A candidate gene-based association study to investigate potentially adaptive genetic variation in European beech (*Fagus sylvatica* L.)

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In Memory of Dr. Sarah Seifert

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Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
bp(s)	Base Pair(s)
DBH	Diameter at Breast Height
DNA	Deoxyribonucleic Acid
FDR	False Discovery Rate
GLM	General Linear Model
GWAS	Genome-wide Association Study
HKA	Hudson-Kreitman-Aguadè
LD	Linkage Disequilibrium
Mb	Mega Bases
MCMC	Markov Chain Monte Carlo
MLM	Mixed Linear Model
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Locus
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SSR	Short Sequence Repeat
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UTR	Untranslated Region

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1 Introduction

1.1 General Introduction

Global warming led to an increase of global surface temperature of 0.85 °C in the time period from 1880 to 2012. A further temperature increase (0.3-1.7 °C to 2.6-4.8 °C) is predicted, depending on the underlying scenario, for the period 2081–2100 relative to 1986–2005 (IPCC 2013). For Europe, the observed warming is even higher. An increase of temperature of 1.3 °C was observed between pre-industrial times and the decade 2002–2011 (EEA 2012), and the annual temperature is projected to increase by 2.5° to 4.0 °C between 2071–2100 compared to the reference period. Thereby, warming is predicted to be greatest in Northeastern Europe and Scandinavia in winter and in Southern Europe in summer (EEA 2012). Also changes in precipitation were observed since pre-industrial times. It increased in Northern and Northwestern Europe and decreased in Southern Europe. For Germany a warming of 2.5° C to 3.5 °C is predicted, depending on the underlying scenario until the end of the century compared to the reference period 1961–1990, whereas the highest warming is expected in South and Southeast Germany in winter (Jacob et al. 2008). Further, a decrease of precipitation during summer is predicted, especially in South and Southwest Germany as well as an increase of precipitation during winter.

These environmental changes may have a strong influence on living systems. As shown by Parmesan and Yohe (2003) it is very likely that the geographic distribution of animals and plants is already affected by climate change. Thereby, the prediction of the influence of global warming on tree species is of major importance, because many of them provide important ecosystem services as well as resources for human use (Aitken et al. 2008). In general, there are three different possibilities how forest trees can react to global warming: migration, adaptation or extirpation (Aitken et al. 2008). Tree species have faced large-scale global environmental changes during their evolutionary history and most of them have survived (Hamrick 2004). However, the predicted changes are much faster and thus, it is questionable if tree species are able to track climatic changes in future (Davis and Shaw 2001, Aitken et al. 2008). An appropriate migration might mainly be possible under moderate warming particularly in mountainous areas by migrating short distances among microsites or elevations (Aitken et al. 2008). Also, for an appropriate adaptation to the new environmental conditions global warming might happen too fast (Jump and Peñuelas 2005).

The adaptation potential of European beech (*Fagus sylvatica* L.) to climate change, one of the most important deciduous forest tree species in Central Europe, is controversially discussed. While some authors classify beech as a sensitive tree species in regard to the predicted environmental changes (Rennenberg et al. 2004, Lüpke 2004) other authors conclude that beech will not lose its importance and adaptedness in future (Ammer et al. 2005, Kölling et al. 2005). However, changes in marginal beech populations have already been observed (e.g., Charru et al. 2010, Peñuelas and Boada 2003), and different modeling studies predict range shifts for this species in context of global warming (Meier et al. 2011, Hanewinkel et al. 2013). Thus, adaptive traits relevant for an adaptation to climate change like bud burst and/or drought stress tolerance might gain in importance in future. Nevertheless, there have been only few studies which investigated genes probably involved in these traits in beech (Seifert et al. 2012).

This study is part of the climate impact research project "KLIFF – climate impact and adaptation research in Lower Saxony" (http://www.kliff-niedersachsen.de). Since in the first phase of the project mainly genes probably involved in the adaptation to drought stress (see Seifert 2012 for results) were investigated, this study focusses on the analysis of variation in candidate genes probably involved in bud burst. Seedlings of different beech populations in Northern Germany were planted together in a translocation experiment to detect differences in their bud burst behavior. This facilitated an analysis of associations between genetic variation and the observed phenotypic trait. In addition, one plot of the translocation experiment was established on a comparatively dry site facilitating the analysis of drought stress tolerance of the differently originated seedlings.

1.2 Investigation of Adaptation

Nowadays, it is common to label loci as "adaptive" if they either affect phenotypic traits that are known or suspected to be under selection or if they show statistical signatures of historical selection (Barrett and Hoekstra 2011). In general, methods for the identification of polymorphisms related to adaptation can be classified into bottom-up and top-down approaches. The bottom-up approach identifies putative adaptive markers and genes which show signatures of selection, whereas top-down approaches try to find the genetic basis of known adaptive traits (Barrett and Hoekstra 2011, Prunier et al. 2013).

A vast number of methods have been developed for the detection of selection (bottom-up). Because of the high number of different methods, only the most common tests based on the review by Nielsen (2001), will be described in the following. In general, tests for selective neutrality can be divided into three different categories (Nielsen 2001, Beaumont 2005): (1) detailed modeling of selection at individual loci or sequences, (2) multilocus comparisons and (3) comparison of patterns of nucleotide substitution among synonymous (substitutions cause no amino acid exchanges) and non-synonymous sites (substitutions cause amino acid exchanges). In the first category, Tajima's *D*-test (Tajima 1989) is the most popular for nucleotide data. It is based on the number of pairwise differences and the number of segregating sites in a sample of nucleotide sequences. Closely related methods based on slightly different statistics are the tests by Fu and Li (1993) and Fay and Wu (2000). In the second category (multiple loci tests), the Lewontin-Krakauer test (Lewontin and Krakauer 1973) (see below) and the Hudson-Kreitman-Aguadè (HKA) test (Hudson et al. 1987) are often applied. In the HKA test the variability within and between species is compared for multiple loci. This method is based on the assumption that in the absence of selection the expected number of segregating sites within species and the expected number of fixed differences between species are proportional to the mutation rate, and their ratio should be constant among loci. A related approach to the HKA test is the McDonald-Kreitman test (McDonald and Kreitman 1991) which falls in the third category of selection detection methods, because different classes of mutations are compared. In this test, the ratio of non-synonymous to synonymous polymorphisms within species is compared to the ratio of the number of non-synonymous and synonymous fixed differences between species. Without selection, the ratio of the number of fixations to polymorphisms should be the same for synonymous and non-synonymous sites (Nielsen 2001).

However, among the several strategies for the detection of selection, F_{ST} outlier approaches are becoming widely used (Antao et al. 2008). The background of these methods is that loci under selection are expected to show significant higher levels of differentiation between populations than neutral ones. Most of these methods are based on the classic Lewontin-Krakauer test (Lewontin and Krakauer 1973) which compares single locus estimates of F_{ST} to an expected neutral distribution of F_{ST} (Helyar et al. 2011). A general concern about this test is that the influence of demographic history may lead to false positive results. Thus, different methods based on the original idea have been developed. One often used approach is the

method by Beaumont and Nichols (1996) which uses a classical island model to generate the expected neutral distribution of F_{ST} estimates. The approaches by Beaumont and Balding (2004) and Foll and Gaggiotti (2008) are Bayesian methods based on logistic regression models of locus and population effects on F_{ST} (Helyar et al. 2011).

As mentioned in the beginning, top-down approaches try to find the genetic basis of known adaptive traits (Barrett and Hoekstra 2011). In this context, different kinds of association studies are applied. These studies attempt to identify patterns of polymorphisms that vary systematically between individuals with different phenotypes (Balding 2006). Stands or experimental populations genetically investigated to find those associations, are called *association populations* (Finkeldey 2010). In general, association studies can be classified into different categories. One of the earliest approaches is *Quantitative trait locus* (QTL) *mapping*. These studies use a large number of individuals from a known pedigree (usually the F_2 generation or a backcrossed family of a known cross) that show substantial variation in the phenotypic trait of interest (Kirk and Freeland 2011). For most forest trees however, the production of F_2 or backcross families is not feasible in a reasonable time span, because of their long generation times. Nevertheless, the high level of heterozygosity in outcrossing forest tree species allows the use of F_1 full- or half-sib progenies for genetic map construction (Gailing et al. 2009). QTL mapping in forest trees has been used for several species and traits for more than 20 years. Nevertheless, with this technique it was not possible to identify the specific underlying genes for complex traits in forest trees as it has been done in model systems or a few crop species (Neale and Kremer 2011). *Candidate polymorphism studies* focus on individual polymorphisms which are suspected to be involved in the manifestation of a special trait (Balding 2006, Foulkes 2009). These polymorphisms are commonly DNA sequence variations of single nucleotides called SNPs (single nucleotide polymorphisms). Usually, SNPs are also analyzed in the *candidate gene approach*. Here, genes are investigated which are potentially involved in the trait of interest. Different techniques are available to identify candidate genes. For instance, gene expression analyses, investigations of mutants showing special phenotypes or the analyses of known genes in model organisms are applied (Pflieger et al. 2001, Zhu and Zhao 2007, Kirk and Freeland 2011). In this context, the relatively recent technique of next-generation sequencing (NGS), offers new opportunities facilitating the concurrent production of thousands to millions sequences within a single sequencing run. Thus, it is possible to analyze whole genomes or transcriptomes in compara-

tively short time. This technique facilitates also the implementation of *genome-wide association studies* (GWAS). Here, the entire genome is covered with genetic markers (commonly SNPs). The strategy is to genotype enough markers across the genome that functional alleles will likely be in linkage disequilibrium (LD) (the correlation between alleles in a population) with at least one of the genotyped markers (Myles et al. 2009). The number of markers varies between species depending on genome size and LD decay. For instance, it is assumed that over two million markers are necessary to cover the grapevine genome (475 Mb) and up to 15 million markers are required for maize (Myles et al. 2009). In most forest tree populations a high marker density would be needed, due to rapid decay of LD. Nevertheless, the advantages of rapid decay of LD is that once a marker-trait association has been discovered and validated, it is likely that such a marker is at close physical distance to the functional variant (Neale and Kremer 2011).

A well known problem in association studies is population structure which can lead to spurious associations and thus cause an elevated false-positive rate (Lander and Schork 1994, Zhao et al. 2007). Different approaches have been developed to solve this problem. For instance, neutral genetic markers or random markers throughout the genome are used to estimate relatedness among individuals of the association population (Myles et al. 2009). Another common approach is to include population structure as a covariate in programs for association analysis.

Bottom-up and top-down approaches are often combined. Thus, promising loci for a following association analysis can be selected by applying tests for neutrality. For instance, Eckert et al. (2009a) selected candidate genes, putatively involved in the tolerance of cold temperatures, applied several tests for neutrality and detected signatures of selection for some loci. In a different study (Eckert et al. 2009b) the authors associated these loci with cold-hardiness related traits.

Recently, amplified fragment length polymorphisms (AFLPs), microsatellites (short sequence repeats – SSRs), isozymes and SNPs have been used to analyze the adaptation to climate change related traits in beech. For instance, Kraj and Sztorc (2009) found differences in genetic variability of early-, intermediate and late-flushing forms of beech using microsatellites. They are short sequence repeats (SSRs) of one to six bp and they are codominant which facilitates a discrimination of homo- and heterozygous individuals. SSRs are thought to be selec-

tively neutral markers, and thus, Kraj and Sztorc (2009) concluded that the observed differences between the phenological forms of beech do not have a direct influence on the fitness of these forms. Nevertheless, neutral loci may show signals of selection when they are linked with adaptive loci (Montgomery et al. 2010). Microsatellite were also used by Bilela et al. (2012) who analyzed adaptation to moisture and temperature in beech and found loci showing signals of selection. In addition, they applied isozyme markers to determine the genetic variation and differentiation of the populations. Isozymes are structurally different molecule forms of an enzyme with the same catalytic function (Kumar et al. 2009). The application of these codominant markers is comparatively cheap and easy, but they might be affected by environmental conditions, and they show a low level of polymorphisms (Kumar et al. 2009). In addition, isozymes might be rather neutral than adaptive markers as summarized by Eriksson (1998). By using a genome scan approach, Pluess and Weber (2012) identified AFLP outliers by analyzing *F. sylvatica* growing at dry and mesic sites. AFLPs were also used by Jump et al. (2006) to investigate temperature-related adaptive differentiation of European beech. The AFLP method is based on a selective amplification of a subset of restriction fragments from a mixture of DNA fragments obtained after digestion of genomic DNA with restriction enzymes (Kumar et al. 2009). This technique allows the investigation of hundreds random markers covering large parts of the genome without the need for prior sequence information (Kuchma 2010). However, the amplified genomic region is usually unknown. Because AFLPs are dominant markers, it is not possible to distinguish homo- and heterozygous individuals with this method.

Seifert (2012) firstly analyzed adaptive genetic diversity in addition to neutral genetic diversity by using a candidate gene approach in European beech. Thereby, comparative sequencing was conducted to identify variation (mainly SNPs) in candidate genes for drought stress tolerance. In comparison to SSRs and AFLPs, SNPs are more valuable markers to study adaptation in plants. The location of a SNP is normally known (in contrast to AFLPs) and unlike SSRs, which are in most cases located in non-coding regions, SNPs are regularly found in coding regions (Seifert 2012, Seifert et al. 2012).

Nowadays, it is possible to analyze and to compare whole genomes of organisms by applying NGS. Nevertheless, this technique still is too expensive to analyze a sufficient number of individuals for the study of adaptation in natural populations (Seifert et al. 2012). In addition,

for most forest trees (including beech) there is no reference genome available. Thus, the selection of promising candidate genes for the trait of interest might be the best alternative to study adaptation in forest trees.

1.3 Taxonomy and Ecology of European Beech

European beech (*Fagus sylvatica* L.) belongs to the genus *Fagus* L. in the family of Fagaceae. Worldwide, there are more than 1,000 species belonging to this family and all of them are woody plants (Kremer et al. 2007). The Fagaceae comprise seven genera: *Castanea*, *Castanopsis*, *Chrysolepis*, *Fagus*, *Lithocarpus*, *Quercus* and *Trigonobalanus* (Govaerts and Frodin 1998). Govaerts and Frodin (1998) included also the genus *Nothofagus* to the Fagaceae, but nowadays this genus is classified to an own family (Nothofagaceae) (APG III 2009). The genus *Fagus* is divided into the two subgenera *Fagus* and *Engleriana* comprising about ten species that are distributed in temperate areas of the Northern Hemisphere (Denk 2003).

European beech is a monoecious diploid ($2n = 24$), late-successional forest tree with a height up to 50 m and a maximum diameter at breast height (DBH) of 2.6 m which can reach an age of 300 years (Wilmanns 1990, Ohri and Ahuja 1991, Comps et al. 2001, Ellenberg and Leuschner 2010). It is a highly outcrossing wind-pollinated species with a selfing rate less than 10 % (Merzeau et al. 1994). The leaves of *F. sylvatica* are simple and alternate with a length of 5-10 cm and a width of 3-7 cm. Flowering and seed production starts at an age of 40-50 years (Wagner et al. 2010). Times of high seed production, so-called mast years, are occurring every 5-8 years, whereas the intervals seem to have become shorter in recent years (Schmidt 2006, Ellenberg and Leuschner 2010). The small triangular seeds (beechnuts) are primarily dispersed by gravity and secondarily by animals (Jensen 1985).

European beech is able to grow on a wide variety of sites (Bolte et al. 2007). Within a wide range, this species is not constrained by soil acidity or humus type. Only extremely dry soils and sites with flooding and waterlogging are less favorable (Ellenberg and Leuschner 2010). Concerning climatic constraints, Bolte et al. (2007) described the minimum requirements for European beech with minimum precipitation rates of 500 mm per year or around 250 mm between May and September, a July mean temperature less than 19 °C, less than 141 frost days with a daily minimum temperature below 0 °C, a January mean temperature above -3° C and more than 217 days with a daily mean temperature of 7 °C or more. In addi-

tion, the absence of extreme drought or heat, winter frosts below -35 °C and strong late frost events are required.

In its rated range, European beech is able to outcompete all or almost all other tree species, mainly due to its high shade tolerance and ability to create deep shadows (Wilmanns 1990, Ellenberg and Leuschner 2010). The potentially natural distribution area of beech forest would comprise 910,000 km^2 in Europe, but this area has strongly decreased due to anthropogenic influences during the last centuries (Knapp et al. 2008). Nevertheless, European beech is presently widely distributed throughout Europe. It ranges from Southern Sweden and Norway in the north to Sicily (Italy) in the south and from the Cantabrian Mountains in Spain to the Carpathians and Balkan Mountains (Ukraine, Romania, Bulgaria) in the east (Bolte et al. 2007). In Germany, the center of the European beech distribution, it is the most frequent deciduous forest tree species (amount of 14.8 %) (Schmitz et al. 2004). Its importance will even increase due to current forest conversion programs in which pure conifer stands are converted into pure beech stands or mixed deciduous stands including beech (Tarp et al. 2000, Scharnweber et al. 2011).

1.4 Genetic Variation and Differentiation of European Beech

Forest trees as sessile and long-living species must survive temporally varying and spatially heterogeneous environmental conditions. For that, the presence and maintenance of sufficient genetic variation is a prerequisite (Vornam et al. 2004).

Most of *F. sylvatica* populations in Central, Eastern and Northern Europe have a very homogeneous genetic structure. In Southern and Western Europe a more inhomogeneous genetic pattern can be found, with several separate populations in the Iberian, Italian and Balkan Peninsulas as well as in Southern France (Magri et al. 2006). The analysis of paleobotanical and genetic data indicates that beech survived in various regions of Europe during the last glacial period explaining this pattern. Slovenia and the eastern Alps, and possibly Southern Moravia and Southern Bohemia are considered as the main source areas for the colonization of Central and Northern Europe by beech (Magri et al. 2006, Magri 2008). In general, beech populations show a relatively low differentiation and most of the genetic variation can be found within them (Hattemer and Ziehe 1996, Konnert et al. 2000, Magri et al. 2006, Buiteveld et al. 2007, Pluess and Weber 2012, Seifert 2012). Further, different studies revealed a high genetic diversity for this species (e.g., Oddou-Muratorio et al. 2011, K.C. 2011, Bilela

et al. 2012). Within populations, limited gene flow can induce local genetic differentiation. Spatial genetic structure up to 40 m has been found in European beech populations, mainly due to limited seed dispersal (Vornam et al. 2004, Oddou-Muratorio et al. 2010, 2011, Jump and Peñuelas 2007).

1.5 Impact of Climate Change on the Growing Season

Woody perennials have evolved control mechanisms of their growth cycle that synchronize them with annual variations in temperature (Caffarra and Donnelly 2011). One of these mechanisms is dormancy, a period without growth, which allows a tree to avoid cold injury (Ueno et al. 2013). The dormancy period can be divided into different states. First, in late summer, trees enter a so-called "endodormancy", in which growth is inhibited even in favorable environmental conditions (Caffarra and Donelly 2011). This state is followed by the "ecodormancy", in which external environmental factors (e.g. cold temperatures) prevent bud burst (Horvath et al. 2003). Thereby, the transition from endodormancy to ecodormancy is jointly controlled by the fulfillment of a chilling requirement (low temperatures) and by photoperiod (Basler and Körner 2012). During ecodormancy warm temperatures accelerate bud development ("forcing temperatures") until bud burst begins (Basler and Körner 2012).

The timing of dormancy and thus bud burst is a tradeoff between an early development in spring providing a longer growing season and a delayed development to minimize the risk of frost damage (Basler and Körner 2012, Augspurger 2013). Thus, bud burst is thought to be strongly related to the fitness of trees (Alberto et al. 2011). Simultaneously, phenology is probably one of the most affected adaptive traits by climate change (Bertin 2008). Several studies detected a prolonged growing season over the last decades (e.g., Menzel and Fabian 1999 (+ 10.8 days from 1959 to 1993), Menzel 2000 (+ 10.8 days from 1959 to 1996) Khanduri et al. 2008 (+ 3.3 days per decade)), whereas higher temperatures are the main factor driving these trends (Legave et al. 2013). The lengthening of the growing season is mainly caused by advances of spring phenology rather than a delay of autumn (Menzel et al. 2003). However, based on the results of a common garden experiment Morin et al. (2010) concluded that the response of trees to climate change may not be linear, because the rate of advancement of leaf unfolding dates decreased with increasing temperature in their study. Also, a modeling analysis of Morin et al. (2009) revealed a higher advancement of leaf unfolding under a colder scenario than under a warmer scenario. The authors suggest that in-

sufficient chilling temperatures to break bud dormancy but also effects of the photoperiod may explain these results. Generally, the influence of photoperiod and temperature on bud burst, and consequently for the length of the growing season, seems to depend on species. Different studies revealed that opportunistic pioneer species mainly react to warmer temperatures while late successional species show a more complex response with a large chilling requirement and enhanced photoperiodic sensitivity (Körner and Basler 2010, Caffarra and Donnelly 2011, Basler and Körner 2012).

These findings are in line with several studies which analyzed the role of temperature and photoperiod for bud burst in European beech. The interplay between chilling temperatures, forcing temperatures and photoperiod seems to be quite complex for this species. Different studies revealed a high chilling requirement for *F. sylvatica* (Murray et al. 1989, Falusi and Calamassi 1990, Caffarra and Donnelli 2011, Vitasse and Basler 2013). After Vitasse and Basler (2013) the high chilling requirement might not be reached during warm winters, resulting in a higher forcing requirement and thus delayed bud burst. The lengthened photoperiod through spring might compensate this delay by increasing the forcing accumulation rate or by decreasing the amount of forcing required for bud burst and in this way counterbalance the lack of chilling. As a consequence, the year to year bud burst dates would be relatively stable. In cooler winters, the chilling requirement might be reached and the interaction between chilling temperature and photoperiod might be removed. Under these conditions bud burst would mainly depend on forcing temperatures and bud burst dates would be more variable between years. In conclusion, bud burst might depend on photoperiod in warmer climates while in cooler environments (e.g., high altitudes) bud burst dates may mainly depend on temperature (Vitasse and Basler 2013). As a consequence, beech (and other photoperiod sensitive species) might not be able to extend the growing season in the same amount as temperature sensitive species (Basler and Körner 2012). In addition, a modeling study of Vitasse et al. (2011) revealed that the extension of the growing season for oak is mainly caused by an earlier bud burst while the extension of the growing season for beech is mainly due to delayed leaf senescence in fall. Because the photosynthetic capacities and day lengths are more beneficial in the beginning of the growing season (Morecroft et al. 2003), oak might gain competitiveness towards beech (Vitasse et al. 2011).

In general, the timing of bud burst has also a great impact on the late frost sensitivity of trees which might become more important in a changing climate. Paradoxically, warming may increase the risk of plant frost damage, because warm temperatures might lead to a premature plant development which would result in the exposure of vulnerable plant tissues to late frosts (Gu et al. 2008). However, different studies came to contrasting results concerning an increasing, decreasing or unchanged late frost risk (Hänninen 1991, Kramer 1994, Linkosalo et al. 2000, Menzel et al. 2003, Scheifinger et al. 2003). The danger of late frost events may depend on the affected species as well as on the mode of warming. If climate change will lead to more variable temperatures in spring, the late frost risk may increase (Rigby and Porporato 2008).

