Molecular mechanisms regulating early cone development in Norway spruce *Picea abies* (Karst)

Shirin Akhter
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2019

Acta Universitatis agriculturae Sueciae
2019:12

Cover: SEM (Scanning electron microscope) of vegetative (left) and female (right) cone.
Photo: Jens Sundström

ISSN 1652-6880
ISBN (print version) 978-91-7760-342-9
ISBN (electronic version) 978-91-7760-343-6
© 2019 Shirin Akhter, Uppsala
Print: SLU Service/Repro, Uppsala 2019

Molecular mechanisms regulating early cone development in Norway spruce *Picea abies* (karst).

Abstract

Flower development progress through distinct phases that are controlled by complex genetic networks, which in turn are regulated by both endogenous and exogenous factors. A large number of functional studies have been conducted over the past decades to understand the genetic networks that regulate flower development in angiosperm model species. In gymnosperms, however, the gene regulatory networks behind distinct phases of cone development are largely unknown due to the lack of functional studies.

In a morphological study of early cone development in *Picea abies*, three distinct growth phases were defined. Transcriptome comparisons of female and vegetative buds in the three growth phases identified members of the MADS-box gene family, LEAFY-orthologs, bZIP-, AP2-, and SBP-domain proteins as being highly expressed in the different phases of female cone development. In a separate study different isoform of the MADS-box gene, *DAL19* were identified. Isoforms specific expression in male, female and vegetative bud meristems provided evidence that alternative splicing may influence cone formation in a bud identity specific manner.

In the early cone-setting *acrocona* mutant, *P. abies* var. *acrocona*, leading shoots often have needles at the base, but ovuliferous scale-like structures in the top. Hence, during shoot development, the leading shoots make a morphological shift and produce transition shoots. RNA sequencing of *acrocona* transition shoots demonstrated that the MADS-box genes *DAL10* and *DAL21,* which previously have been associated with reproductive shoot identity were expressed at high levels in transition shoot meristems before the morphological shift. In addition, genes encoding FT/TFL-like, bZIP-, SOC1-like and gymnosperm specific MADS-domain proteins co-expressed with *DAL10* and *DAL21*, suggesting at a putative role for these genes in the early development of reproductive meristems. In addition, genes encoding F-box protein and ubiquitin were expressed at high levels in late *acrocona* transition shoots, which possibly reflects an involvement of hormonal signalling in the *acrocona* transition shoot phenotype.

Keywords: *Picea abies*, phase change, reproduction, GA, MADS-box, *DAL19*, FT/TFL, bZIP, SOC1, LEAFY, SBP, gymnosperm.

Author's address: Shirin Akhter, SLU, Department of Plant Biology,
P.O. Box 7080, 750 07 Uppsala, Sweden. E-mail: shirin.akhter@slu.se

Dedication

To my mother….

“Imagination is more important than knowledge. Knowledge is limited. Imagination encircles the world.”

---- Albert Einstein ----

Contents*

List of publications*[1]

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Shirin Akhter[1]**, Veronika Nordal[1], Warren W. Kretzschmar[2], Nicolas Delhomme[3], Nathaniel R. Street[4], Ove Nilsson[3], Olof Emanuelsson[2] & Jens F. Sundström[1]. Transcriptome profiling of early cone development in *Picea abies*. (Manuscript).

II **Shirin Akhter[1]**, Warren W. Kretzschmar[2], Veronika Nordal[1], Nicolas Delhomme[3], Nathaniel R. Street[4], Ove Nilsson[3], Olof Emanuelsson[2] & Jens F. Sundström[1*]. (2018). Integrative Analysis of Three RNA Sequencing Methods Identifies Mutually Exclusive Exons of MADS-Box Isoforms During Early Bud Development in *Picea abies*. *Frontiers in Plant Science*, *9*, 1625.

III **Shirin Akhter[1]**, Veronika Nordal[1], Warren W. Kretzschmar[2], Nicolas Delhomme[3], Nathaniel R. Street[4], Ove Nilsson[3], Olof Emanuelsson[2] & Jens F. Sundström[1]. Transcript profiling of *acrocona* transition shoots identifies candidate genes of importance for female bud development in *Picea abies*. (Manuscript)

Paper II is reproduced with the permission of the publishers.

