s. In contrast to our study, Burczyk and Giertych (1991) did not find differences among provenances, they just observed a weak genotype x environment interaction and concluded that selection for drought resistance in Norway spruce might not be successful. This conclusion may rely on the tested genetic material, which consisted only of provenances from Poland and thus represented a small and genetically homogeneous part of the natural range. Actually, the single Polish provenance in our test, d04-Istebna (similar to Prov. 99 in Burczyk and Giertych 1991) neither showed remarkable drought response nor revealed significant repeatability for any of the applied drought measures. This indicates that selection pressures for drought might be rather weak and the response of trees therefore uniform within the northwestern Carpathian Mountains. More consenting evidence of genetic variation in drought response comes from several seedling studies. Schmidt-Vogt (1978) tested drought resistance in seedlings of five Central European provenances and obtained significant differences among the seedling origins. Here, provenances from higher elevations were found to show higher suction potential and this was interpreted as better adaptation to water-limited conditions (Schmidt-Vogt 1978). More recently, this finding was confirmed (Blödner et al. 2005) in one-year old seedlings by investigating physiological traits related to drought and freezing sensitivity. Blödner et al. (2005) found drought-sensitivity and freezing-sensitivity to be co-occurring traits suggesting that the physiological reactions for both environmental extremes are based on comparable oxidative stress protection systems.

Evidence for genetic variation in drought resistance within populations comes from a seedling experiment with 12 clones originating from another single Alpine population (Wagner 1993). Wagner (1993, 1994) measured stress respiration, photosynthesis, needle morphology and drought tolerance on clonal propagules from 12 Alpine trees and found significant variation among clones as well as correlations between the physiological traits and growth performance at tree age of 12. Similar inter-clonal variation of hydraulic properties was found within young and mature trunk wood of 5- and 24-year old Norway spruce trials consisting of vegetatively propagated clonal material (Rosner et al. 2007, 2008). Broad sense heritability for measures of hydraulic conductivity within these studies ranged from 0.14 to 0.31 for the different conductivity parameters (Rosner et al. 2007). Higher broad sense heritabilities between 0.10 and 0.41 were found for wood density peaks initiated by strong water deficits in clonal trials in southern Sweden (Rozenberg et al. 2002). Our study confirms the genetic variation in drought response among individuals within and among provenances. The highest repeatabilities were found for resistance, slightly smaller ones for recovery, resilience and relative resilience. These differences can likely be explained by the complexity of physiological processes underlying the different dendrologically-based drought measures. Resistance captures the ultimate response of a tree to an ongoing drought and is thus the most direct parameter of drought response. The
remaining parameters include the recovery period and are likely affected by climate conditions after drought or the individual allocation of non-structural carbohydrates (NSC). This complex trait architecture is affected by climate conditions after drought or the individual allocation of non-structural carbohydrates (NSC). This complex trait architecture of drought response makes quantitative genetic studies as well as genotype-trait associations difficult.

Besides differences among the drought response indicators, we were able to demonstrate that some provenances exhibit high degrees of genetic determination for one or more of the calculated drought indicators, while others do not reflect any repeatability. For example, provenance d14 from high elevations at the southern fringe of the species range revealed high repeatability of up to 0.35 for all four drought indicators. Although our data do not reveal a clear climatic or geographic trend to drought response or genetic determination, they strongly indicate varying selection pressures within the seed source populations.

This argument is strongly supported by highly variable phenotypic correlations between drought response indicators and morphological traits (Cheverud 1988; Roff 1996). When comparing phenotypic trait correlations among provenances, we observed strongly varying patterns, which differ not only in magnitude, but also in direction (Figures 4 and 5). Generally, theoretical studies and laboratory analysis indicate that correlations between life history traits (and we may consider drought response as such) should rather be negative and correlations among life history traits are typically larger than between life history traits and morphological traits (Roff 1996). However, when tested across different environments, genetic correlations might change from negative to positive even when estimated on the same genetic material (Sgrò and Hoffmann 2004). In this context, our variable correlation patterns might be explained by differences in the genetic system, i.e., the extent of pleiotropy, the demographic history of the provenances or due to differences in selection intensity (Lascoux 1997).