European beech is sensitive to late frost events after leaf flushing (Kreyling et al. 2012). Thereby, late frost damage can strongly affect beech vitality and competitiveness whereas the critical value for frost damage of flushing beech leaves is -3 °C (Dittmar et al. 2006). Kreyling et al. (2012) emphasize that the frost sensitivity of beech strongly depends on timing, whereby the highest sensitivity can be found directly after leaf flushing and as soon as leaves mature the frost tolerance increases again. For tree populations, the timing of bud burst varies between provenances, whereas populations from colder climates tend to flush earlier than populations from warmer climates when grown in common gardens (Alberto et al. 2011). Also, the bud burst timing for beech populations shows a strong geographical trend, whereas provenances from the east and southeastern part of the range of distribution flush early and provenances from the western part of the range flush late and also differ in its late frost sensitivity (Wühlisch et al. 1995a, Višnjić and Dohrenbusch 2004). In addition, populations from high elevations flush earlier than those from low elevations when grown in common gardens (Vitasse et al. 2009a). Several studies revealed a high heritability for the timing of bud phenology (Wühlisch et al. 1995b, Alberto et al. 2011, Gömöry and Paule 2011, Olson et al. 2013). The timing of bud burst is probably the result of selection due to the avoidance of spring frost damage, which can in extreme cases kill the plant (survival adaptation), and the effective utilization of the growing season through early initiation of growth (capacity adaptation) (Leinonen and Hänninen 2002). Nevertheless, a recent study of Soularue and Kremer (2012) revealed that not only divergent selection but also the effects of assortative mating and gene flow can result in the observed clinal variation in the timing of bud burst, and also, epigenetic effects might play a role (Gömöry and Paule 2011, Yakovlev et al. 2012).

However, it is questionable if locally adapted populations to late frost will also perform sufficiently under changed environmental conditions in a future climate. Phenotypic plasticity, which was found to be high for bud burst in European beech (Vitasse et al. 2010), permits a fast but limited response to environmental changes (Alberto et al. 2011). Especially at higher elevations, where earlier leaf unfolding is anticipated, the risk of late frost damage might be increased and thus endanger the survival of *F. sylvatica* (Čufar et al. 2012).

1.6 Climate Change and Drought Stress

Forests may become more vulnerable to mortality due to drought in a future climate even in environments which are normally not considered to be water-limited (Allen et al. 2010). Thereby, not only the mortality rate may increase but also the forest productivity could decrease because it is highly influenced by water availability (Bréda et al. 2006). Even at present, an increase in tree mortality has been observed (Bréda et al. 2006, Bigler et al. 2007, Mantgem et al. 2009). However, tree species are known to differ in their drought sensitivity, whereas European beech is regarded as drought sensitive compared to other deciduous tree species like oak (Aranda et al. 2000, Leuschner et al. 2001). Also, within species the drought sensitivity can vary and it was shown that different beech provenances are differently adapted to water limitations (Schraml and Rennenberg 2000, Peuke et al. 2002). In general, provenances from dryer environments, e.g., the Mediterranean region, are better adapted to drought than provenances from the center of the beech distribution (Czajkowski and Bolte 2006, Fotelli et al. 2009, Rose et al. 2009, Robson et al. 2012). These differences might be explained by modifications of the morphology and physiology of the provenances and underlying genetic constitutions. García-Plazaola and Becerril (2000) showed that differences in biochemical and physiological responses between beech provenances modulate the adaptation to drought stress. Thereby, the observed morphological adaptations (e.g., reduction of leaf area, increase of the leaf area fine roots ratio) had a greater impact than the biochemical differences between the provenances. Furthermore, beech exhibits a high plasticity of the fine root growth and turnover, which might be one reason for the success of this species in dry and wet environments (Meier and Leuschner 2008a, Meier and Leuschner 2008b). Further, beech can benefit from ectomycorrhiza colonization under drought stress (Pena et al. 2013). Schall et al. (2012) found a significant increase of the percentage of belowground compartments as a reaction to drought in beech seedlings and even whole tree water reser-

voirs play a major role in maintaining leaf transpiration under severe drought stress (Betsch et al. 2011). For stress resistance, the developmental stage of a tree plays also a role, whereas the stress resistance increases with ontogeny (Niinemets 2010). But also adult trees within a stand can vary between their drought resistances as a consequence of their social class. Dominant and co-dominant trees were found to be more drought sensitive than intermediate trees, likely because of different transpiration rates (van der Maaten 2012).

However, even drought adapted beech provenances may suffer from increasing water limitations in future. A modeling study of Czúcz et al. (2011) for Hungary revealed that 56 – 99 % of the present-day zonal beech populations might be outside their present bioclimatic niche by the year 2050, albeit the authors comment that these result represent a rather pessimistic scenario. But also at present, different studies revealed changes in marginal beech populations. Thus, Charru et al. (2010) found a decline of vitality of beech populations in Northeast France. On a mountain in the Northeast of Spain, Peñuelas and Boada (2003) observed that beech reached higher altitudes and was partly replaced by *Quercus ilex* in the timespan from 1945 to 2003. Concerning the authors, these effects are mainly caused by higher temperatures but enabled by land-use changes. Finally, Jump et al. (2006) showed a growth reduction of beech in the same region. A growth reduction of beech caused by drought was also shown outside of marginal provenances. Thus, Scharnweber et al. (2011) found a declining growth of beech along a precipitation gradient in Northeast Germany. A growth decline of beech seedlings was also observed by Czajkowski et al. (2005) as an effect of the severe drought in summer 2003. This dry period had also a negative effect on the growth in the following year. The same effect was observed by Granier et al. (2007).

Changing precipitation regimes may alter also the competitive balance between beech and other species. Thus, beech seedlings were able to cope with competition from *Rubus fruticosus* under sufficient water availability. But even under moderate shortage of water, the growth of the seedlings declined (Fotelli et al. 2001). Nevertheless, a mixture of beech with other tree species can also have a positive effect for water availability. For instance, grown in mixture with oak, beech can benefit from a hydraulic lift of water by oak (Pretzsch et al. 2013). However, oak is more drought tolerant than beech, and although both species show a declining growth under drought stress, the effect is higher for beech. Even small changes in precipitation can have a considerable impact on the growth of beech, and thus, more

drought tolerant species like oak might gain competitive advantages under the projected climate changes (Scharnweber et al. 2011).

1.7 Hypothesis and Objectives of the Study

In this study, the genetic basis of adaptation of European beech to climate change relevant traits was investigated in a translocation experiment with offspring of beech populations growing under different environmental conditions in Northern Germany.

The following objectives were set:

- to find phenotypic differences among seedlings of the translocation experiment originating from populations from different environments which are relevant regarding climate change,
- to identify variation (mainly SNPs) in candidate genes for bud burst in beech,
- to detect potentially adaptive genetic markers by conducting association analyses between SNPs and climate change relevant traits observed in the translocation experiment,
- to find further signs of selection by conducting outlier analyses with selected SNPs.

Hypotheses to be tested

Phenotypic Data:

- Different beech populations in the translocation experiment show phenotypic differences in bud burst, drought stress sensitivity, height/growth and general mortality. In detail,
  - populations from higher altitudes flush earlier than populations from lower altitudes,
  - populations from drier environmental conditions are more drought stress tolerant resulting in low mortality and high growth compared to populations from moist environments, especially in the dry experimental plot

Genetic Data:

- The neutral genetic diversity is high for all populations, with low but observable genetic differentiation among the analyzed beech populations.
- Some SNPs identified in the analyzed candidate genes are associated with bud burst, and/or behave as outliers, which can be interpreted as a sign of selection.

2 Material and Methods

2.1 Study Sites

For this study, beech stands along a precipitation gradient in Northern Germany were selected. They are investigated by different working groups within the KLIFF-project (http://www.kliff-niedersachsen.de). At each study site two stands were selected, which differed in their soil properties: one stand on a sandy soil, hereafter termed “sand”, and a stand on a loam-richer soil, hereafter termed “loam”. The different stands were the following: Calvörde sand (CS), Calvörde loam (GL), Göhrde sand (GS), Göhrde loam (GL), Unterlüß sand (US), Unterlüß loam (UL). Additionally, a reference area in the Harz Mountains (Harz, (Ha)) near the village Bad Grund was investigated. The populations were located at elevations of 72 m (CL) to 458 m (Ha). The annual mean temperatures ranged from 7.2 °C (Ha) to 9.2 °C (CS) and the annual mean precipitation ranged from 543 mm (CL) to 1,170 mm (Ha). All populations are between 97 (CS) and 142 years (GL) old and either pure beech stands or beech stands with admixture of a few oak trees. The origin of the stands is unknown. Most likely, they originated from natural regeneration, but planting can not be ruled out. A summary of stand characteristics can be found in Table 1.

2.2 Translocation Experiment

In the first phase of the KLIFF-project, the mature beech stands mentioned above have been genotyped (see Seifert 2012). For that, at least 100 trees per stand were sampled (in total 707 trees). To establish a translocation experiment with offspring of these stands, 100 beechnuts under every sampled tree were collected in fall 2009. The beechnuts were treated as following (Seifert 2012): without cleaning they were dried at room temperature until a moisture content of 10 % (+/- 2 %) was reached. Afterwards, they were stored in plastic bags at -10 °C until the end of January 2010. Starting in February, the beechnuts were stored in darkness at 5 °C for stratification, and they were watered from time to time to prevent them from dehydration for a period of seven weeks. To delay their growth, firstly germinated beechnuts were transferred into plastic bags and stored at 0 °C. After stratification, all seeds (germinated or not) were planted into plastic containers (100 cm^3, HerkuPlast-Kubern GmbH, type QP D 84 T/11,5) using regular potting soil (Fruhstorfer Erde, type P25). The seedlings were grown in a greenhouse and were regularly watered and fertilized

(Wuxal® Super). In July 2010, one leaf per plant was sampled and stored at -20 °C for further analysis.

The seedlings were planted out in a translocation experiment in fall 2010. The experiment consisted of two different experimental plots, one located in the reference area in the Harz Mountains and the other one in the stand Calvörde sand. From each population 400 individuals were planted out (in total, 3,600 individuals). In the plot in the Harz Mountains the populations Harz, Göhrde sand and Calvörde loam were planted out (in total, 1,200 individuals), whereas all populations except Unterlüß loam were planted out in the plot in Calvörde (in total 2,400 individuals). The lower number of populations on the plot in the Harz Mountains as well as the exclusion of the population Unterlüß loam in the plot in Calvörde, resulted from an insufficient number of established seedlings in the greenhouse for these populations. The experiment included four randomized blocks, in which the different populations were planted. In the plot in Calvörde each block contained 600 plants, whereas each block in the plot in the Harz Mountains contained 300 plants. Within blocks, each two populations were planted in "cages" with dimensions of 2 m x 3 m to protect the seedlings against damage by deer. In each block, each population was planted in 20 rows (14 cm distance between them) each containing five plants (11 cm distance between them) (100 plants per population). The space between the rows of the different populations was 0.6 m.

Table 1: Characteristics of the investigated beech populations (Hertel et al. 2013).

Characteristic	Population						
	Calvörde sand	Calvörde loam	Göhrde sand	Göhrde loam	Unterlüß sand	Unterlüß loam	Harz Mountains (Bad Grund)[+]
Position	52°23' N 11°17' E	52°24' N 11°16' E	53°09' N 10°52' E	53°07' N 10°49' E	52°50' N 10°19' E	52°50' N 10°19' E	51°49' N 10°15' E
Elevation (m a.s.l.)	75	72	85	85	117	120	458
Mean annual temperature (°C)	9.2	9.1	8.7	8.7	8.5	8.5	7.2
Mean annual precipitation (mm)	544	543	665	675	766	766	1170
Stand age (years)	97	131	133	142	115	115	136
Mean tree height (m)	23.8	28.3	24.6	30.2	25.3	28.4	n.a.
Stem density (no. ha^{-1})	711	300	289	122	611	411	n.a.
Mean DBH (cm)	23.4	36.6	30.7	51.0	18.6	26.1	n.a.
Water storage capacity mineral soil (mm 120 cm^{-1})	81	140	80	78	79	95	n.a.

n.a.: not available; data only available for populations jointly investigated within the KLIFF-project, [+] Seifert (2012)

2.3 Phenotypic Observations

The height of living translocated plants was measured in fall 2010 (planting), 2011 and 2012. The height was measured to 0.5 cm accuracy from ground to the beginning of the terminal bud for each plant. Plants with damaged terminal shoots were excluded from analysis. Plant increment was calculated by subtracting the plant height at planting (2010) from the plant height in fall 2012 for each seedling in the translocation experiment. For the observation of bud burst behavior, leaf unfolding was divided into five different stages (Figure 1). Bud burst was recorded for each seedling in the translocation experiment. In the experimental plot in Calvörde, bud burst was recorded on five days in 2011 (days of the year: 102; 109; 112; 116; 119), on two days in 2012 (days of the year: 116 and 119) and on three days in 2013 (days of the year: 113, 115, 120). In the experimental plot in the Harz Mountains bud burst was recorded on three days in 2011 (days of the year: 110; 115; 119), on three days in 2012 (days of the year: 119; 121; 124) and on four days in 2013 (days of the year: 115; 120; 123; 127). Mortality data (see below) was used to validate the data, since a differentiation between living and dead buds in the first stage (bud in dormant stage) is difficult. Natural drought stress occurred in the plot in Calvörde in June 2011, which led to damages on the translocated plants. For recording, the damage of the plants was classified into “minor” (at least one leaf slightly damaged), “strong” (at least one leaf strongly damaged) and “dead”. The viability of the translocated plants was recorded in early summer 2011, 2012 and 2013.

Figure 1: Overview of the five stages used for the classification of bud burst, 1: all buds in dormant stage, 2: at least one swollen bud, 3: start of leaf development of at least one bud, 4: leaves distinguishable of at least one bud, 5: all leaves fully developed.

2.4 Selection of Plants for Genotyping

Populations planted in the plot in Calvörde were analyzed with microsatellite markers for the investigation of neutral genetic variation. For that, 100 individuals (25 within each block) per population were randomly selected for genotyping (in total, 600 plants). Microsatellite data for the adult stands (origin of the seedlings) was provided und published before by Seifert (2012).

The adaptive genetic variation was investigated using SNP markers. Since “bud burst” was the main trait investigated in this study, individuals were selected for genotyping in respect to their flushing behavior. Thus, in a first step, the date with the highest variation in bud burst timing in the year 2011 was selected for each population planted in the plot in Calvörde. Because it was necessary to classify the seedlings into early and late flushing individuals and to simultaneously reduce sample size for genotyping, all seedlings showing the intermediate bud burst stage “three” on the particular date were excluded. All residual individuals (those with bud burst stages “one”, “two”, “four” and “five”) were chosen for genotyping (in total 1,407 individuals) (Table 2).

Table 2: Number of individuals selected for SNP-genotyping for the different populations.

Population	No. of early flushing individuals	No. of late flushing individuals	Total no. of individuals
Calvörde sand	158	72	230
Calvörde loam	220	47	267
Göhrde sand	66	185	251
Göhrde loam	53	176	229
Unterlüß sand	110	101	211
Harz	64	155	219
Total	671	736	1,407

2.5 DNA Isolation

Total DNA was extracted from leaves using the DNeasy™ 96 Plant Kit (Qiagen, Hilden, Germany). The amount and the quality of the DNA were analyzed by 1% agarose gel electrophoresis with 1 X TAE as running buffer (Sambrook et al. 1989). DNA was stained with ethidium bromide or Roti®-Safe GelStain (Roth, Karlsruhe, Germany), visualized by UV illumination and compared to a Lambda DNA size marker (Roche, Mannheim, Germany).

2.6 Microsatellite Analysis

In this study, nine highly polymorphic microsatellite markers were used. Only two markers (FS3-04, Pastorelli et al. 2003 and mfs11, Vornam et al. 2004) were originally developed for *F. sylvatica*. The markers sfc0018, sfc0161, sfc1063 and sfc1143 (Asuka et al. 2004) were originally developed for *Fagus crenata*. In addition, three EST microsatellite markers were applied which were originally developed and transferred from *Quercus robur* (GOT066, FIR065, FIR004; Durand et al. 2010).

For analysis, the primers labeled with two different fluorescent dyes (6-Carboxyfluorescein (FAM): sfc0161, sfc1063, FIR004, mfs11; 6-Hexachlorofluorescein (HEX): sfc0018, sfc1143, GOT066, FIR065, FS3-04) were multiplexed. For that, the primers were pooled in different sets (set1: all sfc loci, set 2: FS 3-04 and mfs 11, set 3: GOT066, FIR065, FIR004). PCRs were conducted in a 15 µl volume containing 2 µl of genomic DNA (about 10 ng), 10 x reaction buffer (0.8 M Tris-HCl pH 9.0, 0.2 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20; Solis BioDyne, Tartu,

Estonia), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 unit of *Taq* DNA polymerase (HOT FIREPol® DNA Polymerase, Solis BioDyne, Tartu, Estonia), 0.3 µM of each forward and reverse primer. The thermal cycling conditions were the following: an initial denaturation step of 95 °C for 15 min followed by 30 cycles of 94 °C for 1 min (denaturation), 47 °C (for the EST primer set 3) or 55 °C (for primer set 1 and 2) for 30 sec (annealing), 72 °C for 1 min (denaturation) and a final extension step of 72 °C for 20 min. Microsatellite fragments were separated on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). Data were collected and aligned using the internal size standard GS 500 ROX™ (Applied Biosystems, Foster City, USA) and GeneScan 3.7® (Applied Biosystems, Foster City, USA). Scoring of fragments was conducted with the software Genotyper 3.7® (Applied Biosystems, Foster City, USA).

2.7 Selection of Candidate Genes

A literature search was conducted to select candidate genes which might be involved in bud burst behavior. The ten selected genes are expected to have an impact on bud burst in oak (Derory et al. 2006, Ueno et al. 2010), a genus of the Fagaceae family related to beech. Both, the Evoltree EST database (http://www.evoltree.eu) and the EMBL Nucleotide Sequence Database (http://www.ebi.ac.uk/embl/) were used to find corresponding *F. sylvatica* sequences. These were verified by a BLASTn and BLASTx search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and used for primer design in order to amplify the corresponding genomic regions. Putative functions of the genes were inferred by BLAST searches in the databases of UniProtKB/Swiss-Prot (http://www.uniprot.org/) and *Arabidopsis* Information Resource (TAIR) (http://arabidopsis.org) (Table 3).

Table 3: Selected candidate genes with putative functions.

Gene	Reference (Accession no./Gene)	Description
Auxin response factor	AT1G30330; *auxin response factor 6*	Transcriptional factor binding to the sequence 5'-TGTCTC-3' found in the *auxin-responsive promoter elements* (*AuxREs*); involved in flower development
Alpha amylase/subtilisin inhibitor	OS04G0526600; *alpha amylase/subtilisin inhibitor*	Belongs to the *protease inhibitor I3* family*; alpha-amylase* and *protease inhibitor*
Constans like (1) *Constans like (2)*	AT2G24790; *constans-like 3* AT5G24930; *constans-like 4*	Members of the *CONSTANS-like gene* family; *CONSTANS* gene in *Arabidopsis* with important role in regulation of flowering by photoperiod (Griffiths et al. 2003)
Chloroplast chaperonin like	AT5G20720; *chloroplast chaperonin 10*	Encodes a *chloroplast co-chaperonin*; response to cold, response to salt stress, chloroplast organization
Cysteine proteinase	AT4G39090; *responsive to dehydration 19* (*similar to cysteine proteinases*)	Response to desiccation and response to temperature stimulus
Dof zinc finger protein	AT1G28310; Dof-type zinc finger DNA-binding family protein	*Dof-type zinc finger DNA-binding family protein*; regulation of transcription
Frigida	P0DH90; *FRIGIDA*	In *Arabidopsis*: required for the regulation of flowering time in the late-flowering phenotype
Histone 3 (1) *Histone 3 (2)*	AT4G40040; *Histone 3.3*	*Histone super family protein*; involved in: glucose catabolic process, nucleosome assembly, protein targeting
NAC transcription factor	AT1G52890; *NAC transcription factor*	encodes a *NAC transcription factor* whose expression is induced by drought, high salt, and abscisic acid
Protein phosphatase 2C	AT5G59220; *highly ABA-induced PP2C gene 1*	Encodes a member of the *PP2C* family; functions as a negative regulator of osmotic stress and ABA signaling

2.8 Amplification, Cloning and Sequencing of the Candidate Genes

To reduce ascertainment bias (see discussion chapter 4.2.1), comparative sequencing was carried out in close cooperation with the project BEECHADAPT which is part of the research network biodiversity-exploratories (http://www.biodiversitäts-exploratorien.de). This facilitated the inclusion of beech populations from a wide range over Germany for comparative sequencing. In addition to the populations of the KLIFF project, populations from the following sampling areas were included: Schorfheide-Chorin in North-Eastern Germany, the Hain-

ich-Dün region in Central Germany and the Schwäbische Alb in Southern Germany (Table 4). In total, 24 trees from twelve different populations were used for comparative sequencing (two individuals per population).

The software Primer3, version 0.4.0 (Rozen and Skaletsky 2000) was used to design primers for amplification and direct sequencing of PCR products. Primers were checked for self-annealing, dimer and hairpin formations with the program OligoCalc, version 3.26 (Kibbe 2007).

PCRs were conducted in a 15 µl volume containing 2 µl of genomic DNA (about 10 ng), 10 x reaction buffer (0.8 M Tris-HCl pH 9.0, 0.2 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20; Solis BioDyne, Tartu, Estonia), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 unit of *Taq* DNA polymerase (HOT FIREPol® DNA Polymerase, Solis BioDyne, Tartu, Estonia), 0.3 µM of each forward and reverse primer (Table 5). For amplification of the gene fragment *PP2C* the HotStarTaq® MasterMix (Qiagen, Hilden, Germany) was used. The thermal cycling conditions were the following: an initial denaturation step of 95 °C for 15 min followed by 35 cycles of 94 °C for 1 min (denaturation), between 50 °C and 68 °C for 1 min (annealing, see Table 5 for the different annealing temperatures), 72 °C for 1 min (denaturation) and a final extension step of 72 °C for 20 min. PCR products were analyzed using 1% agarose gel electrophoresis with 1x TAE as running buffer (Sambrook et al. 1989). DNA was stained with Roti®-Safe GelStain (Roth, Karlsruhe, Germany) and visualized by UV illumination. PCR products were excised from gel and purified using the innuPREP Gel Extraction Kit (Analytik Jena, Jena, Germany). The purified products were cloned into a pCR2.1 vector using the TOPO TA Cloning® Kit (Invitrogen, Carlsbad, USA) with slight modifications. Plasmid DNA was extracted using the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich, Steinheim, Germany). The sequencing reaction was carried out for three different clones of the fragments by using the Big Dye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) with both M13 forward and M13 reverse primers. Sequencing reactions were run on an ABI PRISM® 3100*xl* Genetic Analyzer (Applied Biosystems, Foster City, USA), and the sequenced fragments were verified by a BLASTn and BLASTx search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 4: Characteristics of regions (Fischer et al. 2010) additionally included for comparative sequencing.

Region	Altitude [m a.s.l.]	Annual mean temperature [°C]	Annual mean precipitation [mm]
Schorfheide-Chorin	3–140	8–8.5	500–600
Hainich-Dün	285–550	6.5–8	500–800
Schwäbische Alb	460–860	6–7	700–1000

Table 5: Primer sequences and corresponding annealing temperatures for selected candidate genes (candidate gene fragments longer than 1,000 basepairs were divided into two parts for sequencing). For the amplication of the product, the primers F part 1 and R part 2 were used, F: forward, R: reverse.

