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# Differential gene expression related to an epigenetic memory affecting climatic adaptation in Norway spruce

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ABSTRACT

In Norway spruce, the temperature during zygotic embryogenesis appears to adjust an adaptive epigenetic memory in the progeny that may regulate bud phenology and cold acclimation. Conditions colder than normal advance the timing whilst temperatures above normal delay the onset of these processes and altered performance is long lasting in progeny with identical genetic background.

As a step toward unraveling the molecular mechanism behind an epigenetic memory, transcriptional analysis was performed on seedlings from seeds of six full-sib families produced at different embryogenesis temperature–cold (CE) vs warm (WE) under long and short day conditions. We prepared two suppressive subtracted cDNA libraries, forward and reverse, representing genes predominantly expressed in plants from seeds obtained after CE and WE embryogenesis following short day treatment (inducing bud set).

Sequencing and annotation revealed considerable differences in the transcriptome of WE versus CE originated plants. By using qRT-PCR we studied the expression patterns of 32 selected candidate genes chosen from subtractive cDNA libraries analysis and nine siRNA pathways genes by a direct candidate approach. Eight genes, two transposons related genes, three with no match to Databases sequences and three genes from siRNA pathways (*PaDCL1* and *2*, *PaSGS3*) showed differential expression in progeny from CE and WE correlated with the family phenotypic differences. These findings may contribute to our understanding of the epigenetic mechanisms underlying adaptive changes acquired during embryogenesis.



## 1. Introduction

Due to the longevity of their sessile life, trees and perennial woody plants from the temperate and boreal areas have developed systems to modify their phenotype to tolerate seasonal change in weather. Although the plant response to environment are based on natural evolution of mechanisms leading to tolerance, resistance, and avoidance of the environmental constrains, there are studies indicating that adaptive phenomena do not always fit well into traditional Mendelian genetic framework [1–3]. Plants require flexible and efficient strategies based on the adaptive potential of the extant genetic information to withstand changes in their environment.

Temporal and spatial variation in environmental conditions can lead to local adaptation of plant populations. Supplementing the well-studied view that woody plants have adapted to the local climate, there are emerging findings, which posit that, the parental environment during reproduction affect the performance of the progenies. *Arabidopsis thaliana* progenies from a warm parental environment have faster germination rates, faster root elongation growth, higher leaf biomass and increased seed production compared with those from a cold parental environment despite having identical genetic backgrounds [4]. Related findings have been reported by Andalo et al. [5], Munir et al. [6] and Gehring et al. [7]. The seedlings from seeds produced in Norway by the families of Central European origin had on average a bud set more similar to the local Norwegian origin families than to Central European ones [8]. Norway spruce seedlings "remember" the temperatures [9] and photoperiod [10] prevailing during their zygotic embryogenesis and seed maturation. The timing of dehardening and bud burst in spring, leader shoot cessation in summer, bud set and

*Abbreviations:* CE, cold embryogenesis environment (outdoor); WE, warm embryogenesis environment; SSH, suppression subtractive hybridization; CEL, SSH library enriched for genes predominantly expressed in CE plants; WEL, SSH library enriched for genes predominantly expressed in WE plants; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; TE, transposable elements (transposons); $D_T$, difference in transcription (dCt cycles); $D_P$, difference in bud formation.

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increase in cold hardiness in the autumn are all processes that are advanced or delayed as influenced by the temperature during female reproduction in progeny with identical genetic background. Colder reproductive environment advance bud set and cold acclimation during autumn and dehardening and bud burst during spring in their progenies [11–13]. In progeny obtained at different temperatures during zygotic embryogenesis, and then propagated at different temperatures applied during somatic embryogenesis, identical clones expressed a difference in timing of terminal bud formation that was equivalent to a 4–6° latitudinal ecotypic difference [14,15]. The "memory effects" acting on these phenological traits last for more than 20 years after germination, but there is no change in progeny performance when there were differences in temperature and photoperiod during male meiosis and microsporogenesis [12]. Moreover, there is absence of any genetic marker distortion in the progenies [16]. Thus, this memory, affecting the climatic adaptation in this species, is an epigenetic phenomenon [17].

Progenies of *Picea abies* seeds that were produced in cold environments have been reported to have higher nucleic acid methylation level than seeds that were produced from warm environments [18], but it is not known if this methylation is result of changes at the DNA or RNA level, so could potentially be due to post-transcriptional RNA modification of noncoding RNAs. It has been reported in Arabidopsis that, some parent-of-origin effects such as seed mass development correlate with gene-specific differences in DNA methylation [19,20].

Epigenetic memory can be defined as all the heritable changes in gene expression that occur without a change in the primary sequence of nuclear DNA. Thus, changes in methylation or chromatin remodeling are considered the more likely mechanisms behind the epigenetic memory in Norway spruce. Epigenetic effects occur when chromosomal proteins and methylated DNA result in important phenotypic consequences [21]. DNA methylation, chromatin and noncoding RNAs that, in turn, govern changes in DNA methylation and chromatin status, are considered the molecular basis for epigenetic effects [22]. Histone modification has some attributes of an epigenetic process, but according to Bird [23] are not necessarily heritable. Epigenetic modifications provide a means of altering expression states and can determine the phenotypes that contribute to natural variation and be selected upon.