Across provenances, drought resistance (in 1993 and 2000) was found to be positively correlated to mean annual increment, but negatively to wood density and earlywood density. Similar correlation patterns were previously obtained between vulnerability to cavitation of mature wood and hydraulic conductivity to wood density and tree diameter (Rosner et al. 2008) and were thus discussed as tradeoffs between hydraulic and mechanical stress.
resulted mainly in different SNPs associated with drought response

D. Don (Dillon et al. 2012). In contrast, Beaulieu et al. 2013) and

significant markers were found to be responsible for 37 significant genetic associations for drought response, wood property traits and climate-growth correlations. These SNPs explain between 11% and 43% (Table 3) of the trait variation, which is relatively high as compared to other association studies. This pattern indicates strong divergent local selection of populations, which are connected by intermediate to high levels of gene flow as the low FST value at neutral nuclear genes indicates (Le Corre and Kremer 2012). In contrast, Beaulieu et al. (2011) analyzed 492 trees belonging to 165 families from 40 provenances in E. globulus and Pinus radiata, but also in the nucleus (Manna 2015).

Significant genetic markers

29 SNPs were identified to be responsible for 37 significant genetic associations for drought response, wood property traits and climate-growth correlations. These SNPs explain between 11% and 43% (Table 3) of the trait variation, which is relatively high as compared to other association studies. This pattern indicates strong divergent local selection of populations, which are connected by intermediate to high levels of gene flow as the low FST value at neutral nuclear genes indicates (Le Corre and Kremer 2012). In contrast, Beaulieu et al. (2011) analyzed 492 trees belonging to 165 families from 40 provenances in E. globulus and Pinus radiata, but also in the nucleus (Manna 2015).

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| Marker name | Associated Trait | Genomic position | Nucleotide | Exon / Intron | Strand | SNP | SNP type | Codon position | Non synonymous substitution / Missense change | PFAM Description | BLAST-P |
|-------------|------------------|------------------|-------------|---------------|--------|-----|----------|---------------|-----------------------------------------------|----------------|---------|
| GQ03709-C10.1.1133 | Scen1 | No gene - downstream (13 nt) MA_59794g0010 | 21494 | + | [T/C] | UTR | UTR | | | | Unknown |
| GQ03204-K15.1.405 | Scen2 | No gene - upstream (164 nt) MA_10434266-g0010 | 3694 | — | [T/C] | UTR | UTR | | | | Leucine Rich Repeat No significant similarity found. |
| 08Pg07115c | RW; RWt; EW | No gene - downstream (297 nt) MA_39636g0010 | 24594 | — | [T/C] | UTR | UTR | | Transferase family Hydroxycinnamoyl-CoA shikimate hydroxycinnamoyltransferase |
| GQ03709-C10.1.483 | LW | MA_59794g0010 | 20695 | Exon 1 | + | [T/C] | NSS-M | 2 | GCA = A (Alanine) / GTA = V (Valine) | | |
| GQ03121-E24.1.494 | LW | MA_6741659g0010 | 1238 | Exon 2 | + | [T/C] | SS | 3 | CG[T/C]=R (Arginine) | | |
| PaPHYN-Ri420 | LW | MA_73153g0010 | 13476 | Exon 6 | — | [A/C] | NSS-M | 3 | GAA=E (Glutamic acid) GAC=D (Aspartic acid) | | |
| GQ03205-D16.1.1410 | LW | MA_10432182g0010 | 2114 | Intron | — | [A/G] | UTR | | | | |
| GQ00410.B3-M23.4-2998 | LW | MA_17539g0010 | 1887 | Exon 1 | + | [A/G] | SS | 3 | GAT(T/C)=D (Aspartic acid) | | Calcium-binding protein ERBB-3 BINDING PROTEIN 1 |
| WS-2.0-GQ0036.TB-K03.1-397 | RD; RDt | MA_10426694g0020 | 18480 | Exon 3 | — | [T/C] | SS | 3 | GGA/G]=G (Glycine) | | |