Abbreviation	Gene	Primer sequence (5'-3')	Annealing temperature
Asi	*Alpha amylase/ subtilisin inhibitor*	F: GTTGATGAGATCGATTGGAACCCTGAG R: GCCAACGAGGGCAATTACAGAACTA	68 °C
Arf	*Auxin response factor*	F part 1: AGTGATAGCAACTCCACAACCGTACC R part 1: GAGTCTTAGGCTCTGAGATGCAAATG F part 2: GTTGACCGGGAGAATGATGTGCTTC R part 2: GTACTCAAGTGACCCCACAGACGTTA	68 °C
ConsC1	*Constans like (1)*	F part 1: ACTCTCTTCTGCCGTGCCGACTCAG R part 1: GTCGAGAGACGAAGAAGAAACCTG F part 2: ACTCATCAGTGTCTCAGCCAGAGT R part 2: GGCACGAGAGCTTCGCAGTAGTTAAT	68 °C
ConsC2	*Constans like (2)*	F: ACTCTCACTACTCCCACACGTCTAC R: GCTGTCAGTACCCGAACTGTGAAAC	62 °C
Cp10	*Chloroplast chaperonin like*	F part 1: GAGTAGGGAGTGGTCTGTCTCAGAGG R part 1: TCAAGGGCTTGAGATCCTGT F part 2: CTGGCACCCAAGTTGTGTATT R part 2: ATCCACATGCCTTGAGGCACTTTCACC	66 °C
CysPro	*Cystein Proteinase*	F: GACCATGAGTGTGATCCCGAGGAATA R: CTGCATGGCATCAAGCTTCACTTACC	60 °C
DAG	*DOF zinc finger protein*	F part 1: CCTTCTCCTTCTCCAACACACT R part 1: TTCAAGTTCTAGACATTCTTTGTCG F part 2: CCAGTCACTCCTCGGCTTAG R part 2: GTACCGTGCGTGCCAAGTAT	50 °C
Frigida	*Frigida*	F: GCGCGAGACTTAAAATCGAC R: AAAAACCGTCCAATGCAATC	50 °C
His3C1	*Histone 3*	F: GAAGCGAAAAGAGATGGCCCGTACGAA R: GACAGCACAACACCAGTTTGAGATCC	60 °C
His3C2	*Histone 3*	F: CTCTCAGAAAGTCCAGAACCCCAAAAGC R: CGCTTAAGCACGTTCGCCACGGATCCTC	67 °C
NAC	*NAC transcription factor*	F part 1: TTGTAGCCGGAAATGGGTGT R part 1: GACACGTGGCAAAGTGAAGA F part 2: TTGGGTTTTGTGTCGGATTT R part 2: CCCTTTTGGTGCTAAACTCCAG	62 °C
PP2C	*Protein phosphatase 2C*	F part 1: GGGATTTGCTGTGGAGTTGT R part 1:TCTGCAATTGGTGGTTTTGA F part 2: GAAAGAAGAGGTGGAAAGCGTA R part 2: CGTTGTCCGTACTGTGCCTA	50 °C

2.9 SNP Analysis

SNPs occurring in only one individual were excluded after comparative sequencing to avoid the analysis of false SNPs because of sequencing errors. To reduce the number of SNPs for genotyping, they were preselected by using the software HaploBlockFinder version 0.7 (Zhang and Jin 2003). Afterwards, the most promising SNPs (e.g., non-synonymous SNPs) were selected. In total, 56 SNPs (18 non-coding SNPs, 17 synonymous SNPs, 16 non-synonymous SNPs and five SNPs from untranslated regions (UTR)) were chosen for genotyping of the 1,429 beech trees. Surrounding sequences of the selected SNPs were sent to KBiosciences UK Ltd for primer design and analysis of the SNPs using the PCR-based KASP™ genotyping assay (Hoddesdon, UK).

Ten SNPs from two different candidate genes were selected to confirm the results of KBioscience by using the ABI Prism® SnaPshot™ Mulitplex Kit (Applied Biosystems, Foster City, USA) (Appendix 1). Primers were designed by addition of a poly (T) tail of different lengths at their 5' end (Appendix 2). This facilitated an analysis of all SNPs in a single multiplex. Primers were checked for self-annealing, dimer and hairpin formations with the program OligoCalc, version 3.26 (Kibbe 2007). After amplification of the candidate genes (see above), the PCR products were cleaned using 1 unit Exonuclease I (Affymetrix, Santa Clara, USA) and 2.5 units SAP (Shrimp Alkaline Phosphatase; Affymetrix, Santa Clara, USA), and incubated 60 min at 37 °C followed by 75 °C for 15 min. PCR amplifications were carried out in a 10 µl volume containing 5 µl of cleaned PCR product from the different genes, 5 µl Reaction Mix (SnaPshot® Multiplex Kit (Applied Biosystems, Foster City, USA)) and 0.2 µM of each primer. The thermal cycling conditions were the following: 35 cycles of 96 °C for 10 sec, 50 °C for 5 sec and 60 °C for 30 sec. After thermal cycling, the products were post-extension treated using 1 unit SAP (Affymetrix, Santa Clara, USA) and incubated 60 min at 37 °C followed by 75 °C for 15 min. Preparations for the SNP analysis were carried out following the protocol. SNP analysis reactions were run on an ABI PRISM® 3100*xl* Genetic Analyzer (Applied Biosystems, Foster City, USA) and scoring (no automatic scoring) was conducted according to the protocol.

2.10 Data Analysis

2.10.1 Phenotypic Data

Populations were tested for significant differences of the phenotypic characteristics height, growth and bud burst using the non-parametric Kruskal-Wallis test with multiple comparisons as heterogeneity of variance and non-normal distribution of the data was revealed by the Levene test and Kolmogorov-Smirnov test, respectively. Significant differences between the populations concerning drought stress induced damage and mortality were tested using Pearson's chi-square test. Only populations existent in both plots were analyzed for significant differences between the mean height of all populations in the plot in Calvörde and in the plot in the Harz Mountains. To test the statistical differences between bud burst stages of different populations, data of the observation date with highest variation between the populations was used in every analyzed year. Spearman's rank-order correlations coefficient was used to determine correlations between "bud burst behavior within populations between years", "drought induced plant damage" and "precipitation at the original population sites" as well as "drought induced plant damage" and "plant height". All statistical analyses were conducted using the software STATISTICA version 10 (StatSoft Inc., Tulsa, USA).

2.10.2 Microsatellites

Linkage disequilibrium, frequency of null alleles and Hardy-Weinberg proportions were estimated using the software Genepop version 4.2.1 (Rousset 2008). Markov chain parameters for the test of linkage disequilibrium and deviations from Hardy-Weinberg proportions were the following: 10,000 demorization steps, 100 batches and 5,000 iterations per batch. A LD-plot of pair-wise R^2 values was calculated using 1,000 permutations with the software TASSEL version 2.1 (Bradbury et al. 2007). The molecular diversity indices "number of alleles" (N_a), "observed heterozygosity" (H_o), "expected heterozygosity" (H_e) and "fixation index" (F) for adult and juvenile trees were estimated using the software GenAlEx version 6.4.1 (Peakall and Smouse 2006, 2012). Differences between adult and juvenile populations for these parameters were tested using a Kruskal-Wallis test with multiple comparisons implemented in the software STATISTICA version 10 (StatSoft Inc., Tulsa, USA). Additionally, Nei's genetic diversity (Nei 1972) as well as the analysis of molecular variance (AMOVA) among juvenile populations was calculated with the software GenAlEx version 6.4.1 (Peakall and

Smouse 2006, 2012) using 999 permutations. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram, based on Nei´s distance (Nei 1972), was calculated with the software "populations" version 1.2.32 (Langella 1999). Bootstrap values based on 1,000 permutations were also calculated with this program. The dendrogram was visualized with the software TreeView version 1.6.6 (Page 1996) using the phylogram tree style. The Bayesian model-based clustering method implemented in the software STRUCTURE version 2.3.3 (Pritchard et al. 2000) was applied to infer the population structure. The no admixture model and correlated allele frequencies were selected. A burn-in period of 10,000 and Markov chain Monte Carlo (MCMC) replicates of 100,000 were used. Possible clusters (*K*) from one to ten were tested using ten iterations. To determine the optimal value of *K* the delta *K* method by Evanno et al. (2005) was applied calculated with the program STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt 2012).

2.10.3 Candidate Genes

Sequences were edited and aligned using the software BioEdit version 7.1.3.0 (Hall 1999). For alignment the ClustalW Multiple alignment (Thompson et al. 1994) was chosen. Nucleotide diversity, haplotype diversity and Tajimas' *D* were calculated excluding indels with the software DnaSP version 5.10.01 (Librado and Rozas 2009).

2.10.4 SNPs

Linkage disequilibrium and deviations from Hardy-Weinberg Proportions were estimated using the software Genepop version 4.2.1 (Rousset 2008). Thereby, 10,000 demorization steps, 100 batches and 5,000 iterations per batch were used as Markov chain parameters. LD estimates revealed by SNPs were correlated with the LD estimates revealed by SSRs (see above) using Spearman's rank-order correlations coefficient implemented in the software STATISTICA, version 10 (StatSoft Inc., Tulsa, USA). Additionally, a LD-plot of pair-wise R^2 values was calculated with the software TASSEL version 2.1 (Bradbury et al. 2007) using 1,000 permutations. This software was also applied for association analyses between SNP markers and the phenotypic traits height (in 2012) and bud burst. Beside the association analyses based on single populations, corresponding analyses based on pooled individuals from different populations were conducted to increase sample size. For that, all individuals were selected which showed the bud burst stages one, two, four or five on the observation day

with the highest variation in bud burst behavior in the respective year. The choice of a single day ensured comparability between bud burst stages of different populations. In total, 1,202 individuals (423 individuals defined as “late flushing” and 779 defined as “early flushing”) were pooled to an association population for the year 2011. For the year 2012, the association population comprised 925 individuals (288 late flushing, 637 early flushing) and the association population in the year 2013 comprised 869 individuals (536 late flushing, 333 early flushing). For the association analyses, the general linear model (GLM) implemented in TASSEL version 2.1 (Bradbury et al. 2007) was applied using 1,000 permutations for the F-test. The analyses were conducted both with and without the inclusion of population structure (*Q*-matrix) derived from non-coding SNPs (see below). A mixed linear model (MLM) implemented in TASSEL version 2.1 (Bradbury et al. 2007) was additionally applied for the association populations based on pooled individuals using the “EMMA” analysis method. In this model both is included population structure and a kinship matrix, which was also calculated with TASSEL version 2.1 (Bradbury et al. 2007). The Bayesian model-based clustering method implemented in the software STRUCTURE version 2.3.3 (Pritchard et al. 2000) was used to infer the population structure. The no admixture model and correlated allele frequencies were selected. A burn-in period of 50,000 and Markov chain Monte Carlo (MCMC) replicates of 100,000 were used and possible clusters (*K*) from one to ten were tested using ten iterations. To determine the optimal value of *K*, the delta *K* method by Evanno et al. (2005) was applied calculated with the program STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt 2012). The STRUCTURE analysis was conducted with the following subsets of SNPs: the total SNP set, only “silent” SNPs (non-coding SNPs and synonymous SNPs), only non-coding SNPs and only non-synonymous SNPs. Correspondingly, the molecular diversity indices “observed heterozygosity” (H_o), “expected heterozygosity” (H_e) and “fixation index” (F) were calculated based on the determined SNP sets for both the single populations and for the association populations consisting of pooled individuals using the software GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). For association populations consisting of pooled individuals, the molecular diversity indices were additionally calculated only using potentially adaptive SNPs revealed by the association analysis (see above) and/or outlier analysis (see below). F_{ST} outlier analyses were conducted using the Fdist approach (Beaumont and Nichols 1996) implemented in the software LOSITAN version 1.0 (Antao et al. 2008) to detect SNPs under selection. Thereby, all single populations were tested against each other. Further, out-

lier analyses were conducted for the association populations consisting of pooled individuals for the different years. For that, the individuals of each association population were divided into the groups “early flushing” and “late flushing” (see above) and tested against each other. All outlier analyses were conducted using 200,000 simulations and a false discovery rate (FDR) set to 0.1 implemented in the software LOSITAN version 1.0 (Antao et al. 2008).

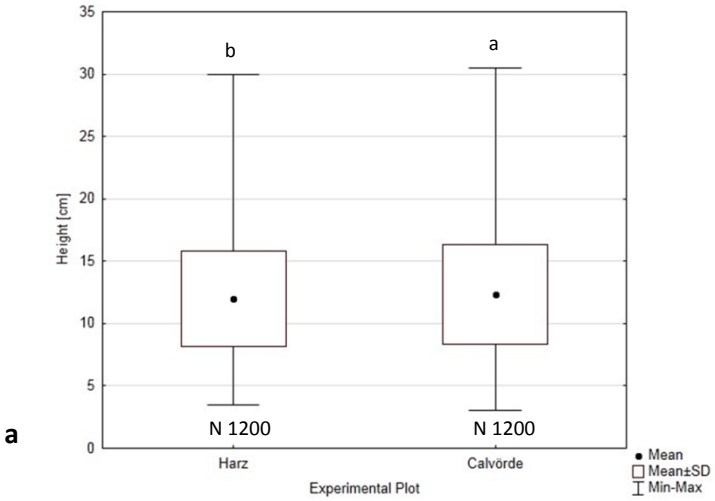
3 Results

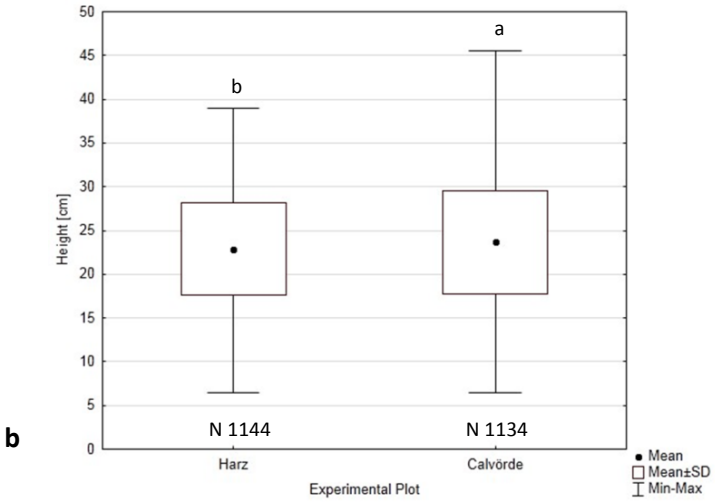
3.1 Phenotypic Data

3.1.1 Height

The seedlings were slightly but significantly higher in the plot in Calvörde than in the plot in the Harz Mountains at planting in fall 2010 (Kruskal-Wallis test $p < 0.05$). The same trend was observed in fall 2011 (Kruskal-Wallis test $p < 0.01$). In contrast, the seedlings were significantly higher in the plot in the Harz Mountains than in the plot in Calvörde in fall 2012 (Kruskal-Wallis test $p < 0.001$) (Figure 2).

Within the two plots, significantly different heights between the seedlings of the different populations were observed (Kruskal-Wallis test $p < 0.01$). At planting, several populations showed significantly different mean heights in Calvörde (Kruskal-Wallis test $p < 0.001$). The values ranged from 11.4 cm for the population Harz to 14 cm for the population Göhrde sand. In fall 2011, the mean height ranged from 21.9 cm for the population Calvörde loam to 25.3 cm for the population Göhrde sand. These two populations showed also the lowest (Calvörde loam 24.7 cm) and highest (Göhrde sand 29.2 cm) plants in fall 2012 (Figure 3). In the plot in the Harz Mountains, all populations showed a significantly different height (Kruskal-Wallis test $p < 0.001$) at planting (Figure 4). The population with the uppermost height was Göhrde sand (13.3 cm) while the lowest plants were observed for the population Harz (10.7 cm). In fall 2011, the same trend was observed. In fall 2012, the tallest seedlings were observed for the population Harz (32.4 cm) and the smallest plants were observed for the population Calvörde loam (30.7 cm).





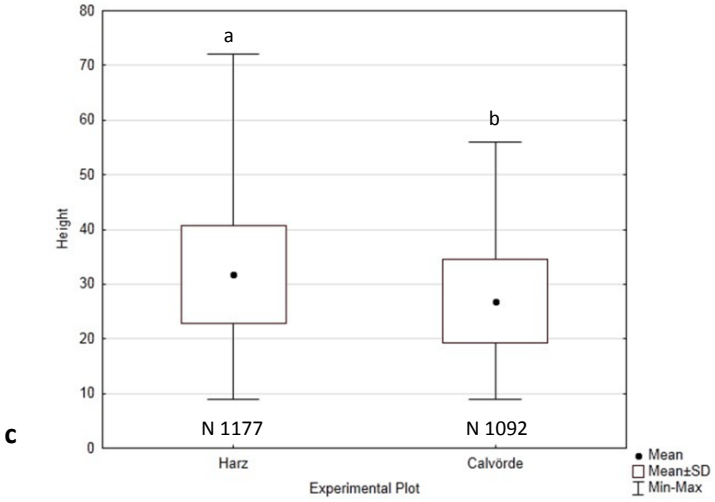
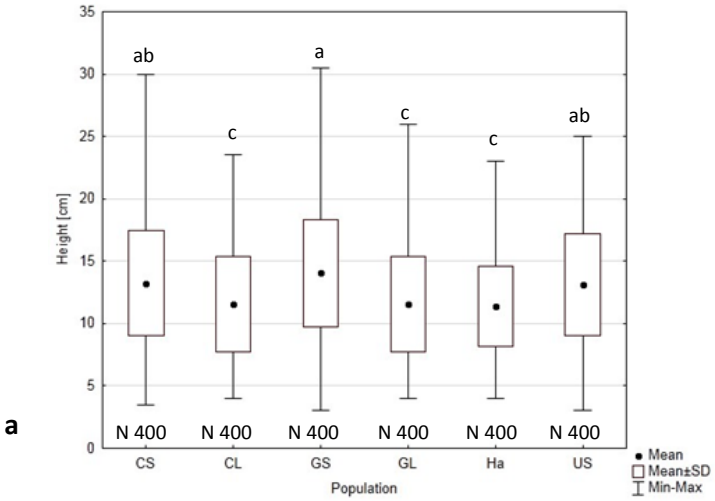
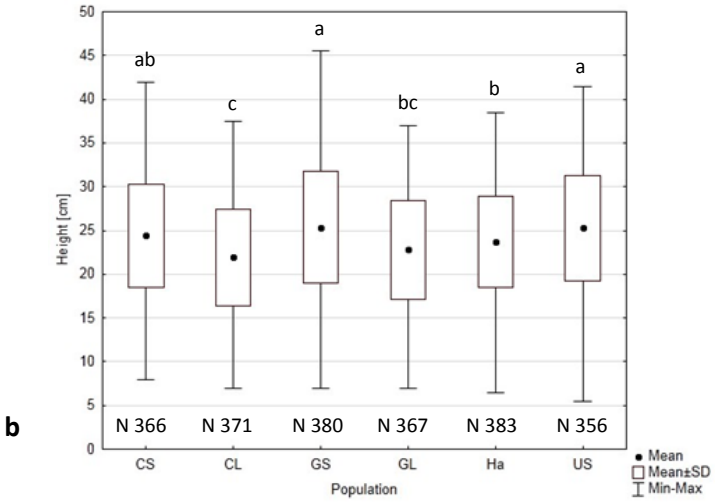


Figure 2: Mean heights of the populations in the plots in Calvörde and the Harz Mountains (Harz) for planting (a), the year 2011 (b) and the year 2012 (c). Different letters indicate significant differences among the experimental plots ($p < 0.05$), N: number of individuals.





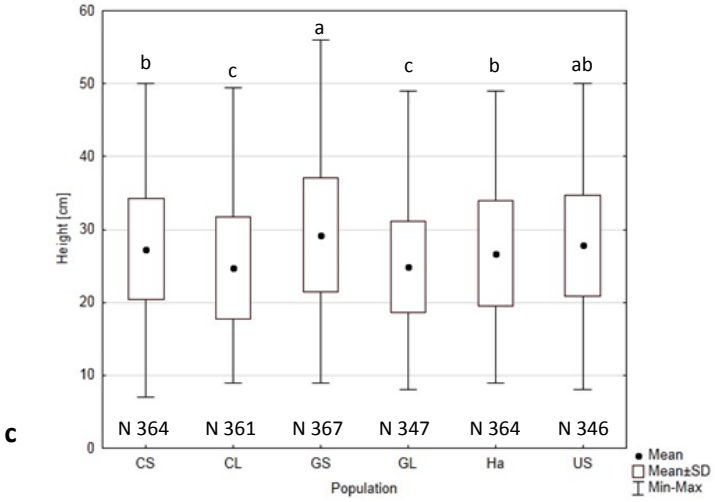


Figure 3: Mean heights of the populations in the plot in Calvörde for planting (2010) (a), the year 2011 (b) and the year 2012 (c). Different letters indicate significant differences among populations ($p < 0.05$), N: number of individuals.

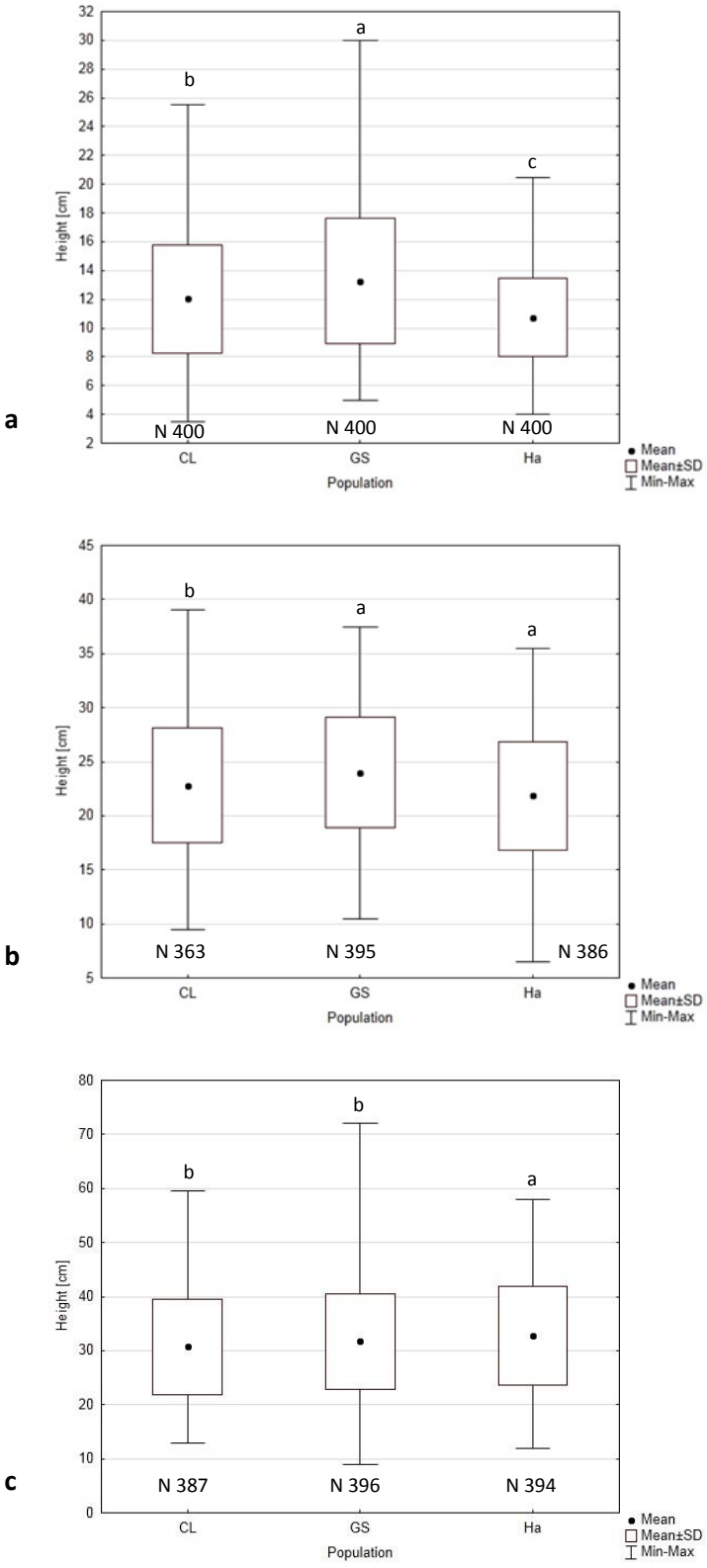


Figure 4: Mean heights of the populations in the plot in the Harz Mountains for planting (2010) (a), the year 2011 (b) and year the 2012 (c). Different letters indicate significant differences among populations ($p < 0.05$), N: number of individuals.