* Corresponding author. *

Other publications outside the thesis?

Daniel Uddenberg, **Shirin Akhter,** Prashanth Ramachandran, Jens F. Sundström*& Annelie Carlsbecker*. Sequenced genomes and rapidly emerging technologies pave the way for conifer evolutionary developmental biology, Front. Plant Sci., 03 November 2015 | https://doi.org/10.3389/fpls.2015.00970

1. Mandatory

The contribution of Shirin Akhter to the papers included in this thesis was as follows:*[2]

I Participated in the planning of the project. Collected the plant material, prepared the RNA, performed filtering, mapping, and differential expression analysis of transcripts. Interpreted the results, wrote the first draft of the manuscript and took part in the final editing of the manuscript.

II Collected the plant material, performed qPCR, and discovered putative MADS-box core sequences. Prepared the RNA and performed the cloning and *in situ* hybridization. Took part in the writing of the draft of the manuscript and the edited the final version of the manuscript.

III Participated in the planning of the project. Collected the plant material, prepared the RNA, performed filtering, mapping, and differential expression analysis of transcripts. Interpreted the results, wrote the first draft of the manuscript and took part in the final editing of the manuscript.

2. Mandatory

1 Introduction

The evolution of land plants from green algae and their colonisation and adaptive radiation in different geologic time-periods have resulted in diverse land plant lineages such as non-vascular plants, non-seed vascular plants, non-flowering, and flowering plants. The major changes in the land plant spore mass (from cryptospores to trilete spores) led to the emergence and adaptive radiation of vascular plants from the early Silurian period (Steemans et al., 2009).

The fundamental difference of seed plants from plants with free spores is the delivery of the male gametophyte directly to the female gametophyte through pollination. In the earliest vascular plants, ovules are not enclosed in an ovary and seeds are exposed to the external environment, these so called "naked seed-plants" are referred to as gymnosperms. The four extant groups of gymnosperms are the conifers, ginkgos, cycads, and gnetophytes. In gymnosperms, pollen grains are mostly adapted to wind or water pollination. In the Cretaceous period, a diverse group of seed plants evolved that protect their seed in an ovary. These plants are referred to as angiosperms. The angiosperms radiated rapidly. According to the theory of evolution by Charles Darwin (Darwin, 1859), which implied that all organisms should increase gradually. The early evolution and diversification of angiosperms was an "abominable mystery" in Darwin's mind.

Angiosperms and gymnosperms separated from their last common ancestor about 310-350 million years ago (Bell, Soltis, & Soltis, 2010; Doyle, 2012). In angiosperms, pollen grains are often adapted to animal pollination, and the co-evolutionary interactions between flowering plants and their pollinators are assumed to have played an essential role in the diversification of many angiosperm lineages (van der Niet & Johnson, 2012). In contrast to the gymnosperms, the angiosperm ovule is enclosed by a carpel, and the female structures are, in turn, arranged in a flower together with the male organs, the stamens and a sterile perianth of sepals and petals (Endress, 2011). While the flower is the central feature of the angiosperm lineage, the origin of the flower and the consequent diversification of angiosperm species remain as key

evolutionary questions. Gymnosperms as a group have been proposed to be the closest relatives of angiosperms (Doyle, 1978). The ovules of most gymnosperms consist of a single integument, except for the Gnetophytes. The ovules in Gnetophytes are enclosed by several nuclear envelopes, which are analogous to the integuments that enclose the angiosperm ovule (Takaso & Bouman, 1986). Based on transcriptome studies of Streptophytes and several sequenced plant genomes and phylogenetic studies, Gnetophytes has recently been proposed to be the closest group of the Coniferales (Wickett et al., 2014; Winter et al., 1999).

A considerable amount of functional studies have been conducted over the past decades to understand the molecular mechanisms that regulate reproductive development in angiosperm model species. In gymnosperms, however, functional studies are challenging to perform. Instead, evolutionary developmental (evo-devo) studies have dominated. Next-generation sequencing technologies, e.g. RNA- *seq* now provide the opportunity to expand our studies of non-model species.

In this thesis, morphological analyses of early reproductive development in *Picea abies* are combined with transcriptome studies in order to understand molecular mechanisms behind reproductive development in conifers. The findings are related to analogous processes in angiosperm model systems in order to enhance our understanding of the evolutionary process leading to distinct reproductive structures in these two seed plant lineages.