(continued)
| Marker name       | Associated Trait | Genomic position | Nucleotide | Exon / Intron | Strand | SNP type | Codon position | Non synonymous substitution / Missense change | PFAM Description | BLAST-P b |
|------------------|------------------|------------------|------------|---------------|--------|----------|----------------|---------------------------------------------|------------------|----------|
| 00930-O17-366    | EDt              | MA_588952g0010   | 731        | Exon 1        | —      | [A/C]    | SS 3           | CT[C/A]=L (Leucine)                           | No significant similarity found. |          |
| ss538948434      | Rc00             | MA_100637g0010   | 3240       | Exon 3        | +      | [A/G]    | NSS-M 1        | GTT = V (Valine)                              | Pentatricopeptide repeat-containing protein |          |
| c89584_g2_i1_197 | Rc00;Rt03        | No gene - downstream (418 nt) MA_90007g0010 | 15514      | —             | [A/C]  | UTR      |                | RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain) | Unknown          |          |
| PGLM2-0082       | Rc00             | MA_8047450g0010  | 2537       | —             | [A/G]  | SS 3     | CA[T/C]=H (Histidine) | Unknown          |          |
| WS-2.0-GQ02827,B7-M03.1-1062 | Rso0           | No gene (MA_75479) | 904        | +             | [A/G]  | UTR      |                | Unknown          |          |
| NODE-12228-length-276-cov-134.847824-162 | Rso0           | Not in 1kb MA_118377 | 17768      | —             | [T/C]  | UTR      |                | Unknown          |          |
| WS-2.0-GQ03417,B7-O19.1-1215 | Rso0,rRso0     | No gene - downstream (104 nt) MA_119424g0010 | 10110      | —             | [T/G]  | UTR      |                | Unknown          |          |
|                  |                  |                  |            |               |        |          |                | Short chain dehydrogenase | Anthocyanidin reductase |          |

(continued)
| Marker name       | Associated Trait    | Genomic position | Nucleotide | Exon / Intron | Strand | SNP type | Codon position | Non synonymous substitution / Missense change | PFAM Description                                                                 | BLAST-P |
|-------------------|---------------------|------------------|------------|---------------|--------|----------|----------------|-----------------------------------------------|---------------------------------------------|---------|
| GQ03812-F08.1.123 | Rs00;Rs00           | No gene (MA_7880)| 20633      | Exon 1        | —      | [A/G]    | UTR            | [---]                                        | NmrA-like family Male sterility protein KR domain NAD(P)H-binding |         |
| MA_475589g0010-111-C_T | Rs03;Rs03       | MA_475589g0010   | 5299       | Exon 1        | —      | [T/C]    | SS             | 3                                            | Leucine Rich Repeat Translocon-associated protein (TRAP), alpha subunit alpha |         |
| ss538948838       | Rt03                | MA_109428g0010   | 15103      | Exon 4        | +      | [T/G]    | NSS-M          | 1                                            | F-box/LRR-repeat protein subunit alpha NICE-3 protein |         |
|                   |                     |                  |            |               |        |          |                |                                              | 2OG-Fe(II) oxygenase superfamily Non-haem dioxygenase in morphine synthesis N-terminal |         |
| GQ03512-P11.2.1194| Rt03                | MA_10427719g0010 | 15051      | Intron        | —      | [T/G]    | UTR            | 2                                           | 2OG-Fe(II) oxygenase superfamily Non-haem dioxygenase in morphine synthesis N-terminal |         |
|                   |                     |                  |            |               |        |          |                |                                              | EGDF domain-specific O-linked N-acetylglucosamine transferase-like Multicopper oxidase |         |
| GQ03013-D24.3.551 | Rt03                | MA_10434370g0040 | 13176      | Exon 2        | —      | [T/C]    | SS             | 3                                            | EGDF domain-specific O-linked N-acetylglucosamine transferase-like Multicopper oxidase |         |
| ss538944271       | Rc03                | MA_161013g0010   | 7889       | Intron        | +      | [A/G]    | UTR            | 2                                           | EGDF domain-specific O-linked N-acetylglucosamine transferase-like Multicopper oxidase |         |
|                   |                     |                  |            |               |        |          |                |                                              | L-ascorbate oxidase homolog Homeodomain protein HB2 |         |
| GQ0197-K07.1.447  | Rc03                | MA_38472g0010    | 6199       | Exon 6        | —      | [T/C]    | SS             | 3                                            | Homeobox domain START domain |         |
| GQ0197-K07.1.345  | Rc03                | MA_38472g0010    | 6301       | Exon 6        | —      | [A/G]    | SS             | 3                                            | Homeobox-leucine zipper protein ANTHOCYANINLESS 2-like isoform |         |