3.1.2 Increment

The increment of the seedlings differed between the populations (Kruskal-Wallis test $p < 0.001$). In the plot in Calvörde, the population Göhrde sand showed the highest increment (21.9 cm) during the observation period from the establishment of the experiment (2010) to fall 2012. The lowest increment was observed for the population Göhrde loam (13.3 cm) (Figure 5). In the plot in the Harz Mountains, the highest increment was observed for the population Harz (21.9 cm). The other two populations in this plot showed similar increment rates which were not significantly different from each other (Figure 6).

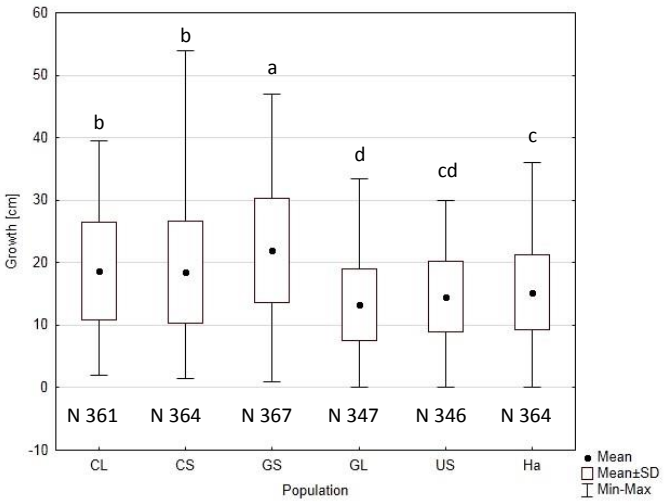


Figure 5: Mean increment of the populations in the plot in Calvörde from establishment (2010) to fall 2012. Different letters indicate significant differences among populations ($p < 0.01$), N: number of individuals.

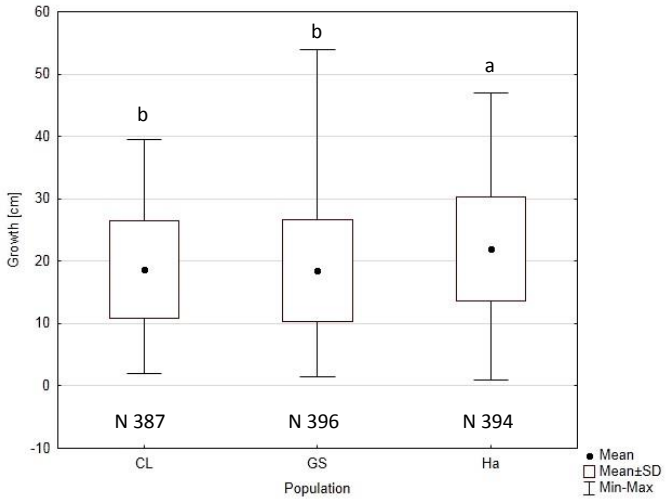


Figure 6: Mean increment of the populations in the plot in the Harz Mountains from establishment (2010) to fall 2012. Different letters indicate significant differences among populations ($p < 0.001$), N: number of individuals.

3.1.3 Bud Burst

Statistically significant differences in the timing of bud burst were observed for the different populations in the translocation experiment (Kruskal-Wallis test $p < 0.001$) (Appendix 3). In the plot in Calvörde, the population with the latest bud burst was Göhrde loam. The populations Göhrde sand and Harz showed a similar bud burst behavior, and they were the populations with the earliest flushing. The rank of bud burst timing between the different populations (from early flushing to late flushing: GS/Ha, CL, CS, US, GL) was very stable between the years (Figure 7). The populations in the plot in the Harz Mountains showed the same trend for timing of bud burst as the populations in the plot in Calvörde (Figure 8). In general, bud burst started later in the plot in the Harz Mountains than in the plot in Calvörde.

The stability of timing of bud burst within populations between the different years was tested using Spearman's rank-order correlation. It resulted in significantly positive, comparatively low to moderate correlations for all tested combinations ($p < 0.05$) (Appendix 4). The lowest correlation coefficient (0.194) was observed for the population Harz for the combination of the years 2011 and 2012 in the plot in the Harz Mountains. The highest correlation (0.586) was observed for the population Calvörde sand for the combination of the years 2012 and 2013 in the plot in Calvörde.

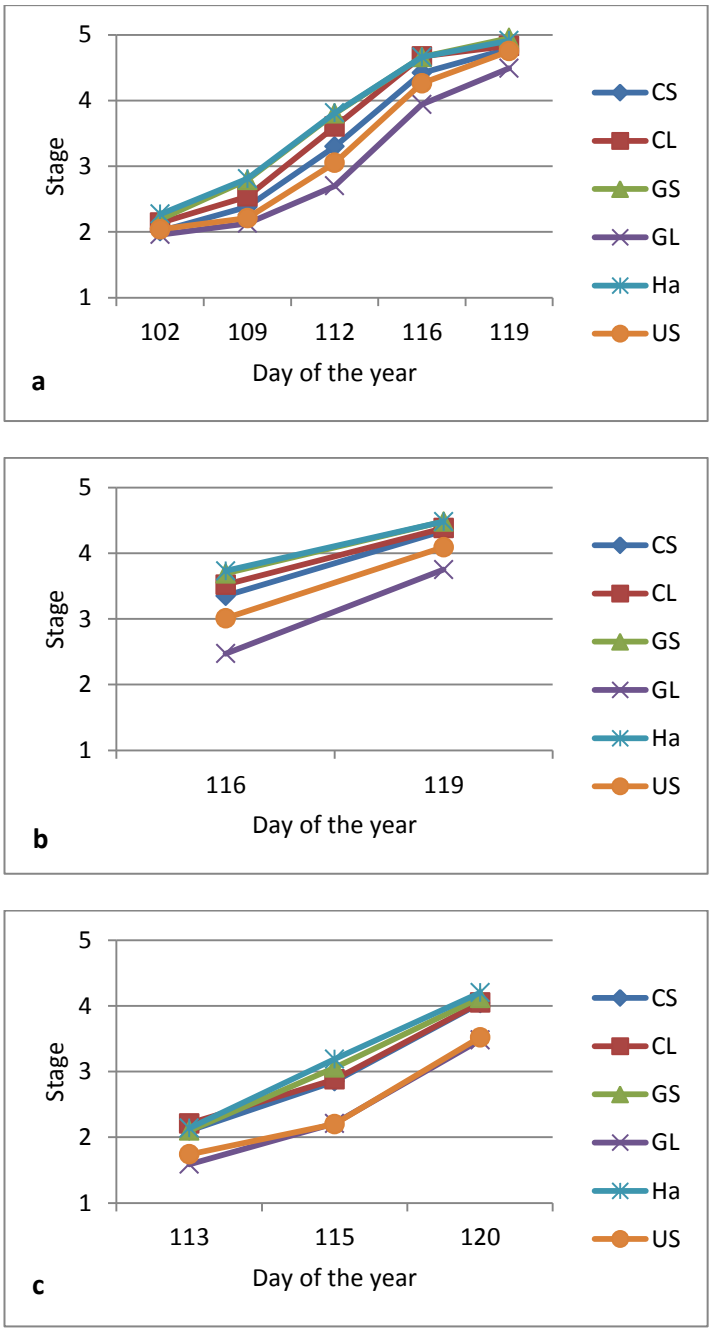


Figure 7: Bud burst of the different populations in the plot in Calvörde for the years (a) 2011, (b) 2012 and (c) 2013. Displayed are mean bud burst stages over days of the year.

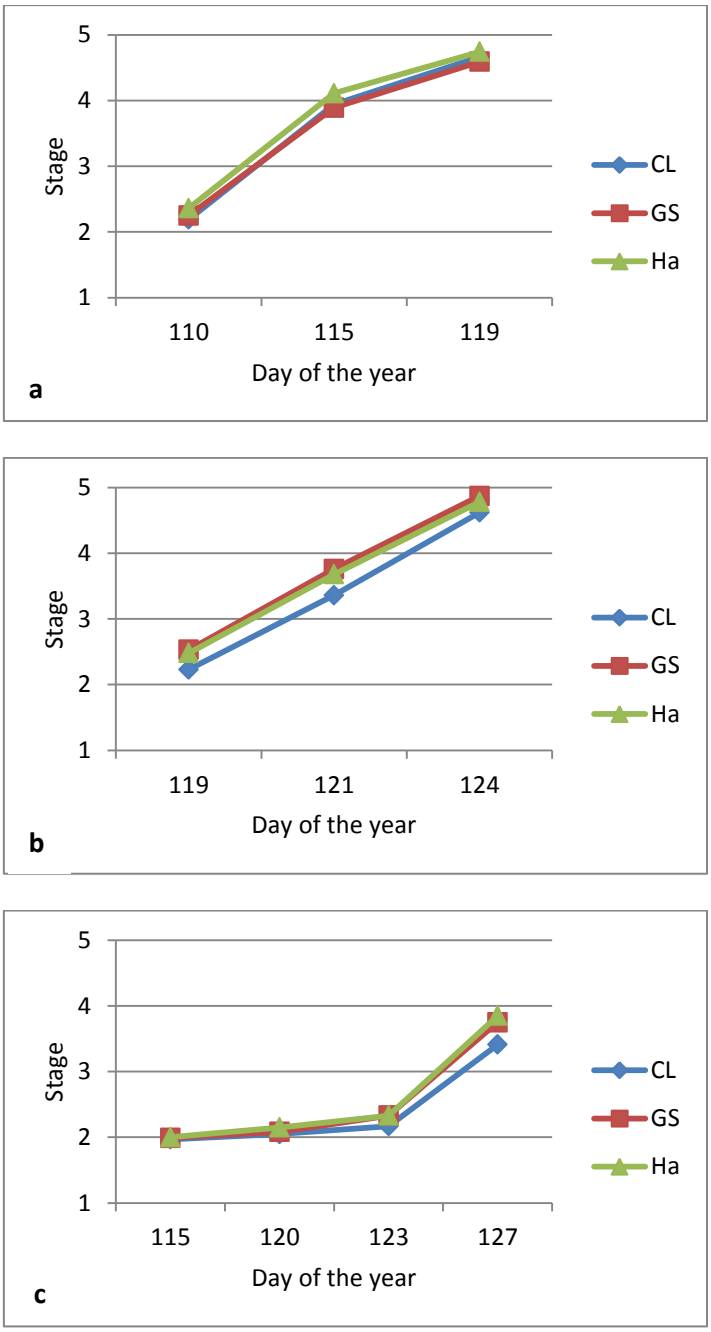


Figure 8: Bud burst of the different populations in the plot in the Harz Mountains in the years (a) 2011, (b) 2012 and (c) 2013. Displayed are mean bud burst stages over days of the year.

3.1.4 Drought Stress Induced Plant Damage

Natural drought stress occurred in the plot in Calvörde in June 2011, which led to damages on the translocated plants. These damages significantly differed between populations ($p < 0.001$). The highest amount of damaged plants was observed in the population Göhrde loam while the neighboring population Göhrde sand exhibited the lowest amount of dam-

aged plants (Figure 9). No statistically significant correlation (Spearman's rank-order) was observed between the annual mean precipitation of the original locations of the populations and damage. In addition, no significant correlations were found between drought stress induced damage and height of the plants. In total, the amount of damaged plants was comparatively low (< 9 %) and most of the seedlings were only slightly damaged.

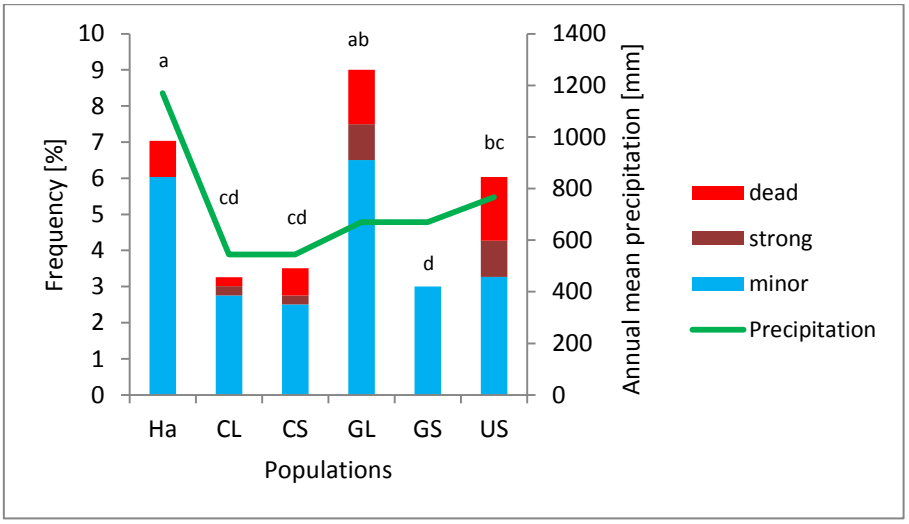


Figure 9: Frequency of drought stress induced damage of the translocated plants in the plot in Calvörde in 2011. Damage of the plants is divided into "minor", "strong" and "dead". Different letters indicate significant differences among populations ($p < 0.05$). The annual mean precipitation of the original site of the different populations is also shown.

3.1.5 Plant Mortality

The observation of mortality in the plot in Calvörde revealed differences between the populations. In spring 2011, only a few plants (1-4) per population were dead. In the year 2012, the mortality rate was low (< 12.5 %) but significantly different between populations ($p < 0.001$). The highest mortality was observed for the population Unterlüß sand (12.3 %) and the lowest one for the population Göhrde sand (4.3 %). In the following year (2013), the mortality rate was clearly higher than in 2012 and still significantly different between populations ($p < 0.001$) in the plot in Calvörde. The highest mortality was recorded for the populations Unterlüß sand (40 %) and Göhrde loam (40.3 %). The lowest mortality was found for the population Harz (25 %) (Figure 10). In the plot in the Harz Mountains, almost all individuals survived until the last observation in the year 2013.

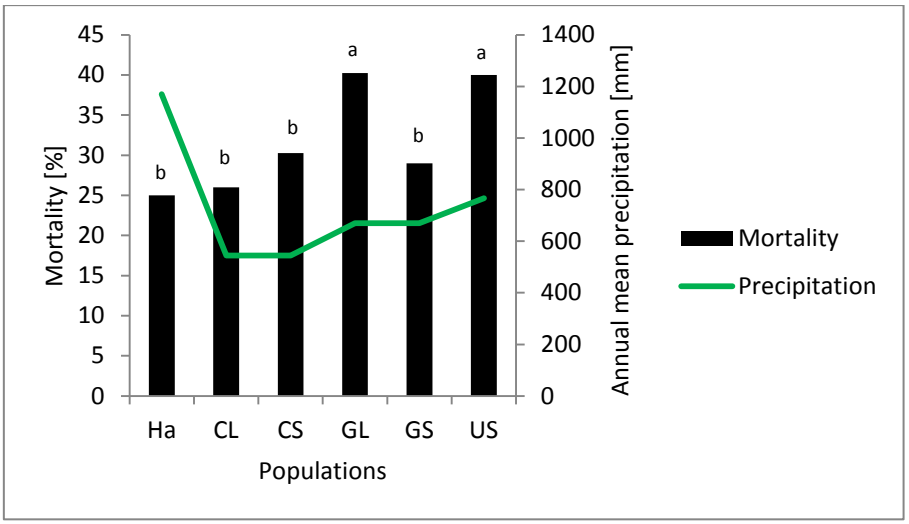


Figure 10: Frequency of the general mortality of the translocated plants in the plot in Calvörde in 2013. Different letters indicate significant differences among populations ($p < 0.01$). Additionally, the annual mean precipitation of the original sites of the different populations is shown.

3.2 Genetic Data

3.2.1 Neutral Genetic Variation (Microsatellites)

3.2.1.1 Linkage Disequilibrium, Null Alleles and Hardy-Weinberg Proportions

The number of null alleles differed among markers and seedling and adult populations (Appendix 5). The highest frequency of null alleles was estimated for the marker "FIR065" (mean 0.170) and was lowest for the marker "mfs11" (mean 0.004). Also, significant linkage disequilibrium ($p < 0.05$) was observed for the markers in different juvenile and adult populations (Table 6). Over all juvenile populations, a significant LD was observed for 11.11 % SSR pairs of all possible marker combinations. For all adult populations, a significant LD was observed for 25 % SSR pairs of all possible marker combinations. No LD was found for the juvenile trees of the population Göhrde sand. The highest LD was estimated for the adult trees of the population Göhrde loam (22.22 %). In general, LD between SSR loci (R^2) was very low (Appendix 6). Only one locus (FIR004) showed deviations from Hardy-Weinberg proportions when tested over all populations. The population-based estimations revealed deviations from Hardy-Weinberg proportions for the juvenile and adult populations Harz and for the adult trees of the population Göhrde sand.

Table 6: Number and percentages of SSRs in LD in the different populations, ($p < 0.05$).

Population	No. of SSR pairs in LD	Percentage [%] of all possible SSR pairs in LD
CL_juvenile	4	11.11
CS_juvenile	4	11.11
GL_juvenile	2	5.56
GS_juvenile	0	0.00
Ha_juvenile	2	5.56
US_juvenile	2	5.56
Juvenile populati-ons combined	4	11.11
CL_adult	4	11.11
CS_adult	1	2.78
GS_adult	4	11.11
UL_adult	1	2.78
US_adult	2	5.56
Ha_adult	7	19.44
GL_adult	8	22.22
Adult populations combined	9	25.00

3.2.1.2 Molecular Diversity Indices

Molecular diversity indices differed among the different SSR loci, whereas the two EST-markers “GOT066” and “FIR004” as well as the SSR locus “FS 3-04” showed lower diversity values than the remaining markers (Table 7). The analysis of the molecular diversity indices revealed no statistically significant differences between the adult and juvenile trees. Only slight differences were observed between the different populations (Table 8). The mean number of alleles was 7.98 for the adult trees and 8.2 for the seedlings. For the adult populations, a mean observed heterozygosity of 0.605 was estimated, very similar to the mean observed heterozygosity for the juvenile populations (0.06). The mean values of the expected heterozygosity were 0.619 for the adult and 0.618 for the juvenile trees. The mean fixation index was 0.024 for the adult and 0.015 for the juvenile trees.

Table 7: Molecular diversity indices for investigated SSR loci over all analyzed populations, N: number of individuals, N_a: number of alleles, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index.

Locus	N	N_a	H_o	H_e	F
sfc0018	100.3	10.231	0.716	0.735	0.024
sfc0161	100.2	15.154	0.794	0.798	0.006
sfc1063	100.0	9.692	0.794	0.805	0.013
sfc1143	100.2	11.000	0.771	0.762	-0.012
GOT066	100.1	3.385	0.160	0.152	-0.038
FIR065	100.3	4.000	0.673	0.688	0.021
FIR004	100.4	7.462	0.509	0.585	0.128
FS 3-04	100.4	3.923	0.304	0.311	0.028
mfs11	100.3	8.154	0.732	0.723	-0.013

Table 8: Molecular diversity indices for the different populations divided into adult and seedling populations, N: number of individuals, N_a: number of alleles, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index.

	Adult Populations					Seedling Populations				
Population	N	N_a	H_o	H_e	F	N	N_a	H_o	H_e	F
CL	101.0	8.000	0.581	0.599	0.019	99.9	8.222	0.594	0.603	0.009
CS	104.0	8.111	0.599	0.619	0.015	98.8	8.444	0.621	0.633	0.012
GL	99.8	7.444	0.643	0.635	-0.020	99.8	7.889	0.615	0.626	0.012
GS	103.0	8.444	0.602	0.638	0.077	100.0	8.556	0.613	0.623	0.010
US	99.4	8.000	0.594	0.595	0.009	99.9	7.889	0.571	0.586	0.043
Ha	98.8	7.889	0.612	0.630	0.042	98.9	8.222	0.624	0.635	0.002
Mean	101	7.981	0.605	0.619	0.024	99.6	8.204	0.606	0.618	0.015

3.2.1.3 Genetic Differentiation, Variation and Population Structure

The genetic distances (Nei 1972) were comparatively low and ranged from 0.007 (CL_juvenile vs. CL_adult) to 0.068 (CL_adult vs. GL_adult). The mean distances were 0.041 among adult stands, 0.031 among juvenile trees and 0.032 between adult and juvenile trees (Appendix 7). All juvenile populations grouped to the adult stands of origin in the dendrogram (Figure 11). The bootstrap values ranged from 52 % for the group GS_adult/GS_juvenile and 100 % for the group CL_adult/CL_juvenile. The mean bootstrap

value for all clusters of the adult/juvenile population pairs was 84 %. The grouping between the different populations originating from different regions was low supported (mean bootstrap 28 %). The AMOVA calculated for the juvenile trees revealed most variation within populations (97 %), and only 3 % of variation was observed among them (Table 9). The STRUCTURE analysis revealed very weak population structure between the different (juvenile) populations. The delta *K* method (Evanno et al. 2005) revealed an optimal value of *K* = 2 (Appendix 8a). The individuals of the different populations showed a similar clustering among populations with a slightly different clustering for the populations Calvörde loam and Göhrde loam (Figure 12).

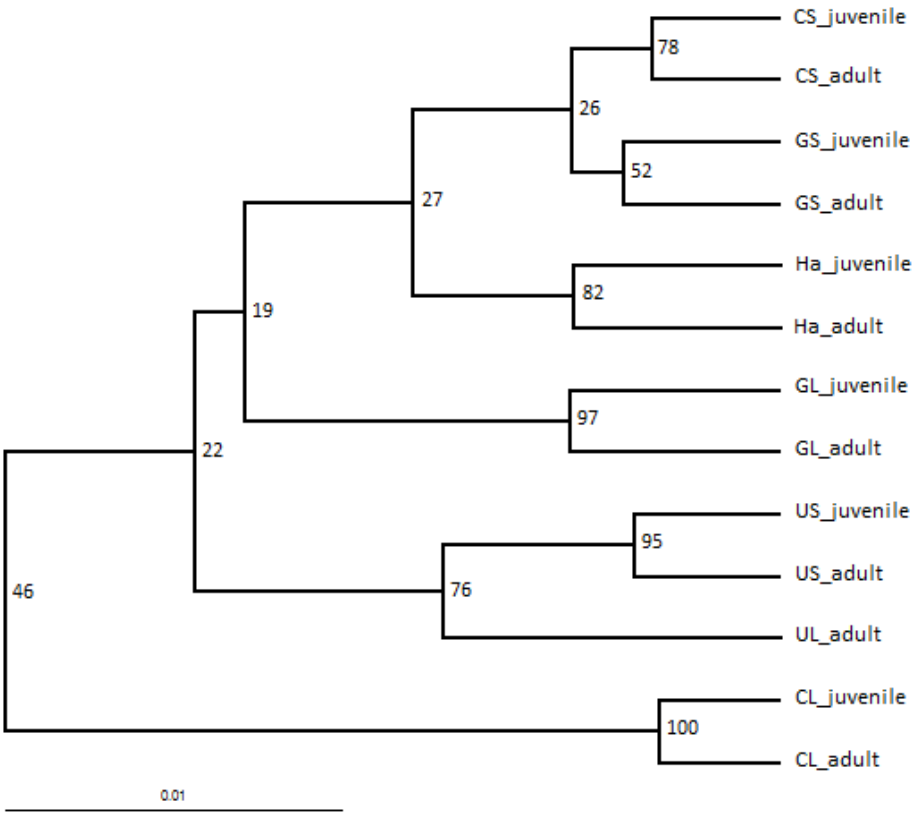


Figure 11: UPGMA dendrogram based on Nei´s genetic distance (Nei 1972) and bootstrap values [%] for the investigated populations.