1.1 Reproductive development

The structural features of the reproductive organs differ between angiosperms and gymnosperms. In angiosperms, the reproductive organs are arranged in flowers, which typically consists of a sterile perianth of sepals and petals that surround the reproductive organs, the stamens and the carpels. Whereas, extant gymnosperms lack the sterile perianth. However, the origin and evolution of the flower is a crucial evolutionary question (Doyle, 2012; Friis, Crane, & Pedersen, 2011) that remains scantily understood. The exact composition of the structural features of the ancestral angiosperm flower is unclear, due to the lack of fossil records from the period when this ancestor existed (Herendeen, Friis, Pedersen, & Crane, 2017).

The flowers of basal angiosperms (*Amborella*, *Nymphaeales*, *Austrobaileyales* and *Magnoliids*) vary significantly in size, in the number of flower organs, and the arrangement of the floral organs in a spiral or in whorls (Tsai, Pan, Su, & Liu, 2014). In *Amborella* and *Austrobaileyales*, the flower organs are spirally arranged with a gradual transition from bracts to tepals, from

tepals to stamens, and finally from stamens to carpels (Tsai et al., 2014). Phylogenetic studies have indicated that *Amborella trichopoda* is a sister species to all other extant angiosperms (P. S. Soltis, 2005). Hence, phylogenetic studies indicate that the flower of early angiosperms was mostly spiral. In a recent study, which utilizes model-based reconstructions of a large data-sets of floral traits from extant angiosperms, it was proposed that the ancestral angiosperm flower was bisexual and radially symmetric. The model also suggested that the ancestral flower consisted of two or more whorls of separate perianth organs (undifferentiated tepals), at least two whorls of stamens, surrounding at least five spirally arranged carpels (Sauquet et al., 2017). Hence, this study suggests that the early evolution of angiosperm flowers were marked by a sequential reduction in the number of whorls in both the perianth and in the androecium. This proposed structure of the ancestral flower is opposite to the traditional view that the early angiosperm flower consisted of mostly spirally arranged floral organs.

The ovule is the common reproductive structural feature between gymnosperms and angiosperms. The fundamental structure of the ovule is similar in all seed plants groups (Endress, 2011; Linkies, Graeber, Knight, & Leubner-Metzger, 2010). Although the characteristic function of the ovule in all seed plants is to produce a seed containing an embryo, ovules in gymnosperms also play a variety of different roles. In particular, at the time of pollination, gymnosperm ovules may secret an aqueous solution that helps to capture (Gelbart & Aderkas, 2002) or to collect pollen from the integuments or surrounding structures (Chandler & Owens, 2004; Leslie, 2010; Leslie & Kevin Boyce, 2012; Tomlinson, Braggins, & Rattenbury, 1991). The ovule in gymnosperms also contains a large cellular megagametophyte that produces archegonia which serves as a nutrient source for the developing embryo (Leslie & Kevin Boyce, 2012). In contrast, the angiosperm megagametophyte is typically a reduced structure, which does not produce archegonia (Leslie & Kevin Boyce, 2012). On the other hand, seeds of angiosperms harbour an endosperm, which serves as a nutrient source to the developing embryo (Leslie & Kevin Boyce, 2012).

In the gymnosperm lineage of seed plants, cone-bearing plants are referred to as conifers. Adaptations of conifers to cold and dry weather explain their superiority at high altitudes and in cold climates. Different conifers vary in their reproductive cycle but the process is broadly similar throughout the Pinaceae. In *P. abies*, male and female cones are formed from separate meristems. The female cones are initiated in the upper part of the tree in apical positions of lateral branches and are larger than male cones. In contrast, male cones are initiated in the lower region of the tree. The buds at the shoot apex of lateral branches tend to initiate as vegetative or female cones, while buds at the base of the shoot are

initiated as pollen cones. Apical buds on leading branches are always vegetative. Allen and Owens, 1972, observed similar patterns of a cone-setting manner in Douglas fir (*Pseudotsuga menziesii*).