(continued)
Table 4, continued

| Marker name                  | Associated Trait | Genomic position | Nucleotide | Exon / Intron | Strand | SNP | SNP type | Codon position | Non synonymous substitution / Missense change | PFAM Description | BLAST-P |
|------------------------------|------------------|------------------|------------|---------------|--------|-----|----------|----------------|-----------------------------------------------|-------------------|---------|
| GQ0068-K02.1.644             | Rc03             | MA_47260g0010    | 3906       | Exon 4        | —      | [T/C] | SS       | 3              | TC[T/C]=S (Serine)                           | Tetrtroicopeptide repeat Region in Clathrin and VPS Coatomer WD associated region Clathrin-H-link WD domain, G-beta repeat Cytochrome D1 heme domain Coatomer WD associated region Eukaryotic translation initiation factor eIF2A Lactonase, 7-bladed beta-propeller Nucleoporin Nup120/160 Clathrin heavy chain 1 |
| GQ02904-L21.2.961            | Rc03             | MA_10432150-g0010 | 33483      | Exon 2        | +      | [T/C] | NSS-M    | 2              | CCG = P (Proline) CTG = L (Leucine)          | WD repeat-containing protein VIP3 |
| GQ03204-O22.1.645            | CorJunT          | MA_6521216g0010  | 1742       | Exon 1        | —      | [A/G] | SS       | 3              | GA[A/G]= E (Glutamic acid)                   | Dihydropyrimidinase |
| FCL808Contig2-402            | CorJunT          | MA_2267030g0010  | 105        | Exon 1        | +      | [A/G] | SS       | 3              | GT[A/G]=V (Valine)                          | CutA1 divalent ion tolerance protein 2OG-Fe(II) oxygenase superfamily Non-haem dioxygenase in morphine synthesis N-terminal |
| MA_10289324g0010-1023-[G_A]  | CorJunT          | MA_10289324g0010 | 1649       | Exon 2        | +      | [A/G] | SS       | 3              | CT[A/G]=L (Leucine)                         | GA2OX3 (Gibberellin 2-beta-dioxygenase 3) |

SS= Synonymous substitution; NSS-M= non synonymous substitution Missense change, UTR= untranslated regions.

Putative gene function assigned by BLAST-P (Table S5 for complete BLAST-P output).

PFAM ID could not be found.

SNP on the complementary strand of a translated protein.
oxidase / L-ascorbate oxidase. This protein is known to oxidize ascorbate and was suggested to affect the plant system in response to stress deleteriously (Batth et al. 2017). Homozygous genotypes GG (of ss538944271) were found to show better recovery and this could be linked to a less effective or less expressed L-ascorbate oxidase. In Norway spruce, MA_161013g0010 belongs to a gene family (consisting of at least 24 members) and is preferentially expressed in cambium and xylem expansion layers (NorWood), where new wood is being formed (Figure S8B) and ascorbate might be needed under drought stress.

We also identified significant associations with climate-growth relationships that were inferred from traditional dendroclimatic analyses. The correlation between June temperature and annual increment is one of the most important climate-growth relationships in Norway spruce growing at lower elevations and at the warm margin of the species range (Schiessl et al. 2010). It demonstrates that high temperatures within the main growing period of spruce have a negative impact on annual increment. Hence, it is strikingly important to estimate the genetic variation in this relationship and to identify candidate genes which are involved in the response: All three SNPs with significant associations to CorJunT are located in exons and in all three markers, heterozygous genotypes show lower correlations between annual increment and June temperature compared to the homozygous genotypes. These heterozygous genotypes seem to be less sensitive to high summer temperatures and might be advantageous for growing in warm habitats under climate change. On the other hand, for all these three SNPs an homozygous genotype is absent (Table S9) but just the SNP GQ03204-O22.1.645 is out of Hardy-Weinberg equilibrium due to some reason we cannot ensure but putatively a deleterious effect of the homozygous absent allele. Among the three linked genes, GA2OX3 (Gibberellin 2-beta-dioxygenase 3) is the most interesting one as this protein is involved in the gibberellin biosynthesis, which is an important plant hormone for wood formation (Aloni 2013) and was found to be involved in the physiological response to drought (Zawaski and Busov 2014).