Table 9: AMOVA within and among the investigated juvenile populations, df: degrees of freedom, SS: sum of squares, EV: estimated variance, PV: percentage of variance, Φ_{pt}: proportion of the variance among populations relative to the total variance, n.a.: not available.

Source	df	SS	EV	PV [%]	Φ_{pt}	p
Among Populations	5	109.135	0.161	3	0.027	0.001
Within Populations	592	3401.106	5.745	97	n.a.	n.a.
Total	597	3510.241	5.906	100	n.a.	n.a.

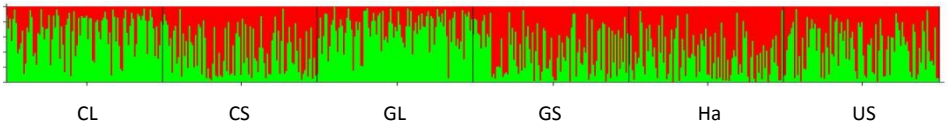


Figure 12: Results of the STRUCTURE analysis based on SSRs for the different seedling populations. Displayed is the clustering of individuals assuming *K* = 2.

3.2.2 Potentially Adaptive Genetic Variation (SNPs)

3.2.2.1 Candidate Genes

Fragments of ten different candidate genes were analyzed with a total length of 12,290 bp (Table 10). From the total length of the analyzed fragments, 7,586 bp accounted for exons, 3,461 bp for introns and 1,243 bp for untranslated regions (UTR). In total, 20 indels and 116 SNPs were identified (Appendix 1). All 19 identified non-synonymous SNPs led to an amino acid exchange and no one caused a stop codon. The number of haplotypes ranged from three (gene fragment *Asi*) to 15 (gene fragment *CP10*), whereas the mean value was 8.42 (Table 11). The nucleotide diversity ranged from 0.57 (gene fragment *ConsC1*) to 4.58 (gene fragment *Arf*). The mean nucleotide diversity over all fragments was higher for non-coding sites than for coding sites (Table 11). Tajima's *D* (Tajima 1989) was significantly positive for the gene fragment *His3C1* ($p < 0.05$).

Table 10: Overview of exons, introns, UTR, indels and SNPs of the analyzed gene fragments.

Gene name	Abbreviation	Total length (bp)	Length (bp) of exons	Length (bp) of introns	Length (bp) of UTR	No. of indels	Total no. of SNPs	No. of non-coding SNPs	No. of SNPs in UTR	No. of synony-mous SNPs	No. of non-synonymous SNPs
Auxin response factor	*Arf*	1058	512	546	0	0	13	13	0	0	0
Alpha amyl-ase/subtilisin inhibitor	*Asi*	873	631	0	242	1	10	0	4	2	4
Constans like	*ConsC1*	1200	935	106	159	0	3	0	0	1	2
	ConsC2	583	551	0	32	1	8	0	1	2	5
Chloroplast chaperonin like	*CP10*	1594	633	917	44	2	19	11	0	6	2
Cysteine proteinase	*CysPro*	920	496	212	212	1	12	3	7	2	0
Dof zinc finger protein	*DAG*	1210	459	627	124	5	15	11	1	3	0
Frigida	*Frigida*	430	430	0	0	1	7	0	0	5	2
Histone 3	*His3C1*	939	394	292	253	2	5	2	1	2	0
	His3C2	716	386	292	38	2	11	7	2	2	0
NAC transcription factor	*NAC*	1357	1030	188	139	5	6	1	2	2	1
Protein phosphatase 2C	*PP2C*	1410	1129	281	0	0	7	2	0	2	3
Total		12,290	7,586	3,461	1,243	20	116	50	18	29	19

Table 11: Nucleotide Diversity, haplotype diversity and results of Tajima's *D* test of the different gene fragments.

Name of Gene fragment	No of haplo-types	Haplotype diversity	Total Nucleotide diversity[+]	Non-coding sites (introns)[+]	Coding sites[+]	UTR[+]	Syn. sites[+]	Non-syn. sites[+]	Tajima's *D*
Arf	12	0.805	4.58	8.87	0	-	0	0	1.974
Asi	3	0.377	3.37	-	2.60	5.58	2.64	2.59	0.804
ConsC1	4	0.568	0.57	0	0.73	0	0.53	0.79	0.012
ConsC2	8	0.807	4.23	-	3.82	11.64	3.95	3.78	1.002
CP10	15	0.841	3.06	3.38	2.42	0	8.51	0.40	0.110
CysPro	5	0.621	3.03	3.12	1.13	7.44	4.75	0	0.094
DAG	10	0.704	3.28	4.89	1.97	2.51	8.58	0	0.492
Frigida	4	0.302	2.14	-	2.14	-	6.07	0.96	-1.131
His3C1	5	0.566	2.25	3.51	2.57	0.32	10.32	0	2.124*
His3C2	13	0.735	2.86	5.35	0.90	9.12	3.68	0	-0.517
NAC	9	0.645	1.44	2.37	1.10	2.85	2.82	0.60	1.119
PP2C	13	0.848	1.57	2.13	1.43	-	3.41	0.82	1.067
Mean	8.42	0.652	2.70	4.20	1.89	5.64	5.02	1.24	0.596

[+] $\pi \times 10^{-3}$, *$p < 0.05$

3.2.2.2 Genotyping, Linkage Disequilibrium and Hardy-Weinberg Proportions

In total, 56 SNPs were chosen for genotyping, but eight SNPs were not processed successfully and two SNPs were monomorphic. Thus, 46 SNPs were used for the final analysis (15 synonymous SNPs, 14 non-coding SNPs, 12 non-synonymous SNPs and five SNPs from UTR) (Table 12). The positive control of the genotyping service with the ABI PRISM® SnaPshot™ Multiplex Kit (Applied Biosystems, Foster City, USA) confirmed all tested SNPs. Significant LD was found for different SNP pairs in the different populations ($p < 0.05$). Over all populations 18.45 % of all possible SNPs pairs were found to be in LD (Table 13). Most SNPs significantly in LD were found within genes and, in general, LD between SNPs (R^2) was low (Figure 13). The lowest amount of SNP pairs significantly in LD was found for the population Göhrde sand (11.01 %), whereas the highest amount of SNP pairs in LD was found for the population Göhrde loam (17.97 %) (Table 13). A positive, but statistically not significant correlation was found between LD revealed by SSRs and LD revealed by SNPs. No deviations of Hardy-Weinberg proportions were detected, when SNPs were tested over all populations.

Table 12: Characterization of the successfully processed SNPs.

SNP no.	SNP name	Gene	Characteristic	Substitution
1	Arf_265	*Auxin response factor*	non-coding	A/G
2	Arf_303		non-coding	A/G
3	Arf_563		non-coding	A/G
4	Arf_573		non-coding	T/C
7	Arf_615		non-coding	A/G
12	Arf_833		non-coding	A/G
13	Arf_878		non-coding	A/G
25	ConsC1_293	*Constans like (1)*	non-synonymous	A/T
26	ConsC1_306		synonymous	A/G
29	ConsC2_51	*Constans like (2)*	UTR	A/G
30	ConsC2_98		non-synonymous	C/G
31	ConsC2_147		synonymous	T/G
32	ConsC2_151		non-synonymous	C/G
33	ConsC2_211		non-synonymous	T/G
34	ConsC2_390		synonymous	T/C
36	ConsC2_488		non-synonymous	T/C
38	CP10_65	*Chloroplast Chaperonin like*	synonymous	T/C
39	CP10_67		non-synonymous	T/C
45	CP10_377		non-coding	T/G
47	CP10_442		non-coding	C/G
48	CP10_503		synonymous	C/G
50	CP10_749		synonymous	C/G
55	CP10_1317		non-coding	A/G
56	CP10_1428		non-synonymous	T/C
58	CysPro_118	*Cystein proteinase*	synonymous	C/G
59	CysPro_202		synonymous	A/G
63	CysPro_728		UTR	C/G
65	CysPro_783		UTR	T/G
71	DAG_81	*Dof zinc finger protein*	UTR	A/G
72	DAG_289		non-coding	A/T
89	DAG_1059		synonymous	T/G
91	Frigida_54	*Frigida*	synonymous	T/C
92	Frigida_104		non-synonymous	A/G
93	Frigida_179		non-synonymous	A/G
101	His3C1_292	*Histone 3 (1)*	non-coding	T/C
108	His3C2_104	*Histone 3 (2)*	synonymous	A/C
110	His3C2_186		non-coding	T/C
112	His3C2_260		synonymous	A/G
123	NAC_854	*NAC transcription factor*	non-synonymous	A/C
124	NAC_962		synonymous	A/G
129	NAC_1300		UTR	A/G
131	PP2C_315	*Protein phosphatase 2C*	non-synonymous	C/G
132	PP2C_391		synonymous	T/G
134	PP2C_791		non-synonymous	A/G
135	PP2C_941		non-coding	T/G
136	PP2C_1200		synonymous	A/G

Table 13: Number and percentages of SNPs in LD in the different populations, ($p < 0.05$).

Population	No. of SNP pairs in LD	Percentage [%] of all possible SNP pairs in LD
CL	133	12.85
CS	149	14.40
GL	186	17.97
GS	114	11.01
Ha	148	14.30
US	125	12.08
Populations combined	191	18.45

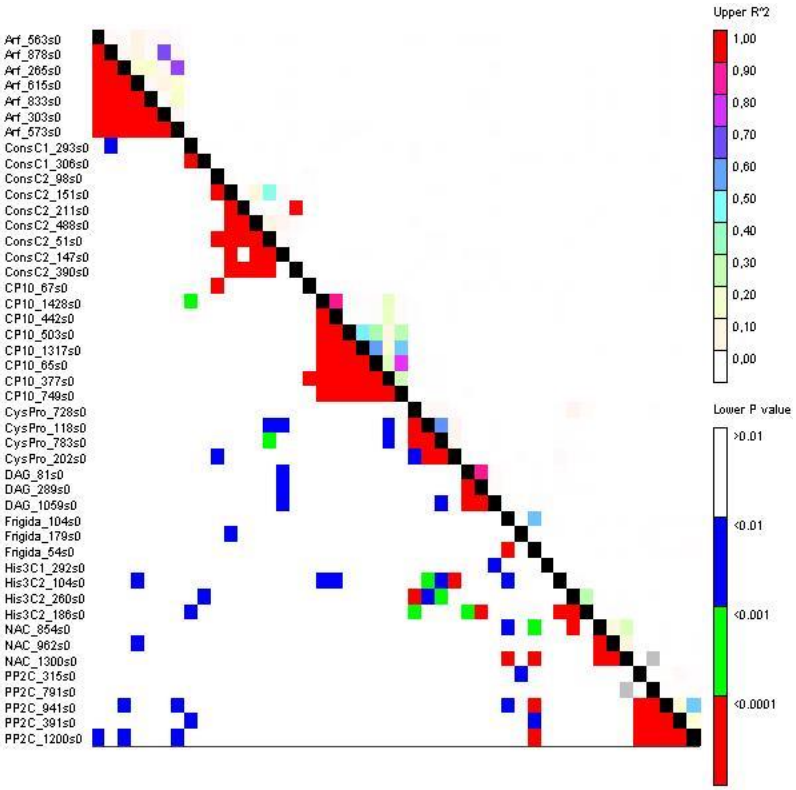


Figure 13: LD Plot of pair-wise R^2 values (upper diagonal) between all SNP pairs with corresponding p-values (lower diagonal).

3.2.2.3 Molecular Diversity Indices and Population Structure

The observed heterozygosity estimated with the total SNP set differed between populations and ranged from 0.251 for Calvörde loam to 0.277 for Unterlüß sand (Table 14). The population Calvörde loam showed the lowest expected heterozygosity (0.252), whereas the population Harz showed the highest one (0.280). The highest mean observed and expected heterozygosities were estimated with non-coding SNPs, the lowest values were found with non-synonymous SNPs. The fixation index was slightly above or below zero (Table 14). The molecular diversity indices differed between single SNPs (Appendix 9). Pooling of individuals to association populations turned out to have only minor effects on the molecular diversity indices. A higher expected heterozygosity was observed for late flushing individuals compared to early flushing ones for all analyzed years. This trend was stronger using only potentially adaptive SNP markers revealed by the association and/or outlier analysis conducted in this study (Table 15). The same tendency was detected for the observed heterozygosity except for the pooled individuals of the year 2013, which showed a higher observed heterozygosity for early flushing individuals compared to late flushing ones. Both the observed and expected heterozygosities among late and early flushing individuals strongly differed among potentially adaptive single SNPs markers (Appendix 10).

The STRUCTURE analysis revealed only weak population structure among populations. When the populations were analyzed with the total SNP set, the delta *K* method (Evanno et al. 2005) revealed an optimal value of $K = 3$ (Appendix 8b). Analyzing the populations with different subsets of SNPs, the delta *K* method revealed an optimal value of $K = 2$ for “non-coding” and “non-synonymous” SNPs and an optimal value of $K = 3$ for “silent SNPs” (Appendix 8b). In general, only slight differences were detectable among different SNP sets. The most pronounced difference was observed for non-synonymous SNPs for the population Calvörde loam compared to the others (Figure 14).

Table 14: Molecular diversity indices based on SNPs for the different populations. Results are displayed for the total SNPs, non-coding SNPs, synonymous SNPs and non-synonymous SNPs, N: number of individuals, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index.

	Total SNPs				Non-coding SNPs				Synonymous SNPs				Non-synonymous SNPs			
Population	N	H_o	H_e	F	N	H_o	H_e	F	N	H_o	H_e	F	N	H_o	H_e	F
CL	258.1	0.251	0.252	0.018	258.2	0.311	0.311	0.012	257.3	0.212	0.214	0.012	259	0.205	0.205	0.035
CS	221.5	0.275	0.276	0.009	220.1	0.353	0.349	-0.006	222.1	0.226	0.228	0.006	222.9	0.213	0.219	0.034
GL	221.5	0.264	0.272	0.012	220.5	0.346	0.363	0.039	221.7	0.216	0.222	0.006	223	0.194	0.189	-0.024
GS	247.7	0.266	0.274	0.020	247.1	0.347	0.356	0.025	247.9	0.204	0.215	0.031	248.3	0.214	0.218	-0.001
Ha	216.8	0.274	0.280	0.020	216.1	0.354	0.357	0.014	216.9	0.245	0.247	0.016	217.9	0.183	0.199	0.034
US	207.8	0.277	0.276	-0.004	207.1	0.376	0.37	-0.014	207.7	0.206	0.213	0.013	209	0.208	0.206	-0.011
Mean	228.9	0.268	0.271	0.012	228.2	0.348	0.351	0.011	228.9	0.218	0.223	0.014	230	0.203	0.206	0.011

Table 15: Molecular diversity indices calculated with the total SNP set and potentially adaptive SNPs revealed by association and/or outlier analysis for the pooled individuals of the different years divided into "early" flushing and "late" flushing, N: number of individuals, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index.

		Total SNPs				Potentially adaptive SNPs			
Year	Individuals	N	H_o	H_e	F	N	H_o	H_e	F
2011	early	758.9	0.267	0.274	0.031	759.3	0.260	0.269	0.047
	late	413.0	0.269	0.279	0.024	412.8	0.282	0.286	0.007
2012	early	622.9	0.265	0.273	0.030	620.4	0.330	0.339	0.029
	late	280.7	0.267	0.276	0.030	279.8	0.339	0.356	0.051
2013	early	326.0	0.270	0.276	0.024	324.5	0.286	0.281	0.000
	late	523.5	0.266	0.278	0.035	522.3	0.267	0.289	0.063

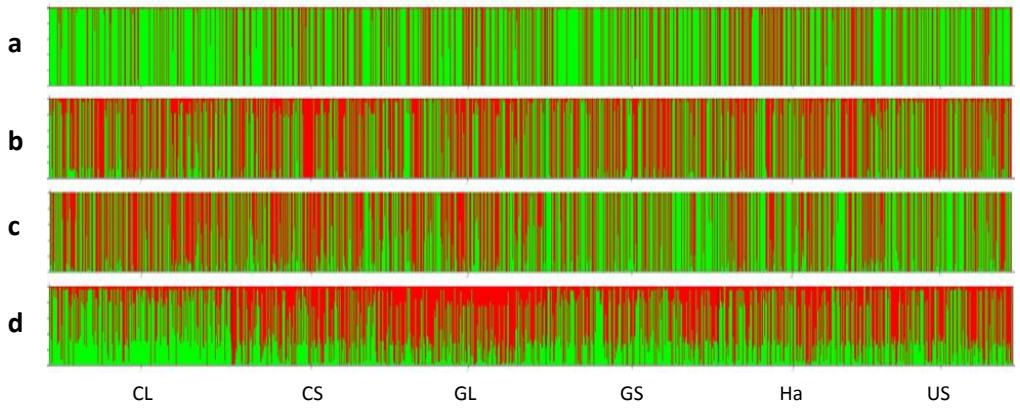


Figure 14: Results of the STRUCTURE analysis for the different populations. Displayed is the clustering of individuals based on (a) the total SNP set , (b) non-coding SNPs, (c) "silent" SNPs and (d) non-synonymous SNPs assuming *K* = 2.

3.2.2.4 Association and Outlier Analysis

The association analysis under a GLM based on single populations revealed several significantly associated SNPs with bud burst, albeit the repeatability between years was low (Table 16).

The following results of the association analyses are based on pooled individuals to association populations for the different years. In total, 23 out of all analyzed 46 SNPs showed sig-

nificant associations with bud burst in at least one year using a GLM (Table 17). Several SNPs were significantly associated with bud burst in more than one year and two SNPs (CysPro_728 and His3C2_104) were significantly associated with bud burst in all three analyzed years. The inclusion of population structure did not affect the results in most cases. The phenotypic variation explained by the marker was comparatively low ($R^2 <$ 2.2 %). Under a MLM, 19 out of the 46 analyzed SNPs were significantly associated with bud burst in at least one year (Table 18), and the two SNPs “CysPro_728” and “His3C2_104” were also significantly associated with bud burst in all three years. All 19 SNPs were also significantly associated with bud burst under the GLM.

The outlier analysis revealed evidence for both balancing and directional selection for several SNPs (Table 19). The outliers differed between the tested populations.

The association analysis between SNPs and height of the plants revealed two significant associations of the non-synonymous SNPs “CP10_1428” and “PP2C_315” (R^2: 0.006; $p < 0.05$).

Table 16a: Results of the association analysis for the different populations for different years under a GLM. All SNPs of the total SNP set are displayed which are significantly associated with bud burst in at least one year, N: number of individuals, *p < 0.05, **p < 0.01, ***p < 0.001, blank cells: not significant.

SNP name	Characteristic	Population/Year																	
		CS			CL			GS			GL			Ha			US		
		2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
			p			p			p			p			p			p	
Arf_265	non-coding												*		*				
Arf_303	non-coding			*	*														
Arf_573	non-coding												*		*				
Arf_833	non-coding					***													
Arf_878	non-coding				*														
ConsC1_293	non-synonymous															*			
ConsC2_51	UTR				*														
ConsC2_147	synonymous						*												
ConsC2_211	non-synonymous				*					*									
ConsC2_488	non-synonymous							*								*			
CP10_503	synonymous						*												
CysPro_118	synonymous											*							
CysPro_202	synonymous				*														
CysPro_728	UTR		*		*														*
CysPro_783	UTR											*							

Table 16b: Results of the association analysis for the different populations for different years under a GLM. All SNPs of the total SNP set are displayed which are significantly associated with bud burst in at least one year, N: number of individuals, *p < 0.05, **p < 0.01, ***p < 0.001, blank cells: not significant.

SNP name	Characteristic	Population/Year																	
		CS			CL			GS			GL			Ha			US		
		2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
		p			p			p			p			p			p		
Frigida_54	synonymous					*													
Frigida_104	non-synonymous	*											*						
Frigida_179	non-synonymous														*			*	
His3C2_104	synonymous					*													***
His3C2_186	non-coding		*				*												
NAC_854	non-synonymous				*												*	*	
NAC_962	synonymous	*																*	
PP2C_315	non-synonymous																	**	
PP2C_791	non-synonymous														*				
PP2C_941	non-coding						*				*				*				
PP2C_1200	synonymous						*				*				*				

Table 17: Results of the association analysis for the pooled individuals of the different years under a GLM. All SNPs of the total SNP set are displayed which are significantly associated with bud burst in at least one year, (s): population structure included, R^2: phenotypic variation explained by marker, N: number of individuals, ns: not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

		2011 (N 1202)				2012 (N 925)				2013 (N 869)			
SNP	Characteristic	p	p (s)	R^2	R^2 (s)	p	p (s)	R^2	R^2 (s)	p	p (s)	R^2	R^2 (s)
Arf_265	non-coding	*	ns	0.0057	ns	**	ns	0.0105	ns	ns	ns	ns	ns
Arf_573	non-coding	ns	ns	ns	ns	*	ns	0.0084	ns	ns	ns	ns	ns
ConsC1_306	synonymous	ns	ns	ns	ns	ns	ns	ns	ns	*	*	0.008	0.0080
ConsC2_51	UTR	*	**	0.0072	0.0076	*	*	0.0076	0.0078	ns	ns	ns	ns
ConsC2_98	non-synonymous	ns	ns	ns	ns	ns	ns	ns	ns	*	*	0.0079	0.0079
ConsC2_147	synonymous	***	**	0.0148	0.0149	**	**	0.0107	0.0104	ns	ns	ns	ns
ConsC2_151	non-synonymous	ns	ns	ns	ns	*	*	0.009	0.0093	**	**	0.0133	0.0133
ConsC2_488	non-synonymous	***	***	0.0148	0.0156	**	**	0.0102	0.0105	ns	ns	ns	ns
CP10_67	non-synonymous	*	*	0.0065	0.0065	ns	ns	ns	ns	ns	ns	ns	ns
CP10_377	non-coding	*	*	0.0054	0.0057	*	*	0.0071	0.0073	ns	ns	ns	ns
CP10_442	non-coding	ns	ns	ns	ns	*	*	0.0093	0.0094	ns	ns	ns	ns
CP10_503	synonymous	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	0.0075
CP10_1428	non-synonymous	ns	*	ns	0.0051	*	*	0.0085	0.0087	ns	ns	ns	ns
CysPro_118	synonymous	ns	ns	ns	ns	*	*	0.009	0.0091	***	***	0.0216	0.0216
CysPro_728	UTR	**	**	0.0084	0.0086	**	**	0.0115	0.0116	*	*	0.0076	0.0076
CysPro_783	UTR	ns	ns	ns	ns	**	**	0.0099	0.0101	***	**	0.0207	0.0207
DAG_81	UTR	ns	ns	ns	ns	ns	ns	ns	ns	*	*	0.0078	0.0078
DAG_289	non-coding	ns	ns	ns	ns	ns	ns	ns	ns	*	*	0.0089	0.0089
His3C2_104	synonymous	*	*	0.0067	0.0070	**	***	0.0162	0.0183	**	**	0.0123	0.0124
NAC_854	non-synonymous	*	*	0.007	0.0072	ns	ns	ns	ns	ns	ns	ns	ns
NAC_1300	UTR	*	**	0.0073	0.0077	*	*	0.0104	0.0102	ns	ns	ns	ns
PP2C_941	non-coding	ns	ns	ns	ns	ns	ns	ns	ns	**	**	0.0115	0.0115
PP2C_1200	synonymous	ns	ns	ns	ns	ns	ns	ns	ns	*	*	0.0094	0.0095

Table 18: Results of the association analysis for the pooled individuals of the different years under a MLM. All SNPs of the total SNP set are displayed which are significantly associated with bud burst in at least one year, ns: not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

		2011	2012	2013
		N 1202	N 925	N 869
SNP	Characteristic		p	
Arf_265	non-coding	ns	ns	*
ConsC1_306	synonymous	ns	ns	*
ConsC2_51	UTR	ns	*	ns
ConsC2_98	non-synonymous	ns	ns	*
ConsC2_147	synonymous	***	**	ns
ConsC2_151	non-synonymous	ns	**	**
ConsC2_488	non-synonymous	**	**	ns
CP10_67	non-synonymous	*	ns	ns
CP10_377	non-coding	*	ns	ns
CP10_442	non-coding	ns	*	ns
CysPro_118	synonymous	*	ns	***
CysPro_728	UTR	*	**	*
CysPro_783	UTR	*	ns	**
DAG_81	UTR	ns	ns	*
DAG_289	non-coding	ns	ns	*
His3C2_104	synonymous	*	***	*
NAC_1300	UTR	ns	*	ns
PP2C_941	non-coding	ns	*	*
PP2C_1200	synonymous	ns	**	ns

Table 19: Results of the outlier analyses for the pooled individuals and population comparisons.