The female cone of *P. abies* consists of spirally arranged ovuliferous scales, each subtended by a sterile bract. Each of the ovuliferous scales carries two ovules. Hence, the female cone is a compound structure, and can be viewed as a reproductive shoot, analogous to the angiosperm inflorescence. In contrast, the male cone of conifers have historically been considered to be a simple structure or a short shoot, more similar to the angiosperm flower (Florin, 1951). However, based on morphological studies that include species from all extant conifer genera, it has recently been suggested that the Taxaceae, Podocarpus, Prumnopitys, and Retrophyllum possess compound male cones analogous to inflorescences (Schulz et al., 2014). Generally, the male cone can be categorised either as solitary or fascicular (clustered) (Schulz et al., 2014). In some conifer species, the fascicular male cone can either be a simple male cone analogous to a flower or a compound cone similar to an inflorescence (Schulz et al., 2014). In *P. abies*, the male cone consists of spirally arranged microsporophylls formed from a single meristem and the entire cone represents a single reproductive short shoot. Also buds that later will form pollen cones can initiate at various positions along a vegetative shoot, both in basal parts and in lateral and apical positions.

Initiation and early bud development in Douglas fir (*Pseudotsuga menziesii*) have been thoroughly described by Owens & Smith (1964) and the further development of vegetative shoots and reproductive cones have been described by Allen and Owens (1972). Date and timing of distinct differentiation stages have been well established for specific locations, *i.e.* Victoria in Canada, but the progress of cone development may vary with geographical position, environment and between trees. It has been reported that the time of bud development progress later as elevation increases (Silen, 1967). In Douglas fir (*Pseudotsuga menziesii*), lateral buds initiate in the shoot meristem axils from cortical cells in early April. At this point, the identity, vegetative or reproductive, cannot be distinguished (Owens & Smith, 1964). The apical zone of the meristems becomes apparent by mid-May in female and vegetative buds, but the male buds remain distinctly smaller during that time (Owens & Smith, 1964). The transition from undetermined bud primordial to reproductive forms occurs during April and May (Allen and Owens, 1972). The lateral organs (needles, bracts and microsporophylls) initiates at the end of July. Lateral organs differentiate and further elongate and initiation ends early in October and the cones become dormant in early November (Owens & Smith, 1964). The dormant phase of cones extends until February the following year. The female cones continue their growth from March to June. In male cones, the developing pollen

undergoes meiosis at the end of the February. Pollination and fertilization occur during April and June. Seeds are developed at the August after fertilization. Mature cones form in September when the cones open and seed dispersal occurs. An overview of conifer reproductive cycle is illustrated in **Figure 1**.