The epigenetic memory in Norway spruce most likely has a genetic basis and since there are family variations in memory response, it should possess the specific genetic mechanism underplaying this phenomenon. In addition to Norway spruce, similar effects have been demonstrated in progeny from white spruce and *Picea glauca* × *Picea engelmannii* crosses [24,25], Scots pine [26], *Larix* spp. [27] and shortleaf pine [28]. Moreover, there are data indicating that land race formation in conifers is much faster that expected [8], but there is so far lack of substantial evidence of comparable phenomena in angiosperm trees, leaving the generality of the phenomenon an open issue [17]. In the context of global climate changes, this phenomenon is of great scientific and practical significance because any process which in this way regulates vegetative bud phenology of forest trees, may affect their productivity, adaptability and distribution during climate change that would give these plants an adaptive advantage.

The objective in this study was to identify candidate genes that are primarily correlated to the phenotypic expression of the "epigenetic memory" in Norway spruce (*P. abies*), by using a transcriptomic approach. We constructed two suppression subtractive hybridization (SSH) libraries using plants originated after cold and warm zygotic embryo development. Then we used real-time reverse transcription-polymerase chain reaction (RT-PCR) to study the transcription profiles of selected ESTs in progenies from six families showing family differences in the "epigenetic memory" measured as the timing of terminal bud formation in one-year-old seedlings under natural photoperiods.

## 2. Materials and methods

### 2.1. Plant material, growth conditions and sample collection

Grafts were cultivated, and crosses were performed as described previously [10]. Crosses and seed were production were done in 2004 both inside (warm environment; WE) and outside (natural-cold environment; CE) a heated glasshouse at Biri Nursery and Seed Improvement Center (Norway – 60.9°N). Crosses were made by using clonally propagated parents (potted grafts) and seeds were obtained from different grafts of mother clones in environments with different temperatures. We used progenies from six full-sib families of Norway spruce (Table 1) which were preselected based on their differences in the timing of bud set, as assessed by growing the seedlings in a greenhouse experiment at our experimental farm at Hoxmark (Norway – 59.7°N) in the autumn of 2005 identical to that we have reportedearlier [10]. Within each family two types of seeds were used: seeds from plants obtained after zygotic embryogenesis in cold environment (CE) and after zygotic embryogenesis in warm environment (WE), giving a total 12 seed types. Family 1 expressed the lowest average difference in terminal bud formation between CE and WE and was termed "the epigenetically indifferent family". Progenies of family 6 showed the highest difference in bud formation between the CE and WE, and family 6 thus called the "the epigenetically responding family" Families 2–5 showed intermediate response (Table 1) [10]. Otherwise, the six families were preselected because they had a gradual increase in the expression of the epigenetic memory that could be used as a phenotypic response variable in the regressions, where transcrip-

**Table 1**
Origin of parents of the studied full-sib families and proportions of plants with a terminal bud (%) on two dates in August 2005, observed in progenies originated from seed production in a warm and cold environment. The number of plants recorded per combination of family and seed production environment was 90 (30 plants per replicate; three replicates). Standard error of mean was 4.6% on August 18, and 3.3% on August 22.

| Full-sib family number (♀ × ♂) | Female origin (♂, latitude, altitude) | Male origin (♀, latitude, altitude) | Proportion of plants (%) with terminal buds | | | | Maximum difference (days) |
|---|---|---|---|---|---|---|---|
| | | | August 18 | | August 22 | | |
| | | | Cold | Warm | Cold | Warm | |
| 1 (5433 × 7292) | 60.2°N, 320 m | 60.1°N, 235 m | 79 | 76 | 97 | 95 | 3 |
| 2 (1641 × 1960) | 60.0°N, 230 m | 60.7°N, 160 m | 53 | 46 | 96 | 92 | 7 |
| 3 (2474 × 7292) | 59.8°N, 415 m | 60.1°N, 235 m | 50 | 35 | 97 | 88 | 15 |
| 4 (6172 × 1957) | 65.9°N, 125 m | 60.7°N, 200 m | 68 | 44 | 99 | 92 | 24 |
| 5 (6170 × 1960) | 66.0°N, 80 m | 60.7°N, 160 m | 86 | 55 | 99 | 94 | 31 |
| 6 (1957 × 7436) | 60.7°N, 200 m | 60.2°N, 330 m | 17 | 2 | 84 | 48 | 36 |

Recording of the terminal bud formation was done during August 2005. The proportion of plants with a visible terminal bud are shown for August 18 and 22. At August 18, we found the maximal differences between the cold and the warm performance of full-sib families 1–5, and for family 6, maximal differences was found at August 22 (late family performance).