Reference	SNP	Selection
Pooled Individuals 2011*	ConsC2_98	balancing
Pooled Individuals 2012*	His3C2_104	directional
Pooled Individuals 2013*	-	-
GS vs. GL	Frigida_104	balancing
GL vs. Ha	Frigida_54	balancing
CL vs. CS	ConsC2_98	directional
	CP10_1317	directional
CL vs. GL	ConsC2_98	balancing
	CP10_503	balancing
	His3C2_104	balancing
CL vs. GS	Frigida_54	directional
CL vs. US	Frigida_54	directional
CL vs. Ha	ConsC2_98	balancing
CS vs. GL	ConsC2_98	balancing
	Frigida_54	balancing
CS vs. GS	-	-
CS vs. US	ConsC2_98	directional
	His3C2_104	directional
CS vs. Ha	-	-
GL vs. US	ConsC2_98	balancing
	Frigida_104	balancing
	Frigida_54	balancing
	His3C2_104	balancing
GS vs. US	CysPro_728	directional
	Frigida_54	balancing
GS vs. Ha	Frigida_54	balancing
US vs. Ha	ConsC2_98	balancing
	Frigida_54	balancing
	His3C2_104	directional

* early vs. late flushing individuals

4 Discussion

4.1 Phenotypic Data

4.1.1 Height

Significant differences of height and increment were detected for the different populations of the translocation experiment, albeit the absolute values were small. Different to expectations, the precipitation gradient of the sampling areas was not reflected by the growth of the plants on the dry experimental plot in Calvörde. Nevertheless, higher plants were observed for the populations growing in the experimental plot in the Harz Mountains than for the same populations growing in the plot in Calvörde. This might be a result of reduced (drought) stress in the Harz Mountains (see below). Variation in height among beech provenances has been observed in other studies (Liesebach et al. 2011, Liesebach 2012, Wühlisch et al. 2008). Gömöry and Paule (2011) detected a positive correlation between plant height and the length of the vegetation period, whereas the growth cessation had a greater influence than bud burst. In this study, the populations with the earliest bud burst (Harz and Göhrde sand) were also among the populations containing the highest plants. These populations may benefit from a longer vegetation period due to earlier bud burst, but growth cessation has not been measured in this study, and thus, no reliable conclusions are possible in this context. Several studies revealed a correlation between seed mass and growth of seedlings especially in the first years (Oleksyn et al. 1998, Vitasse et al. 2009a, Landergott et al. 2012). Thus, different seed weights among populations might have influenced plant height in this study. Nevertheless, plants of the same populations showed different increments rates in the two different experimental plots in Calvörde and the Harz Mountains. This indicates that other parameters than seed weight were more important for plant development in this study (see below).

4.1.2 Bud Burst

As hypothesized, significant differences in bud burst were identified among the populations in the translocation experiment. Thereby, the order of late and early flushing populations was stable between years. Only in 2013, the population Unterlüß sand showed a later bud burst than in previous years, which can be explained by the high mortality rate for this population (40 %). These results are in accordance with several studies, which detected differ-

ences in bud burst among beech provenances and a high genetic control of this trait (e.g., Wühlisch et al. 1995b, Gömöry and Paule 2011, Liesebach 2012). In general, beech populations from high elevations flush earlier than those from low elevations, when they are grown under similar conditions in a translocation experiment (Vitasse et al. 2009a). This relationship was partly confirmed in this study. Thus, the population from the highest altitude "Harz" was indeed the earliest flushing one in the translocation experiment. The population Göhrde sand from a low altitudinal origin, however, showed almost the same bud burst as the population Harz. However, the greatest altitude difference in this study was 386 m, and thus relatively low in comparison to other studies (Vitasse et al. 2009a, ca. 1,400 m; Liesebach 2012, ca. 980 m), which might explain the results. Interestingly, the neighboring populations Göhrde sand and Göhrde loam exhibited the most different flushing times among all investigated populations. This surprising result is difficult to explain, because the two populations have been grown under nearly the same environmental conditions and should have experienced the same selective forces. Establishment of the stands with different plant material would be a possible explanation, but no indications of planting activities were found, albeit it can not totally be ruled out. The highest differences between the two stands are tree age (GS: 133 years, GL: 142 years), stem density (GS: 289 trees ha^{-1}, GL: 122 trees ha^{-1}) and DBH (GS: 30.7 cm, GL: 51 cm). In short, the stand Göhrde loam exhibits older and bigger trees and as a result a lower number of trees. Thus, there might have been a selection against early flushing individuals in the (late flushing) population Göhrde loam, but this remains speculative.

As expected, bud burst occurred later in the experimental plot in the Harz Mountains than in the experimental plot in Calvörde, but the order of flushing time of the populations was the same between the two plots. This is most likely the result of lower temperatures in the Harz Mountains. Although, known as a species with high photoperiodic control of bud burst, a critical temperature sum is also required for flushing of beech, which was reached later in the Harz Mountains than in Calvörde. A different duration until the required temperature sum was reached also explains the different onset of bud burst on the same experimental plots between years. Because of the high heritability of the trait bud burst mentioned above, a high stability of flushing behavior within populations between years was expected. Nevertheless, the correlation analysis revealed low to moderate positive correlation coefficients, which implies a comparatively high variability of bud burst for single trees between years.

This is most likely caused by observations of bud burst at different time points. Due to field conditions, it was not possible to observe bud burst after a fixed temperature sum and/or photoperiod every year. However, mean bud burst stages of the different populations were very stable, and thus, a classification in late and early flushing populations was possible. Although significantly different, the timing of leaf unfolding varied only in a timespan of a few days in this study. Nevertheless, as revealed by Kreyling et al. (2012), a late frost event damaging leaves immediately after leaf flushing can appear negligible a few days earlier or later. This emphasizes the importance of even slight variation in bud burst behavior among populations. In contrast, Vitasse et al. (2011) revealed that the extension of the growing season for oak is mainly caused by an earlier bud burst while the extension of the growing season for beech is mainly due to delayed leaf senescence in fall. Thus, oak might gain competitiveness towards beech, since photosynthetic capacities and day lengths are more beneficial in the beginning of the growing season (Morecroft et al. 2003, Vitasse et al. 2011). In this context, the identification of higher variation of bud burst among beech populations would be required to be able to plant early flushing populations strengthening the competitiveness of beech if necessary.

4.1.3 Drought Stress and Mortality

Drought stress naturally occurred in the experimental plot in Calvörde and led to damage on the translocated plants differently pronounced among populations. Different drought stress sensitivity among beech provenances was revealed by several studies (e.g., Czajkowski and Bolte 2006, Fotelli et al. 2009, Rose et al. 2009, Robson et al. 2012). Interestingly, the precipitation gradient of the sampling sites was not reflected by drought stress sensitivity among populations in this study. Thus, there might have been only minor adaptation to the precipitation regime at the sampling sites of the different populations. However, these results should be interpreted carefully for some reasons. In general, plant damage occurred only in a low number of plants (< 9 %), which reduced the reliability of the statistical tests. Although drought stress was not correlated with plant height, the below ground biomass has not been measured in the experiment. Thus, differences in drought stress sensitivity may be a result of different rooting depths. In addition, plant damage might also have been indirectly caused by drought. Stressed plants may have been more susceptible to damage by, e.g., insects. Under field conditions it can be difficult to reliable differentiate between different causes of

damage, because of the interaction between several environmental factors. This may also explain that the mortality rate was not increased due to drought stress for the populations from moister environments on the dry plot in Calvörde in contrast to expectations. In 2013, the highest mortality rate was observed for the populations Göhrde loam and Unterlüß sand, whereas the population from the moistest environment "Harz" showed statistically the same mortality rate as populations from dry environments. However, a direct or indirect role of drought stress on the survival of the plants is very likely, since almost all seedlings survived until the end of observations in the experimental plot in the wet reference area in the Harz Mountains. The effect of drought was intensified since half of the plants on the experimental plot in Calvörde were exposed to artificial drought stress (because of absence of natural drought) in late summer 2012. This experiment had to be cancelled, because of a huge infestation by insects. The damage by insects was not distinguishable from damage by drought. The artificial drought stress might have led to an increased mortality rate on the experimental plot in Calvörde. However, different mortality rates have already been observed between the two plots under natural conditions in the years before.

4.1.4 Methodical Aspects

Some aspects of field experiments should be considered carefully before conclusions are drawn for natural populations. Thus, seedlings are normally grown under optimal conditions in a greenhouse before planted in the field experiment. This circumvents natural selection during seed germination, establishment and early growth (Aitken et al. 2008). Further, competing vegetation is often controlled, seedlings are planted at a wide spacing, and trials are often fenced against large herbivores (Aitken et al. 2008).

Additionally, there are practical limitations using field experiments. For instance, it was not possible to conduct height measurements as precisely as under controlled conditions in this study. Different planting depths of the seedlings might have caused variations. Nevertheless, these inaccuracies should be negligible because of the block design of the translocation experiment and the high number of plants.

The translocation experiment was not completely reciprocal due to an insufficient number of established seedlings in the greenhouse for some populations. Thus, a comparison of phenotypic characteristics between the two experimental plots in Calvörde and the Harz Moun-

tains was not possible for each population. Nevertheless, populations existent in both plots represented the entire precipitation gradient (low precipitation: Calvörde loam, moderate precipitation: Göhrde sand, high precipitation: Harz).

In comparison to experiments under controlled environmental conditions, field experiments provide growing conditions for plants that are simultaneously more variable, more resource rich and more stressful (Leakey et al. 2009). Thus, the investigation of climate change relevant traits is more realistic using field experiments than climate chamber experiments. Combined stresses can influence survival of trees even more than chronic exposure to a single predictable stress such as drought (Niinemets 2010).

For practical reasons it is often necessary to investigate seedlings instead of adult plants of forest tree species, but extrapolations from experiments with seedlings to adult trees should be made carefully, due to different responses of the age stages. Thus, it is known that stress resistance increases with ontogeny (Niinemets 2010), and the increment of beech differs between juvenile age stages (Liesebach 2012). Furthermore, juvenile trees flush earlier compared to adult trees (Augspurger and Bartlett 2003), which is a result of ontogenic changes but not of the vertical thermal profile that exists within forests (Vitasse 2013). Nevertheless, the juvenile stage is of high importance for the natural regeneration of forest stands, and high selective pressure of single extreme events (e.g., frost or drought) can determine the genetic composition of future stands (Kreyling et al. 2012). Thus, knowledge about adaptation potential of different seedling populations is of great importance.

4.2 Genetic Data

4.2.1 Ascertainment Bias

Ascertainment bias is the systematic deviation from the expected allele frequency distribution resulting from sampling processes used to find marker loci (Helyar et al. 2011). Typically SNPs are identified in a small panel of individuals from a part of the species' range (ascertainment set). In this case, SNPs with low allele frequencies might not be detected. If a large set of individuals is genotyped with these SNPs, an ascertainment bias can occur affecting any statistical measure that relies on allele frequency (Nielsen 2000, Nielsen et al. 2004, Helyar et al. 2011). To avoid ascertainment bias, a relatively large sample of individuals for SNP detection should be chosen, which represents all populations included in the final genotyp-

ing (Morin et al. 2004). For that reason, comparative sequencing was carried out in the present study in close cooperation with the project BEECHADAPT which is part of the research network biodiversity-exploratories (http://www.biodiversitäts-exploratorien.de). This facilitated the inclusion of individuals of six additional beech populations from distinct regions in Germany in the ascertainment set to minimize the bias.

4.2.2 Linkage Disequilibrium

Linkage disequilibrium is the non-random association of alleles at different loci (Flint-Garcia et al. 2003). LD was observed with both microsatellites and SNPs in the present study, whereas more LD was detected with SNPs than with SSRs (among all juvenile populations: SNPs: 18.45 %; SSRs: 11.22 %). Different patterns of LD are expected between the two types of markers due to the different number of alleles and different mutations rates (Johansson et al. 2005, Lee et al. 2007), and usually, more LD is detected with SSRs compared to SNPs (Chapman and Wijsman 1998). A higher amount of LD observed for SNPs can be expected in the present study, since several SNPs occur in same genes (in contrast to the SSRs), and a rapid decay of LD was often reported for forest trees (Neale 2007, González-Martínez et al. 2006). Higher levels of LD were detected in the adult populations than in the juvenile populations using microsatellite markers. This might be explained by the lower frequency of rare alleles identified in the adult populations than in the juvenile populations (data not shown). However, LD between both SSRs and SNPs (pair-wise R^2) was low, and is expected for a highly outcrossing, wind-pollinated species as *Fagus sylvatica* (Jump et al. 2006). In contrast, Lalagüe et al. (2013) found comparatively high levels of LD in a beech population in Southern France, which was suggested to be an effect of relatively small effective population size. In general, the pair-wise R^2 values should be interpreted cautiously in the present study, since they were not calculated using phased data as actually required for the software.

4.2.3 Genetic Diversity

As hypothesized, the neutral genetic diversity estimated by microsatellite markers was high for all analyzed populations and showed no statistically significant differences between adult and seedling populations (mean H_e: 0.619). Other studies revealed even higher mean diversity values for beech also using microsatellites (Vornam et al. 2004 H_e: 0.765; Oddou-Muratorio et al. 2011 H_e: 0.72; Bilela et al. 2012 H_e: 0.777). However, different loci have

been used in these studies, and estimates of genetic diversity strongly depend on the applied loci. One study (K.C. 2011) applied the same markers as in the present study and revealed comparable results for different beech stands in Germany (mean H_e: 0.622). Although, null alleles were found in the present study, results are most likely not affected, since they occurred in only low frequencies. This assumption is well supported by low fixation indices (F) close to zero. No statistically significant differences were found for the molecular diversity indices among adult and seedling populations, suggesting a sufficient sampling in this study. Further, the treatment of the seedlings in the greenhouse had no influence on their genetic structure.

High genetic diversity was also detected using SNP markers (mean H_e: 0.271). The estimated genetic diversity is lower compared to microsatellites, since SNPs are less polymorphic than microsatellite markers. Seifert (2012) reported a slightly higher genetic diversity for the same populations (mean H_e: 0.327). However, different SNPs derived from different genes were applied, which may explain these differences. Interestingly, a higher expected heterozygosity was observed for late flushing individuals compared to early flushing ones for all analyzed years. This trend was stronger using only potentially adaptive SNP markers revealed by the association and/or outlier analysis in this study. The same tendency was also detected for the observed heterozygosity except for the pooled individuals of the year 2013. This pattern may be a result of heterzygote advantage (balancing selection) in late flushing individuals or directional selection in early flushing ones. Nevertheless, an increase of heterozygots in late flushing individuals was not observed in each of the potentially adaptive SNP markers, and thus no general conclusion can be drawn.

In general, high levels of genetic diversity within populations are expected for outcrossing, wind-pollinated (tree) species (Hamrick et al. 1992, Hamrick and Godt 1996, Petit and Hampe 2006). Such a high genetic diversity is a good basis for adaptation. Beech forests with a wide genetic basis are more likely to be able to cope with warmer and drier conditions, and thus, to adapt successfully under new environmental conditions (Bilela et al 2012). However, the pressure for adaptation will be intense in face of rapid climate change, and genetic variation within populations might only facilitate a short-term adaptation making gene flow necessary from better adapted populations (Jump and Peñuelas 2005, Jump et al. 2006). Thereby, habitat fragmentation can hinder this process. Nevertheless, Pluess and Weber (2012)

found genetic differentiation in relation to water availability in neighboring stands, which were genetically well connected, and also, this study revealed small-scale differences in populations for climate change relevant traits. Thus, a dispersal across large distances is not needed for the spread of pre-adapted genes in beech (Pluess and Weber 2012).

4.2.4 Nucleotide Diversity

The mean nucleotide diversity was 0.0027 in the present study and very similar to the investigations of Seifert et al. (2012) and Lalagüe et al. (2013) despite analyzed in different genes. Other studies revealed higher values for different species in most cases (e.g., Ingvarsson 2005 (*Populus tremula*) π: 0.0111; Krutovsky and Neale 2005 (*Pseudotsuga menziesii*) π: 0.00655; Heuertz et al. 2006 (*Picea abies*) π: 0.00208; Vornam et al. 2007 (*Quercus petraea*) π: 0.00542, (*Pinus pinaster*) π: 0.00351). The results of the present study might be comparatively conservative, since all SNPs occurring only in one individual were excluded from analysis (to avoid an analysis of false SNPs), and only a limited number of trees and clones per tree (*Escherichia coli* transformants) were used for comparative sequencing. In addition, the nucleotide diversity strongly depends on the investigated genes ranging from 0.00057 to 0.00458 in this study, and thus, a comparison between different studies may be complicated. Tajima's *D* test was applied to test the sequence data for selective neutrality. The test was statistically significant for only one gene (*His3C1*) in the analysis. The positive value of Tajima's *D* obtained for that gene indicates balancing selection, but the parameter is known to be highly sensitive to sample size (Larsson et al. 2013). Since the estimations of Tajima's *D* were based on a low number of individuals in this study, the results should be interpreted cautiously.

4.2.5 Genetic Differentiation

All applied methods revealed low genetic differentiation between the investigated beech populations. Nei's genetic distances (Nei 1972) were low among adult stands (mean 0.041), juvenile stands (mean 0.031) as well as among adult and juvenile populations (mean 0.032). Accordingly, the STRUCTURE analysis revealed almost no differentiation between the different populations, either using microsatellite or SNP markers. Additionally, the AMOVA revealed that 97 % of the variation is found within populations and only 3 % between them. The results were expected according to several other studies of beech populations in Central

Europe using different genetic markers (e.g., Demesure et al. 1996, Konnert et al. 2000, Sander et al. 2001, Gailing and Wuehlisch 2004, Magri et al. 2006). Low genetic differentiation between stands from different regions was confirmed by low bootstrap values for the clustering shown in the dendrogram (Figure 11). Furthermore, the grouping of the different beech stands in the dendrogram did not reflect the geographic distances between them. The divergence between geographic and genetic distance was also found by Konnert et al. (2000). K.C. (2011) was able to reliably differentiate between regions of *F. sylvatica* in Germany, but the regions, however, were separated by around 300 km, which is much more than in the present study. Nevertheless, all juvenile populations grouped to the corresponding source stands in the UPGMA dendrogram supported by high bootstrap values, again validating the sampling design of this study.

In the STRUCTURE software, Hardy-Weinberg equilibrium within populations and linkage equilibrium between loci within populations is required (Pritchard et al. 2000). For the SNP data, the former requirement was met in this study, while the second one was not totally fulfilled. However, the linkage disequilibrium among SNPs was only weak and mainly within different genes assuming a reasonable performance of the modelling approach (Pritchard et al. 2007). Almost no difference to the STRUCTURE results was observed by SSRs with one population deviating from Hardy-Weinberg proportions. For most data (SSR as well as SNP data), the STRUCTURE analysis revealed an optimal value of $K = 2$ using the ΔK method by Evanno et al (2005). Nevertheless, concerning Eckert et al. (2010) the use of ΔK to choose an optimal value of $K = 2$ is difficult, since ΔK in this case compares the lack of structure ($K = 1$) to some structure ($K = 2$ or more). A higher value ($K = 3$) was only revealed by applying the "total" and "silent" SNP sets. Since almost no differences were observed between the clustering of the individuals under these and the remaining SNP sets (non-coding and non-synonymous SNPs) as well as the SSR data, this might be an artifact. Additionally, the result of low population structure is supported by the low observed genetic distances among populations discussed above. Thus, the choice of $K = 2$ seems to be reasonable.

4.2.6 Association and Outlier Analysis

Several SNPs significantly associated with bud burst were identified in this study. Thereby, most significant associations were detected based on separate analysis of each of the populations. However, the repeatability of the associations was very low between the different

years. Also, there was low accordance of significant associated SNPs with bud burst between populations. These results are most likely an effect of low sample size. Concerning Long and Langley (1999), association studies have a low repeatability unless sample sizes are in the order of 500 individuals. In the present study, the sample size is around 230 individuals per population, and thus, it is most likely too low for reliable associations. Individuals from the different populations were therefore pooled to increase sample size resulting in association populations with sample sizes of 869 to 1,202 individuals. Association analyses still revealed several significantly associated SNPs with bud burst, and the repeatability was clearly increased as many SNPs were associated with bud burst in more than one year. Two SNPs were significant in all three analyzed years. The "general linear model" (GLM) and the "mixed linear model" (MLM) revealed almost the same results. Only four SNPs were exclusively significant under the GLM, whereas the remaining 19 significant SNPs were revealed by both models. The accordance between the two models increases the reliability of the results. The inclusion of population structure did not affect the results of the association analyses in most cases. In general, population structure can cause spurious associations (Lander and Schork 1994). For instance, the inclusion of population structure in the association analysis resulted in a much lower number of significant associations and also lower R^2 values in the study of Vidalis et al. (2013). However, only weak population structure was detected in the present study, which explains the results. The phenotypic variation explained by significantly associated SNPs with bud burst was low ($R^2 < 2.2$ %). Comparable R^2 values (between 1.5 % and 5 %) were also revealed in other studies for different traits and tree species (e.g., González-Martínez et al. 2007, Ingvarsson et al. 2008, Eckert et al. 2009b, Vidalis et al. 2013). In general, complex traits in trees are controlled by many genes, whereas the individual effects of these genes on the phenotype are small (Neale and Kremer 2011).

F_{ST} outlier analyses were also conducted in addition to the association analyses. Compared with the association analyses, these analyses revealed a lower number of significant SNPs, whereas the majority was found to be under balancing selection. In total, seven different outlier SNPs were identified, whereas the SNPs "ConsC2_98", "His3C2_104" and "Frigida_54" occurred frequently. Four out of the seven outlier SNPs were also significant in the association analysis (without single population based association tests) including the two SNPs "His3C2_104" and "CysPro_728", which were associated with bud burst in all three years under the GLM and MLM in the association analysis. The four SNPs (ConsC2_98,

His3C2_104, CP10_503, CysPro_728) revealed by both the outlier and association analysis might be those with the highest probability of being involved in the manifestation of bud burst in this study. However, several potential adaptive SNPs identified in this study are non-coding or synonymous SNPs. Thus, they are not thought to be the "true" adaptive SNPs, but rather linked to them. Nevertheless, LD was found to be low in this study and it was strongest within genes. The "true" causative SNPs may therefore be in close vicinity. Potentially adaptive SNPs were distributed over all analyzed candidate genes (except "*Asi*", since no SNP in this gene could be used for analysis). Most of them are putatively involved in flowering, temperature response and stress response. These functions have been associated with bud burst before. Thus, the pathway regulating bud development may be common to vegetative and sexual buds (Horvath 2009, Alberto et al. 2013). Additionally, several stress related genes were expressed during bud burst in Norway spruce, suggesting that trees need to protect themselves from unfavorable abiotic factors during bud development (Yakovlev et al. 2006). In the same study, genes associated with temperature were expressed, which is expected, since temperature plays in important role in spring phenology. Alberto et al. (2013) found a *CONSTANS* and *Auxin induced protein* gene associated with bud burst in oak. Interestingly, SNPs out of the *Constans like* and *Auxin repsonse factor* genes were also significantly associated with bud burst in the present study. Since beech and oak are related tree species, this accordance increases the reliability of the results.