08P070115c is associated with two important wood traits in SubsetQD: EW and RW. This SNP marker is located 297 nucleotides (nt) downstream of a gene (MA_39636g0010) which is a transferase putatively involved in the lignin biosynthesis pathway (Table 4). Previous studies suggested that drought stress effects might be mitigated by plant secondary metabolism and lignification (Cabane et al. 2012). A BLASTP search of this gene indicated alignments to a hydroxycinnamoyl-CoA shikimate/quininate hydroxycinnamoyl transferase (HCT) within Larix kaempferi, Pinus massoniana, Pinus pinaster and P. radiata (Table S8). Compared to other tree species, Norway spruce has a large genome size (Nystedt et al. 2013) and this putatively HCT gene belongs to a family of 10 genes all putatively coding for HCT (Figure S9), probably with different levels of lignin biosynthesis activities and different substrate preferences (Kiselev et al. 2016). To test whether MA_39636g0010 coding protein still have the described function, more research would be necessary (Wagner et al. 2007). Nevertheless, there is strong evidence linking drought stress to modifications in lignification in different plants (Yoshimura et al. 2008), probably as a strategy to increase mechanical strength and/or water impermeability (Hu et al. 2009; Jubany-Mari et al. 2009; Fromm 2013). Drought apparently can result in wall thickening and thickening, as observed in the tracheids of drought-stressed Norway spruce roots (Elkhust et al. 2013). Thickening appears to be caused by a number of mechanisms, including lignification of wall polymers (Moore et al. 2008). An increased amount of lignin improves the mechanical strength of cell walls in a dry environment, and cell wall lignification helps to minimize water loss and cell dehydration (Cabane et al. 2012; Fang and Xiong 2015). So far, the MA_39636g0010 expression profile is not available at the NorWood platform, but HCT genes were found to be highly expressed during secondary growth and wood formation in conifers (Wagner et al. 2007). It should be noted that this SNP is out of Hardy-Weinberg equilibrium (Table S9) due to some reason we cannot ensure but putatively a deleterious effect of the homozygous genotype “CC”.

Wood density is likely to be influenced by lignin content and may be an important driver of the hydraulic properties in trees. It has been shown to affect conductivity and survival during drought in conifers such as Douglas-fir (Martinez-Meier et al. 2008; Dalla-Salda et al. 2009). For SubsetQD, wood density (RD) was associated to a SNP (WS-2.0-GQ0036.TB-K03.1-397) directly located in the third exon of a putatively ERBB-3 binding protein 1, a gene that promotes organ growth by stimulating both cell proliferation and expansion (Horváth et al. 2006). Finally, for all provenances SNP 00930-O17-366 was associated to earlywood density (ED) resulting into a synonymous substitution being located in the unique exon of an unknown protein with no significant similarity found by BLASTP at present.

Breeding Norway spruce in a changing climate

Norway spruce is the economic basis for sawnwood and pulp/paper production in many parts of Europe, particularly in Northern and Central European countries. Apart from Scandinavia, its high importance is not yet considered in breeding activities for more productive and climate resilient trees in Central Europe. The present study shows that the species’ Central and Southeastern European range harbors high genetic variation in drought response and in important wood characteristics, which could be further exploited in breeding schemes and genetic conservation programs. Given the high vulnerability of coniferous forests at the southern fringe of species ranges (e.g., Schueier et al. 2014) and the low perception of such forests in nature conservation, the underlying basis of this variation is highly endangered. Thus, the given analysis is not only a step forward in the knowledge of the Norway spruce drought resistance and wood properties traits, but also in raising awareness to important adaptive variation that could get lost in the future. The restriction of our association study to provenances with significant genetic within-provenance variation is expected to avoid spurious false positive marker-phenotype associations. It allows us to provide a set of relevant genetic markers that could be useful (after validation in other populations) for breeding by marker assisted selection (MAS) and conservation genetics in a changing climate (Holiday et al. 2010) with future drought scenarios (Hamamishi and Campbell 2011). Moreover, we show that drought resistance as estimated from dendrochronological time series harbors sufficient degree of genetic determination and should be used as phenotypic trait within tree breeding and seed selection programs.

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