In the present study, an association analysis was also conducted between SNPs and height, which was observed in the translocation experiment. In total, two non-synonymous SNPs (CP10_1428 and PP2C_315) were significantly associated with this trait. Since, no explicit candidate genes for plant growth were analyzed, this result is surprising. As phenotypic traits are controlled by many genes with small effects each, this outcome is reasonable. However, Scotti-Saintagne et al. (2004) found that the trait „height" depends on fewer QTLs with moderate to strong effects compared to the trait "bud burst". Thus, it might be less likely to analyze a gene involved in plant height just by chance. Nevertheless, Scotti-Saintagne et al. (2004) found also that some QTLs of bud burst and height growth are located in the same regions explaining the association of SNPs in candidate genes for bud burst with plant height in this study.

4.3 Conclusions and Outlook

The present study provided insights into the genetic adaptation potential of European beech to global change as well as to the genetic basis of climate change relevant phenotypic traits.

As hypothesized, the different beech populations in the translocation experiment showed variation in all investigated phenotypic traits, albeit the observed variations were partly different from expectations. Especially, the good performance of the population Harz from the moistest environment was surprising in the dry experimental plot in Calvörde. Also, the distinct differences in almost all phenotypic traits were unexpected for the neighboring populations Göhrde sand and Göhrde loam. These are interesting results concerning the regeneration of beech stands. Populations with higher geographic distances can show more similar phenotypes than neighboring stands. Thus, the origin of seed material for planting should be chosen carefully. However, the results especially for drought stress sensitivity should be confirmed in further experiments. Additionally, a long-term observation of the translocation experiment would provide insights in the further development of the plants.

The analysis of neutral genetic variation revealed high genetic diversity for all populations. In general, this is a good basis for an adaptation to changing environmental conditions due to global warming. Nevertheless, high genetic variation within populations might only facilitate a short-term adaptation to climate change (Jump and Peñuelas 2005). Thus, it is important to gain insights in the genetic basis of climate change relevant traits. In this study, candidate genes for the trait “bud burst” were analyzed and both bottom-up and top-down approaches (see chapter 1.2 for explanation) were successfully used to identify potentially adaptive markers. These SNPs have to be confirmed in further experiments with additional populations. Since different genes and SNPs could be involved in the manifestation of a given trait in different ages (Prunier et al. 2013), mature populations should also be included in those surveys.

In this study, a candidate gene approach was used to investigate adaptive genetic variation in beech, although the emerging NGS technique facilitates to cover whole genomes with genetic markers. For instance, genome-wide association studies (GWAS) attempt to genotype enough markers across the genome so that functional alleles will likely be in LD with a least one of the genotyped markers (Myles et al. 2009). Nevertheless, there are advantages

and disadvantages for both candidate gene and genome-wide association studies. Candidate gene studies tend to have a rather high statistical power, but may miss important genes. GWAS can identify relevant genes regardless of whether their function was known before, but have lower statistical power (Amos et al. 2011, Yoo et al. 2010). However, a high marker density would be needed for GWAS in forest tree species due to rapid decay of LD. Further, no reference genomes are available for most forest tree species. Thus, the candidate gene approach might be the best alternative to study adaptation in forest trees. This situation will persist until reference genome sequences are available and very high density SNP genotyping or full-genome resequencing becomes cost-effective (Neale and Kremer 2011).

5 Summary

Climate change models predict higher annual mean temperatures as well as a decrease of precipitation during summer months for Germany. Possible consequences for trees are a prolonged growing season, a higher risk of late frost events and higher drought stress during summer. These changing environmental conditions may lead to shifts in tree species competition. European beech (*Fagus sylvatica* L.) is one of the most important deciduous tree species in Central Europe. Thus, the genetic adaptation potential of this species to climate change is of great interest.

Both the neutral and adaptive genetic variation of beech were investigated in this study. A translocation experiment was established with progenies of beech populations growing under different environmental conditions in Northern Germany. Repeated observations of important phenotypic traits (height, bud burst, drought stress sensitivity, mortality) revealed significant differences among populations. Interestingly, populations with a greater geographic distance partly showed more similar phenotypes than neighboring stands.

The neutral genetic variation of the investigated seedling populations was analyzed with nine different microsatellite markers. Only low genetic differentiation was detected among the investigated beech populations. The genetic diversity was high for all populations and statistically not different from the adult stands of origin (mean H_e: 0.619). The high genetic diversity is a good basis for adaptation, albeit it may only facilitate a short-term adaptation to climate change. Therefore, it is important to gain insights into the genetic basis of climate change relevant traits. Thus, bud burst-related candidate genes were investigated in the present study, whereas fragments of ten different candidate genes were analyzed with a total length of 12,290 bp. From the total length of the analyzed fragments, 7,586 bp accounted for exons, 3,461 bp for introns and 1,243 bp for untranslated regions (UTR). In total, 20 indels and 116 SNPs were identified. The mean nucleotide diversity was 0.0027, but substantially varied between the analyzed gene fragments. The nucleotide diversity is lower in comparison with other forest tree species, but in the same range as in other studies with *F. sylvatica*.

In total, 46 SNPs were successfully used for genotyping of more than 1,400 individuals out of the translocation experiment, which were selected based on their bud burst timing (“early”

and “late” flushing individuals). Association analyses were conducted to identify potentially adaptive SNP markers. Association analyses based on single populations revealed several significantly associated SNPs with the trait “bud burst” in different years. Nevertheless, the repeatability among years was low, which was most likely an effect of low sample size. Thus, association analyses were also conducted using pooled individuals from the different populations to increase sample size. These revealed 23 significantly associated SNPs with bud burst under a “general linear model”. An additionally applied “mixed linear model” revealed similar results. The phenotypic variation explained by the significantly associated SNPs with bud burst was low (R^2 < 2.2 %), but in accordance with other studies in forest tree species. In addition to the association analyses, F_{ST} outlier analyses were conducted revealing seven different SNPs, which are potentially under balancing or directional selection. In total, four potentially adaptive SNPs were simultaneously revealed by both outlier and association analyses. These might have the highest probability of being involved in the manifestation of bud burst behavior. However, several potential adaptive SNPs identified in this study are non-coding or synonymous SNPs, and thus, they are not thought to be the causative SNPs, but rather linked to them. Nevertheless, linkage disequilibrium was found to be low in this study suggesting that the causative SNPs might be in close vicinity. The potentially adaptive SNPs identified in this study, should be confirmed in further experiments with additional populations.

The emerging next-generation sequencing techniques facilitate to cover whole genomes with genetic markers and to conduct genome-wide association studies. Nevertheless, these techniques are still cost-intensive and due to the rapid decay of linkage disequilibrium in forest trees a high density of markers would be required. Further, no reference genomes are available for most forest tree species (including beech). Thus, the analysis of promising candidate genes for the trait of interest might be the best alternative to study adaptation in forest tree species.

6 Zusammenfassung

Klimawandelmodelle sagen für Deutschland sowohl höhere Jahresdurchschnittstemperaturen als auch eine Abnahme von Niederschlägen in den Sommermonaten voraus. Mögliche Konsequenzen für Bäume sind eine verlängerte Vegetationsperiode, ein erhöhtes Spätfrostrisiko und mehr Trockenstress während des Sommers. Diese veränderten Umweltbedingungen könnten zu Veränderungen der Konkurrenzverhältnisse zwischen Baumarten führen. Die Rotbuche (*Fagus sylvatica* L.) ist eine der wichtigsten Laubbaumarten Mitteleuropas. Daher ist das genetische Anpassungspotential dieser Baumart an den Klimawandel von großem Interesse.

In dieser Studie wurden sowohl die neutrale als auch die adaptive genetische Variation der Buche untersucht. Dafür wurde ein Translokationsexperiment mit Nachkommen von Buchenpopulationen, die unter verschiedenen Umweltbedingungen in Norddeutschland wachsen, etabliert. Wiederholte Aufnahmen wichtiger phänotypischer Merkmale (Höhe, Austrieb, Trockenstresssensitivität, Sterblichkeit) zeigten signifikante Unterschiede zwischen den Populationen. Interessanterweise zeigten Populationen mit einer größeren geographischen Distanz teilweise ähnlichere Phänotypen als benachbarte Populationen.

Die neutrale genetische Variation der untersuchten Sämlingspopulationen wurde anhand neun verschiedener Mikrosatellitenmarker analysiert. Zwischen den analysierten Buchenpopulationen wurde nur eine geringe genetische Differenzierung ermittelt. Die genetische Diversität war hoch und statistisch nicht signifikant unterschiedlich von den Altbeständen, aus denen sie stammten (durchschnittliche H_e: 0,619). Die hohe genetische Diversität ist eine gute Basis für Adaption, allerdings könnte sie wahrscheinlich nur eine kurzfristige Anpassung an den Klimawandel ermöglichen. Daher ist es wichtig, Einblicke in die genetische Basis von klimawandelrelevanten Merkmalen zu gewinnen. Deshalb wurden in dieser Studie Kandidatengene für das Austriebsverhalten untersucht, wobei Fragmente von zehn verschiedenen Kandidatengenen mit einer Gesamtlänge von 12.290 bp analysiert wurden. Von der Gesamtlänge der analysierten Fragmente entfielen 7.586 bp auf Exons, 3.461 bp auf Introns und 1.243 bp auf untranslatierte Bereiche (UTR). Insgesamt wurden 20 Indels und 116 SNPs identifiziert. Die durchschnittliche Nukleotiddiversität betrug 0,0027, variierte jedoch beträchtlich zwischen den untersuchten Genfragmenten. Im Vergleich zu anderen Waldbaumarten

ist die Nukleotiddiversität geringer, aber im selben Bereich wie bei anderen Studien mit *F. sylvatica*.

Insgesamt wurden 46 SNPs erfolgreich zur Genotypisierung von über 1.400 Individuen aus dem Translokationsexperiment, die aufgrund ihres Austriebsverhaltens ausgewählt wurden („früh"- und „spätaustreibende" Individuen), verwendet. Assoziationsanalysen wurden durchgeführt, um potentiell adaptive SNP-Marker zu identifizieren. Assoziationsanalysen, die auf Einzelpopulationen basierten, ergaben zahlreiche, in verschiedenen Jahren signifikant mit dem Merkmal „Austrieb" assoziierte SNPs. Jedoch war die Wiederholbarkeit zwischen den Jahren gering, was wahrscheinlich ein Effekt der geringen Stichprobengröße war. Daher wurden außerdem Assoziationsanalysen mit „gepoolten" Individuen aus den verschiedenen Einzelpopulationen durchgeführt, um den Stichprobenumfang zu erhöhen. Diese ergaben unter einem „generalisierten linearen Modell" 23 signifikant mit dem Austrieb assoziierte SNPs. Ein zusätzlich verwendetes „gemischtes lineares Modell" ergab nahezu gleiche Ergebnisse. Die phänotypische Variation, die durch signifikant mit dem Austrieb assoziierte SNPs erklärt wird, war niedrig ($R^2 < 2{,}2$), aber in Übereinstimmung mit anderen Studien mit Waldbaumarten. Zusätzlich zu den Assoziationsanalysen wurden auch F_{ST}-Outlier-Analysen durchgeführt. Diese ergaben sieben verschiedene SNPs, die potentiell unter ausgleichender oder gerichteter Selektion stehen. Insgesamt wurden vier potentiell adaptive SNPs gleichzeitig durch Assoziations- und Outlier-Analysen identifiziert. Diese könnten die höchste Wahrscheinlichkeit aufweisen, an der Ausprägung des Austriebsverhaltens beteiligt zu sein. Allerdings sind viele potentiell adaptive SNPs, die in dieser Studie identifiziert wurden, nichtkodierend oder synonym und somit nicht die kausativen SNPs, sondern eher gelinkt mit ihnen. Allerdings wurde in dieser Studie ein geringes Kopplungsungleichgewicht (linkage disequilibrium) gefunden. Somit könnten die kausativen SNPs in naher Umgebung liegen. Die in dieser Studie identifizierten potentiell adaptiven SNPs sollten in weiteren Studien mit zusätzlichen Populationen bestätigt werden.

Die neu aufkommenden Methoden der Hochdurchsatzsequenzierung (next-generation sequencing) ermöglichen es, ganze Genome mit genetischen Markern abzudecken und genomweite Assoziationsstudien durchzuführen. Allerdings sind diese Techniken noch immer kostenintensiv und durch die rasche Abnahme des Kopplungsungleichgewichtes in Waldbaumarten wäre eine hohe Markerdichte erforderlich. Außerdem sind für die meisten Wald-

baumarten (inklusive Rotbuche) keine Referenzgenome vorhanden. Somit ist die Analyse vielversprechender Kandidatengene für das interessierende Merkmal die wahrscheinlich beste Alternative, um Anpassung in Waldbaumarten zu untersuchen.

7 References

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8 Appendix

Appendix 1

Characterization of all SNPs and indels identified in the analyzed genes. Bold: SNPs genotyped by KBiosciences UK Ltd (KASP™ Genotyping Assay; Hoddesdon, UK), * SNPs analyzed with ABI PRISM® SnaPshot™ Multiplex Kit (Applied Biosystems, Foster City, USA).

SNP/ indel no.	Gene	Position (bp)	Characteristic	Substitution/Indel sequence	Amino acid change
1*	*Auxin response factor (ARF)*	239	non-coding	A/G	
2*		277	non-coding	A/G	
3		537	non-coding	A/G	
4		547	non-coding	T/C	
5		565	non-coding	A/G	
6		576	non-coding	T/C	
7		589	non-coding	A/G	
8		619	non-coding	A/G	
9		736	non-coding	A/T	
10*		771	non-coding	A/C	
11		803	non-coding	A/G	
12		807	non-coding	A/G	
13*		852	non-coding	A/G	
14	*alpha Amyl-ase/subtilisin in-hibitor (ASI)*	84	non-synonymous	T/C	leucine/ serine
15		313	synonymous	T/C	
16		325	synonymous	A/G	
17		363	non-synonymous	A/G	aspartic acid/ glycine
18		467	non-synonymous	A/C	arginine/ serine
19		473	non-synonymous	T/G	alanine/ serine
20		646	3'UTR	T/C	
21		656-663	3'UTR	deletion AA; insertion: TTGT-CAAC	
22		707	3'UTR	A/T	
23		787	3'UTR	A/G	
24		788	3'UTR	A/G	
25	*Constans like (1)*	268	non-synonymous	A/T	phenylalanine/tyrosine
26		281	synonymous	A/G	
27		390	non-synonymous	A/G	threonine/ alanine

28		6	5'UTR	A	
29		26	5'UTR	A/G	
30		73	non-synonymous	C/G	glycine/ alanine
31		122	synonymous	T/G	
32		126	non-synonymous	C/G	valine/ leucine
33	*Constans like (2)*	186	non-synonymous	T/G	alanine/ serine
34		365	synonymous	T/C	
35		452	non-synonymous	C/G	glutamic acid/ aspartic acid
36		463	non-synonymous	T/C	valine/ alanine
37		27	synonymous	A/C	
38		39	synonymous	T/C	
39		41	non-synonymous	T/C	threonine/ isoleucine
40		68	non-coding	A	
41		78	non-coding	T/C	
42		116	non-coding	T/C	
43		275	non-coding	A/T	
44		319	non-coding	T/G	
45		351	non-coding	T/G	
46	*Chloroplast chaperonin like (CP10 like)*	354	non-coding	T	
47		416	non-coding	C/G	
48		477	synonymous	C/G	
49		659	non-coding	C/G	
50		723	synonymous	C/G	
51		772	synonymous	T/C	
52		876	non-coding	T/C	
53		909	non-coding	T/C	
54		978	non-coding	A/T	
55		1291	non-coding	A/G	
56		1402	non-synonymous	T/C	proline/ leucine
57		1499	synonymous	T/C	
58		118	synonymous	C/G	
59		202	synonymous	A/G	
60		292	non-coding	T/G	
61		372	non-coding	T/C	
62	*Cysteine proteinase*	408	non-coding	A/T	
63		728	3'UTR	C/G	
64		750	3'UTR	G	
65		783	3'UTR	T/G	
66		830	3'UTR	A/C	

67		833	3'UTR	A/G	
68		841	3'UTR	A/G	
69		887	3'UTR	A/C	
70		890	3'UTR	T/G	
71		58	5'UTR	A/G	
72		266	non-coding	A/T	
73		272	non-coding	A	
74		284-291	non-coding	TTCAA	
75		345	non-coding	A/G	
76		350	non-coding	A/C	
77		361	non-coding	C/G	
78		551	non-coding	A/G	
79		623	non-coding	A/T	
80		632	non-coding	A/G	
81	*Dof zinc finger protein (DAG)*	660-665	non-coding	microsatellite motif: GTA, three different alleles	
82		700	non-coding	A	
83		716	non-coding	A/G	
84		718	non-coding	A/T	
85		719	non-coding	A/T	
86		764	non-coding	T	
87		792	non-coding	T/G	
88		811	synonymous	T/C	
89		1036	synonymous	T/G	
90		1171	synonymous	T/C	
91		34	synonymous	T/C	
92		84	non-synonymous	A/G	serine/asparagine
93		159	non-synonymous	A/G	serine/asparagine
94		197	synonymous	T/C	
95	*Frigida*	239-244	coding	microsatellite motif: GAA, three different alleles	glutamate
96		343	synonymous	C/G	
97		370	synonymous	A/G	
98		430	synonymous	T/C	
99		128-129	non-coding	TG	
100		230	synonymous	T/C	
101		292	non-coding	T/C	
102	*Histone 3 (1)*	387	synonymous	T/C	
103		434	non-coding	A/G	
104		457	non-coding	G	
105		866	3'UTR	A/G	
106	*Histone 3 (2)*	20	5'UTR	T/G	
107		24	5'UTR	T/C	

108		104	synonymous	A/C	
109		155	non-coding	T/C	
110		186	non-coding	T/C	
111		188	non-coding	A/G	
112		260	synonymous	A/G	
113		301	non-coding	A/C	
114		334	non-coding	G	
115		520	non-coding	A/C	
116		533	non-coding	T/C	
117		557	non-coding	T/C	
118		*566*	*non-coding*	*T*	
119		88	synonymous	A/T	
120		259-260	non-coding	microsatellite motif: T, three different alleles	
121		553	non-coding	A/T	
122		*619*	*non-coding*	*T*	
123		834	non-synonymous	A/C	glutamate/aspartic acid
124	*NAC transcription factor*	942	synonymous	A/G	
125		985-987	coding	microsatellite motif: AAT, two different alleles	asparagine
126		1229	3'UTR	T	
127		1241-1245	3'UTR	complex indel consisting of A and T	
128		1274	3'UTR	A/G	
129		1280	3'UTR	A/G	
130*		220	non-synonymous	T/G	lysine/ asparagine
131		315	non-synonymous	C/G	alanine/glycine
132*	*Protein phospha-tase 2C (PP2C)*	391	synonymous	T/G	
133		538	non-coding	T/G	
134*		791	non-synonymous	A/G	asparagine/ aspartic acid
135*		941	non-coding	T/G	
136*		1200	synonymous	A/G	

Appendix 2

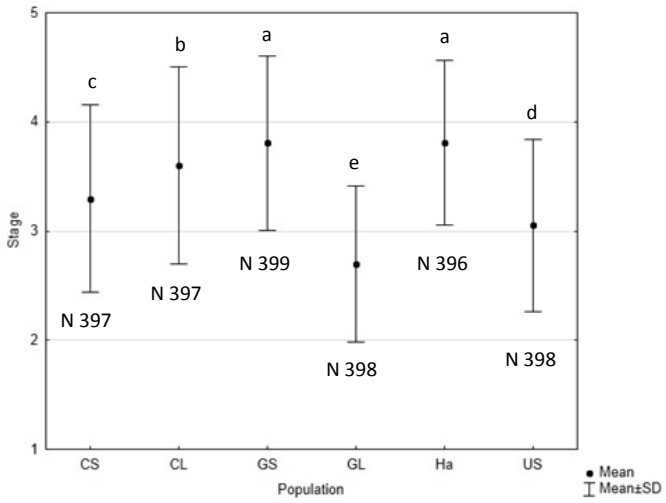
Primers used for the analysis with the ABI Prism® SnaPshot™ Mulitplex Kit (Applied Biosystems, Foster City, USA).

SNP	Gene	Primer sequence 5'-3'	Direction
Arf_797		T(14)ATATAGACCTCCATGACCACCA	reverse
Arf_878		T(19)CTATCCTGATGTCCACACTTAA	forward
Arf_265	*Auxin response factor*	T(26)GAAAGAATGCTGAAGGCAAC	reverse
Arf_303		T(29)CTTTCTTGTTTCTGATTTGACA	forward
Arf_615		T(33)GCTCTGAGATGCAAATGAATACT	reverse
PP2C_220		T(37)CGCTTCGTTCTTATTCGTCTTCTT	reverse
PP2C_791		T(44)CGAGAGTCACCGCAGTTAGAGA	reverse
PP2C_941	*Protein phosphatase 2C*	T(49)GTGCGAATGGTGCTGACGTGTT	forward
PP2C_391		T(54)AATTATTTGAAACCGAAGGGTG	reverse
PP2C_1200		T(60)CGTGATATCGGAACCGGAGGT	forward

Appendix 3

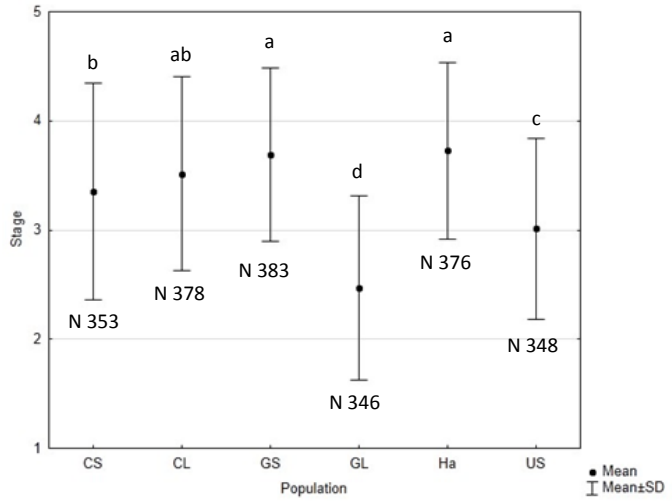
Appendix 3a

Mean bud burst stages of the different populations on the plot in Calvörde on day 112 of the year 2011. Different letters indicate significant differences among populations ($p < 0.05$), N: number of individuals.



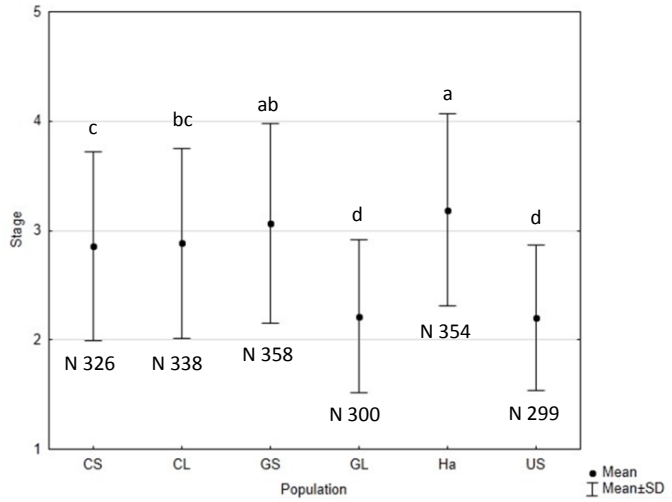
Appendix 3b

Mean bud burst stages of the different populations on the plot in Calvörde on day 116 of the year 2012. Different letters indicate significant differences among populations ($p < 0.001$), N: number of individuals.



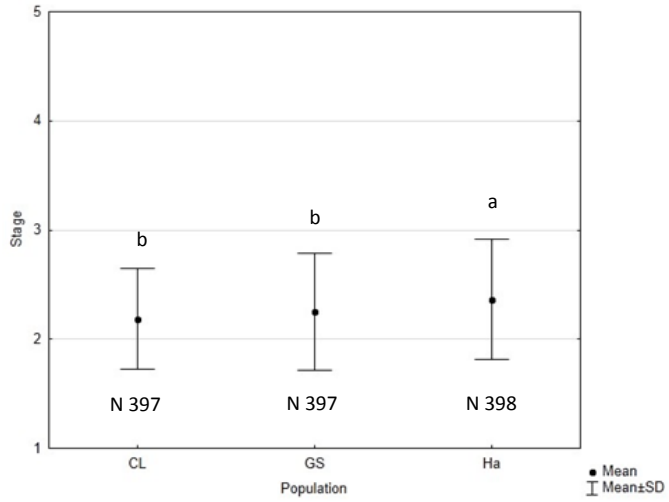
Appendix 3c

Mean bud burst stages of the different populations on the plot in Calvörde on day 115 of the year 2013. Different letters indicate significant differences among populations ($p < 0.05$), N: number of individuals.



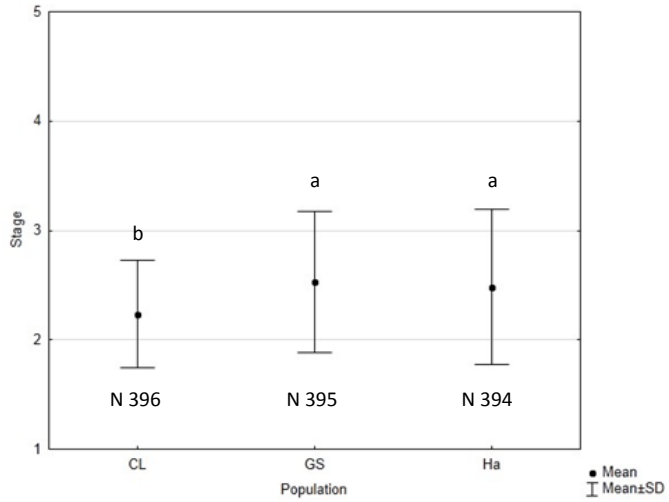
Appendix 3d

Mean bud burst stages of the different populations on the plot in the Harz Mountains on day 110 of the year 2011. Different letters indicate significant differences among populations ($p < 0.01$), N: number of individuals.



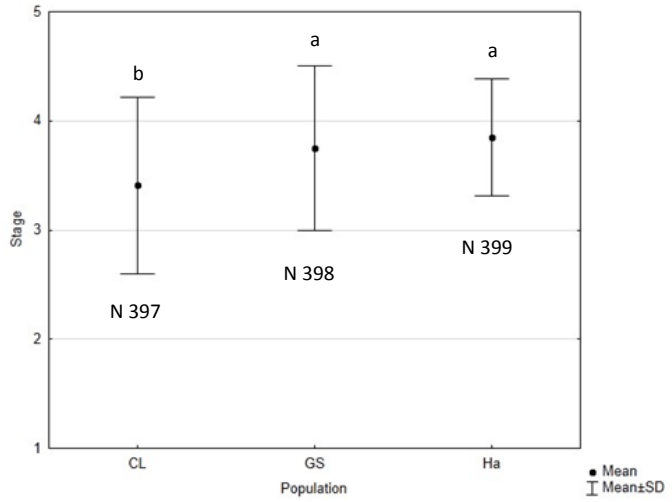
Appendix 3e

Mean bud burst stages of the different populations on the plot in the Harz Mountains on day 119 of the year 2012. Different letters indicate significant differences among populations ($p < 0.001$), N: number of individuals.



Appendix 3f

Mean bud burst stages of the different populations on the plot in the Harz Mountains on day 127 of the year 2013. Different letters indicate significant differences among populations ($p < 0.001$), N: number of individuals.



Appendix 4

Spearman's rank-order correlation coefficients for bud burst between years within populations. All correlations are statistically significant at $p < 0.05$.

Plot	Population	2011 vs. 2012	2011 vs. 2013	2012 vs. 2013
Calvörde	CL	0.428	0.333	0.483
	CS	0.506	0.442	0.586
	GL	0.369	0.275	0.397
	GS	0.477	0.408	0.388
	Ha	0.244	0.316	0.374
	US	0.335	0.266	0.291
Harz	CL	0.314	0.312	0.455
	GS	0.280	0.367	0.438
	Ha	0.194	0.269	0.319

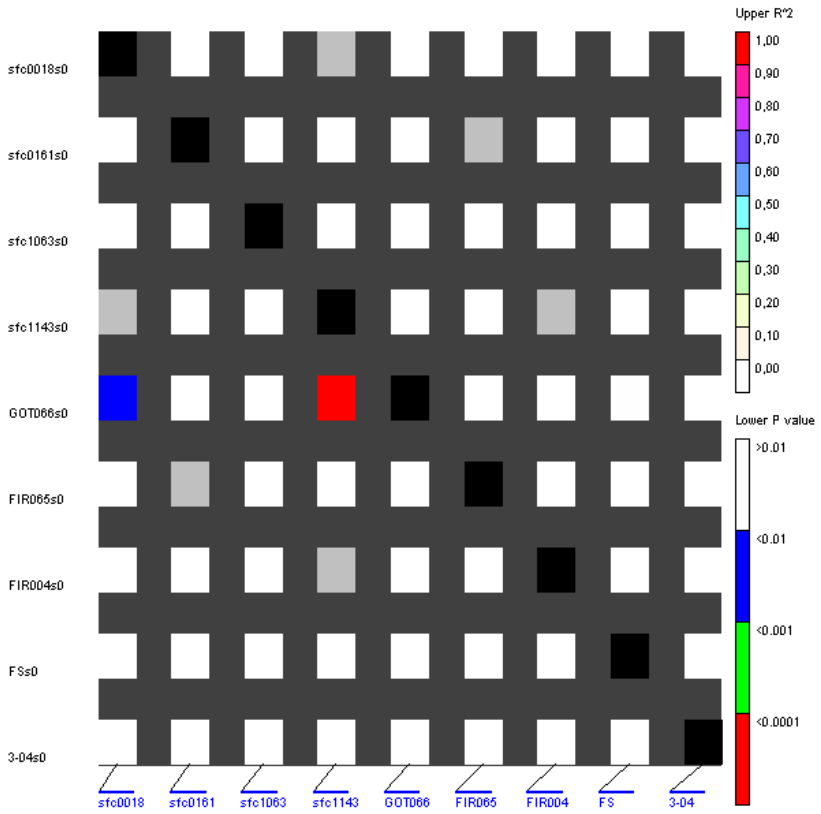
Appendix 5

Estimates for frequency of null alleles for the different microsatellite markers and populations.

Marker	Population												
	CL (juvenile)	CS (juvenile)	GL (juvenile)	GS (juvenile)	Ha (juvenile)	US (juvenile)	CL (adult)	CS (adult)	GS (adult)	UL (adult)	US (adult)	Ha (adult)	GL (adult)
sfc0018	0.0081	0.0340	0.0190	0.0000	0.0000	0.0107	0.0093	0.0024	0.0359	0.0000	0.0281	0.0234	0.0076
sfc0161	0.0023	0.0006	0.0000	0.0130	0.0178	0.0062	0.0612	0.0101	0.0253	0.0000	0.0000	0.0118	0.0000
sfc1063	0.0499	0.0313	0.0000	0.0030	0.0446	0.0000	0.0273	0.0141	0.0000	0.0249	0.0061	0.0000	0.0000
sfc1143	0.0000	0.0000	0.0157	0.0092	0.0000	0.0112	0.0000	0.0103	0.0000	0.0192	0.0000	0.0000	0.0000
GOT066	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0712	0.0000	0.0000	0.0297	0.0000
FIR065	0.1959	0.2395	0.2209	0.1596	0.1331	0.0782	0.1456	0.1722	0.1968	0.1166	0.1150	0.1859	0.2461
FIR004	0.0661	0.0299	0.0454	0.0895	0.1095	0.0797	0.0452	0.0378	0.0843	0.0987	0.0746	0.1047	0.0150
FS3-04	0.0000	0.0193	0.0222	0.0311	0.0000	0.0833	0.0108	0.0000	0.0504	0.0000	0.0907	0.0730	0.0472
mfs11	0.0174	0.0077	0.0068	0.0038	0.0132	0.0000	0.0000	0.0000	0.0045	0.0000	0.0000	0.0000	0.0000

Appendix 6

LD Plot of pair-wise R^2 values (upper diagonal) between all SSR loci with corresponding p-values (lower diagonal).



Appendix 7

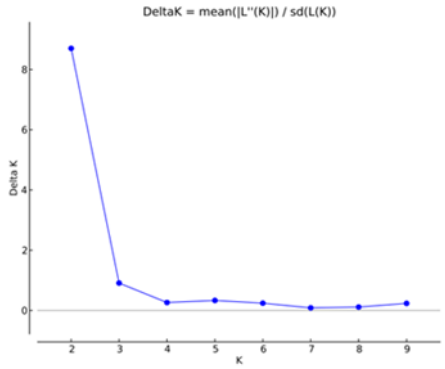
Nei's genetic distances (Nei 1972) for the analyzed adult (ad.) and juvenile (juv.) trees of the different populations.

CL_juv.	CS_juv.	GL_juv.	GS_juv.	Ha_juv.	US_juv.	CL_ad.	CS_ad.	GS_ad.	UL_ad.	US_ad.	Ha_ad.	GL_ad.	
0.000													CL_juv.
0.031	0.000												CS_juv.
0.043	0.027	0.000											GL_juv.
0.028	0.009	0.028	0.000										GS_juv.
0.030	0.021	0.036	0.016	0.000									Ha_juv.
0.055	0.038	0.035	0.034	0.039	0.000								US_juv.
0.007	0.041	0.051	0.039	0.035	0.062	0.000							CL_ad.
0.036	0.008	0.028	0.010	0.025	0.038	0.048	0.000						CS_ad.
0.029	0.015	0.026	0.009	0.022	0.042	0.043	0.015	0.000					GS_ad.
0.044	0.029	0.040	0.027	0.024	0.020	0.046	0.032	0.037	0.000				UL_ad.
0.062	0.043	0.042	0.042	0.042	0.009	0.066	0.043	0.053	0.016	0.000			US_ad.
0.038	0.023	0.042	0.018	0.012	0.037	0.048	0.024	0.028	0.032	0.046	0.000		Ha_ad.
0.054	0.036	0.012	0.032	0.046	0.043	0.068	0.033	0.034	0.056	0.054	0.048	0.000	GL_ad.

Appendix 8

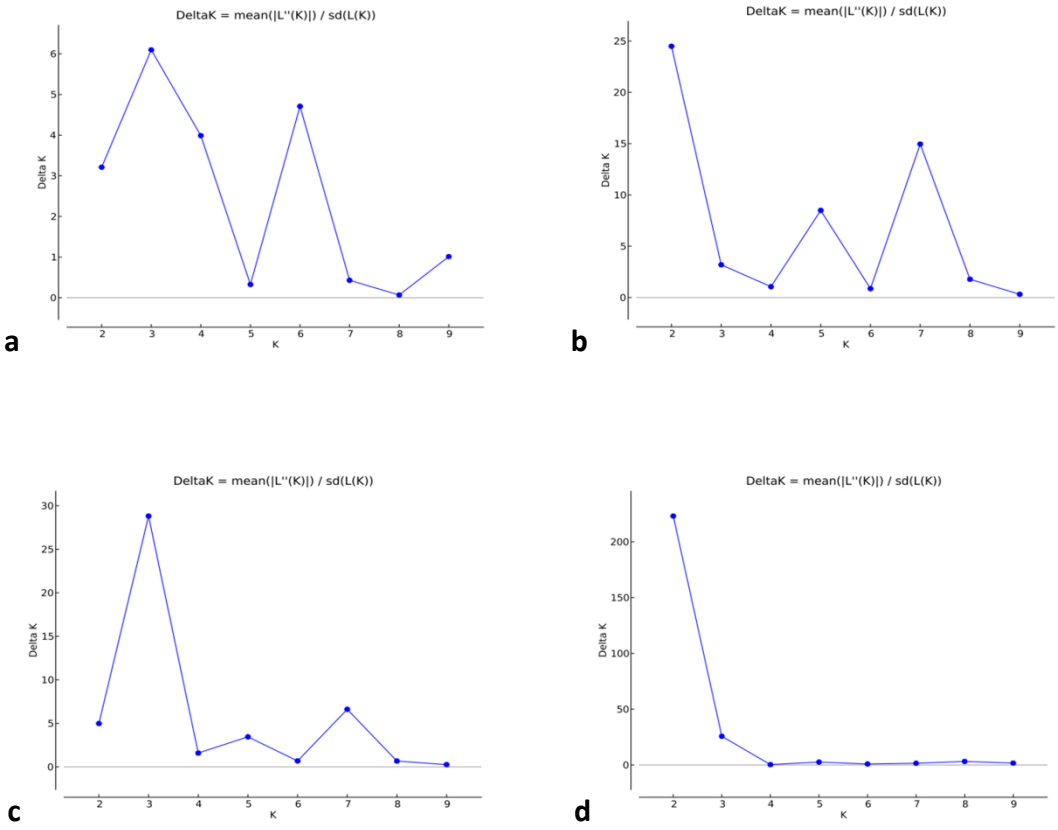
Appendix 8a

Plot of delta *K* (Evanno et al. 2005) based on microsatellite markers.



Appendix 8b

Plots of delta *K* (Evanno et al. 2005) based on (a) total SNP set (b) non-coding SNPs, (c) “silent” SNPs and (d) non-synonymous SNPs for the different populations.



Appendix 9

Mean molecular diversity indices for every SNP locus over all populations, N: number of individuals, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index.

SNP	Characteristic	N	H_o	H_e	F
Arf_265	non-coding	223.3	0.415	0.403	-0.032
Arf_303	non-coding	225.0	0.399	0.404	0.012
Arf_563	non-coding	229.5	0.297	0.309	0.045
Arf_573	non-coding	224.0	0.410	0.401	-0.023
Arf_615	non-coding	228.2	0.483	0.495	0.025
Arf_833	non-coding	228.5	0.197	0.191	-0.006

Arf_878	non-coding	231.7	0.395	0.401	0.015
ConsC1_293	non-synonymous	228.5	0.385	0.395	0.029
ConsC1_306	synonymous	228.2	0.118	0.114	-0.026
ConsC2_51	UTR	229.8	0.377	0.384	0.018
ConsC2_98	non-synonymous	230.2	0.057	0.062	0.024
ConsC2_147	synonymous	229.8	0.229	0.234	0.027
ConsC2_151	non-synonymous	226.5	0.422	0.428	0.013
ConsC2_211	non-synonymous	232.3	0.071	0.072	0.013
ConsC2_390	synonymous	231.0	0.071	0.072	0.015
ConsC2_488	non-synonymous	229.7	0.457	0.472	0.026
CP10_65	synonymous	228.2	0.248	0.263	0.057
CP10_67	non-synonymous	231.2	0.082	0.081	0.045
CP10_377	non-coding	228.8	0.400	0.421	0.048
CP10_442	non-coding	228.7	0.226	0.237	0.045
CP10_503	synonymous	228.0	0.119	0.123	0.013
CP10_749	synonymous	227.0	0.256	0.275	0.070
CP10_1317	non-coding	230.5	0.196	0.209	0.066
CP10_1428	non-synonymous	230.2	0.230	0.237	0.026
CysPro_118	synonymous	228.8	0.424	0.441	0.037
CysPro_202	synonymous	228.5	0.109	0.105	-0.029
CysPro_728	UTR	232.0	0.174	0.174	-0.008
CysPro_783	UTR	225.5	0.419	0.437	0.041
DAG_81	UTR	229.8	0.237	0.241	0.019
DAG_289	non-coding	228.3	0.235	0.238	0.012
DAG_1059	synonymous	231.0	0.274	0.286	0.042
Frigida_54	synonymous	230.2	0.061	0.060	-0.019
Frigida_104	non-synonymous	228.3	0.094	0.092	-0.012
Frigida_179	non-synonymous	231.7	0.112	0.108	-0.025
His3C1_292	non-coding	228.0	0.515	0.493	-0.045
His3C2_104	synonymous	228.3	0.099	0.105	0.026
His3C2_186	non-coding	227.2	0.35	0.357	0.017
His3C2_260	synonymous	228.2	0.197	0.191	-0.028
NAC_854	non-synonymous	228.3	0.368	0.371	0.008
NAC_962	synonymous	231.2	0.117	0.120	0.019
NAC_1300	UTR	227.2	0.395	0.388	-0.02
PP2C_315	non-synonymous	232.0	0.075	0.076	0.015
PP2C_391	synonymous	230.2	0.470	0.473	0.006
PP2C_791	non-synonymous	231.3	0.079	0.077	-0.027
PP2C_941	non-coding	229.2	0.489	0.485	-0.009
PP2C_1200	synonymous	225.5	0.482	0.484	0.005

Appendix 10

Molecular diversity indices based on potentially adaptive SNPs revealed by association and/or outlier analysis. Displayed are the pooled individuals of the different years divided into "early" flushing and "late" flushing, N: number of individuals, H_o: observed heterozygosity, H_e: expected heterozygosity, F: fixation index.

		Late flushing individuals				Early flushing individuals			
Year	SNP	N	H_o	H_e	F	N	H_o	H_e	F
2011	Arf_265	401	0.372	0.385	0.036	737	0.434	0.410	-0.059
	ConsC2_51	415	0.412	0.424	0.028	761	0.359	0.370	0.030
	ConsC2_98	414	0.056	0.054	-0.029	764	0.052	0.063	0.173
	ConsC2_147	415	0.292	0.310	0.059	763	0.198	0.215	0.080
	ConsC2_151	406	0.448	0.459	0.024	752	0.414	0.423	0.022
	ConsC2_390	417	0.072	0.069	-0.037	767	0.065	0.068	0.040
	CP10_67	415	0.106	0.100	-0.056	770	0.066	0.071	0.071
	CP10_377	410	0.437	0.439	0.006	762	0.374	0.411	0.091
	CP10_1428	414	0.258	0.270	0.042	766	0.202	0.216	0.064
	CysPro_728	420	0.200	0.221	0.093	769	0.163	0.160	-0.015
	NAC_854	414	0.338	0.340	0.006	753	0.384	0.400	0.042
	NAC_1300	413	0.395	0.363	-0.087	748	0.408	0.420	0.029
2012	Arf_265	274	0.332	0.364	0.087	610	0.434	0.414	-0.049
	Arf_573	273	0.333	0.365	0.086	609	0.427	0.412	-0.035
	ConsC2_51	281	0.431	0.418	-0.031	624	0.364	0.369	0.015
	ConsC2_147	284	0.282	0.299	0.058	628	0.218	0.219	0.003
	ConsC2_151	276	0.478	0.458	-0.044	617	0.402	0.416	0.034
	ConsC2_488	283	0.484	0.484	0.001	622	0.473	0.500	0.055
	CP10_377	279	0.405	0.448	0.096	623	0.377	0.402	0.061
	CP10_442	279	0.258	0.29	0.109	624	0.194	0.210	0.078
	CP10_1428	281	0.263	0.283	0.070	628	0.199	0.209	0.048
	CysPro_118	278	0.442	0.48	0.079	624	0.429	0.447	0.038
	CysPro_728	285	0.175	0.215	0.186	630	0.162	0.159	-0.016
	CysPro_783	281	0.434	0.479	0.094	610	0.426	0.443	0.038
	His3C2_104	282	0.053	0.052	-0.027	622	0.124	0.136	0.087
	NAC_1300	281	0.370	0.352	-0.052	614	0.397	0.416	0.044
2013	ConsC1_306	518	0.097	0.092	-0.051	325	0.148	0.142	-0.040
	ConsC2_98	527	0.057	0.055	-0.029	329	0.082	0.095	0.139
	ConsC2_151	516	0.450	0.455	0.013	324	0.367	0.400	0.081
	CP10_503	521	0.088	0.102	0.132	322	0.137	0.154	0.111
	CysPro_118	519	0.395	0.476	0.170	328	0.448	0.436	-0.027
	CysPro_728	531	0.169	0.198	0.142	330	0.191	0.173	-0.106
	CysPro_783	517	0.393	0.474	0.172	317	0.435	0.432	-0.008
	DAG_81	526	0.274	0.274	-0.001	328	0.216	0.212	-0.022
	DAG_289	520	0.273	0.271	-0.008	326	0.209	0.206	-0.013
	His3C2_104	526	0.070	0.078	0.103	323	0.149	0.153	0.030
	PP2C_941	526	0.470	0.498	0.058	325	0.529	0.488	-0.085
	PP2C_1200	521	0.470	0.498	0.056	317	0.517	0.487	-0.062

Curriculum Vitae

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Presentations and Posters

Müller M, Seifert S, Finkeldey R (2013) Analysis of Candidate Genes for Bud Burst in European Beech (*Fagus sylvatica* L.), presentation at the meeting "GOEvol Symposium 2013", October 30, 2013, Göttingen, Germany

Müller M, Seifert S, Vornam B, Finkeldey R (2013) Genetische Anpassungspotentiale an den Klimawandel: Variation in Kandidatengenen für das Austriebsverhalten und die Trocken-

stresstoleranz bei der Buche (*Fagus sylvatica* L.), poster presentation at the conference „Vom globalen Klimawandel zu regionalen Anpassungsstrategien“, September 2-3, 2013, Göttingen, Germany

Müller M, Seifert S, Finkeldey R (2013) Variation in Kandidatengenen für das Autriebsverhalten bei der Buche (*Fagus sylvatica* L.), presentation at the conference „Forstgenetische Forschung im Klimawandel – Ergebnisse aus Feld- und Laborversuchen, August 27-29, 2013, Treis-Karden, Germany

Seifert S, **Müller M**, Vornam B, Finkeldey R (2012) Analysis of Candidate Genes related to Climate Change in European Beech (*Fagus sylvatica* L.), poster presentation at the conference “Genetics of Fagaceae & Nothofagaceae”, October 9-12, 2012, Talence, France

Müller M, Seifert S, Vornam B, Finkeldey R (2012) Genetische Variation der Buche entlang eines Umweltgradienten, presentation at the conference "Forstwissenschaftliche Tagung“, September 19-22, 2012, München/Freising, Germany

Seifert S, Vornam B, **Müller M**, Finkeldey R (2012) Genetic Variation of Beech (*Fagus sylvatica* L.) Along an Environmental Gradient, poster presentation at the conference “Plant & Animal Genome XX”, January 14-18, 2012, San Diego USA

Language skills

German (native language), English