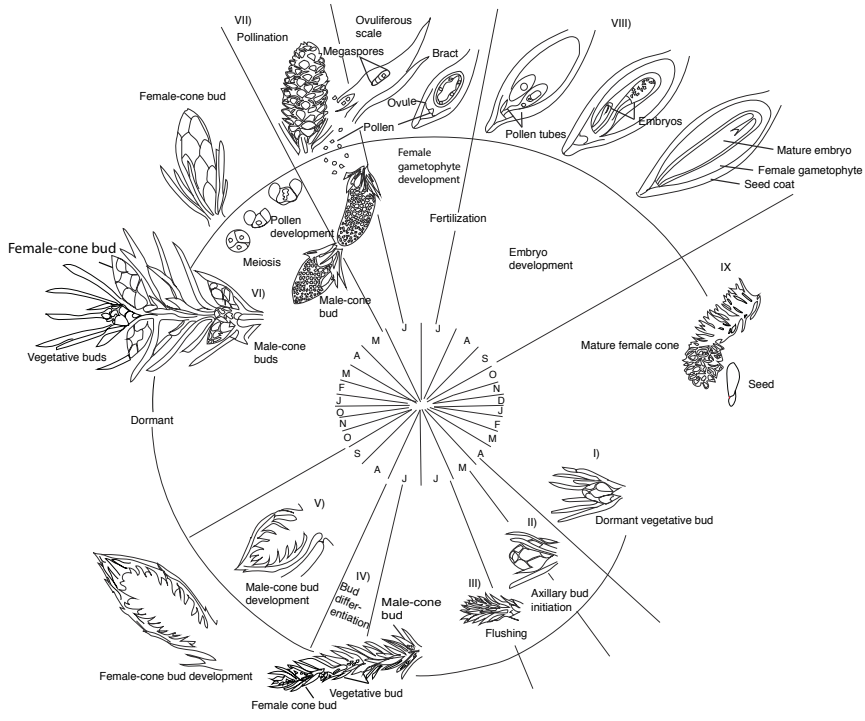

Figure1. Schematic overview of the reproductive cycle in a conifer. This figure is representing reproductive development of *Picea glauca*, which follow a two-year typical conifer reproductive cycle in Northern hemisphere. However, the differentiation of shoots apices as reproductive buds may be differed by the geographical regions. Buds initiate at the shoot apex from about at the late April. Buds differentiate as vegetative, female or male cone buds around mid-July. All microsporophylls, microsporangia, bracts and ovuliferous scales, and needles initiate at the early October, when they become dormant. Pollination of these cones occur in the next year June, and mature seed cone develops in October. Roman numbers represent various stages and letters represent different months of the year. This figure is a re-drawing adopted from Owens and Molder, 1984.

The early bud development has also been studied in different species of spruce and it has been reported that the lateral buds of Black spruce (*Picea mariana*) initiate around the beginning of June in the localities of Chalk river, Ontario (Fraser, 2011). The buds are distinguishable as vegetative or reproductive by the end of July (Eis, 1967).

1.1.1 Initiation of cone-setting in conifers

Conifers undergo a long juvenile period before they reach a reproductive phase. However, suitable conditions depending both on endogenous signals and the environment influence cone initiation, and even after entering the reproductive phase *Picea abies* do not produce cones every year. Female cones are formed in apical positions of a lateral shoot and since the apical meristem terminates within the cone, the initiation indirectly causes a stop in further growth of the shoot. Hence, cone-setting influence the overall growth of a branch, which needs at least two years to reconstitute its morphology after cone-setting (Tiren, 1935). Therefore, after a good cone-year, initiation of new female-cones the following year are limited, even if the environmental conditions are favourable for ample cone-setting. The balance between environmental signals and the need to reconstitute the growth of the tree after ample cone-setting explains the periodicity of cone production in *P.abies.*

The success of cones production is not only dependent on the initiation of reproductive buds, the subsequent differentiation of buds into mature cones is also crucial for a good cone year. Based on studies in Douglas fir (*Pseudotsuga menziesii*), it has been suggested that the number of cones produced is not entirely dependent on the production of primordia, but is also determined by the number of primordia that continue to develop as reproductive buds (Eis, 1973; Owens, 1969; Silen, 1967). A detailed anatomical study in Douglas fir (*Pseudotsuga menziesii*) by Owens, 1969 stated that, environmental and physiological factors influence reproductive bud development and involves different developmental pathways, but that these pathways also can be reversed. Hence, in a good year of bud initiation, possible male cone buds can become latent or abort, and possible female cone buds can revert to vegetative growth due to environmental and physiological factors.

1.1.2 Molecular regulation of reproductive development

The genetic and molecular mechanisms underlying reproductive organ development have been studied thoroughly in angiosperms model species. The transition from a vegetative shoot apical meristem (SAM) to an inflorescence meristem, initiation of floral meristems and floral organogenesis are sequential steps in plant reproductive development. The transition from vegetative to reproductive phase in plants is controlled by several developmental programs that are regulated by both endogenous and exogenous cues (Simpson, 2002; Westerman & Lawrence, 1970). During this transition, the vegetative SAM changes its identity to an inflorescence meristem. To complete these transition

processes, both correct timing and suitable climatic conditions are needed. The coordination of the genetic pathways together with environmental cues and the developmental state of the plant influence flowering, and are commonly referred to as the flowering pathways. The Flowering pathways include: *i*) the vernalization pathway, which dependent on prolonged exposure to cold, *ii*) the photoperiod pathway, which dependent on day-length and light quality, *iii*) the hormonal pathway, which dependent on GA for normal flowering, *iv*) the autonomous pathway that dependent on endogenous regulators independent of photoperiod and GA, v) the age-dependent pathway, which involves miRNA156 and vi) the species-specific pathway which dependent on ambient temperature response and an accumulation of carbohydrate assimilates in the shoot apex (reviewed in Andrés & Coupland, 2012; Ó'Maoiléidigh, Graciet, & Wellmer, 2013). Here, an overview of flowering time regulation based on studies in Arabidopsis is given in **Figure 2**.

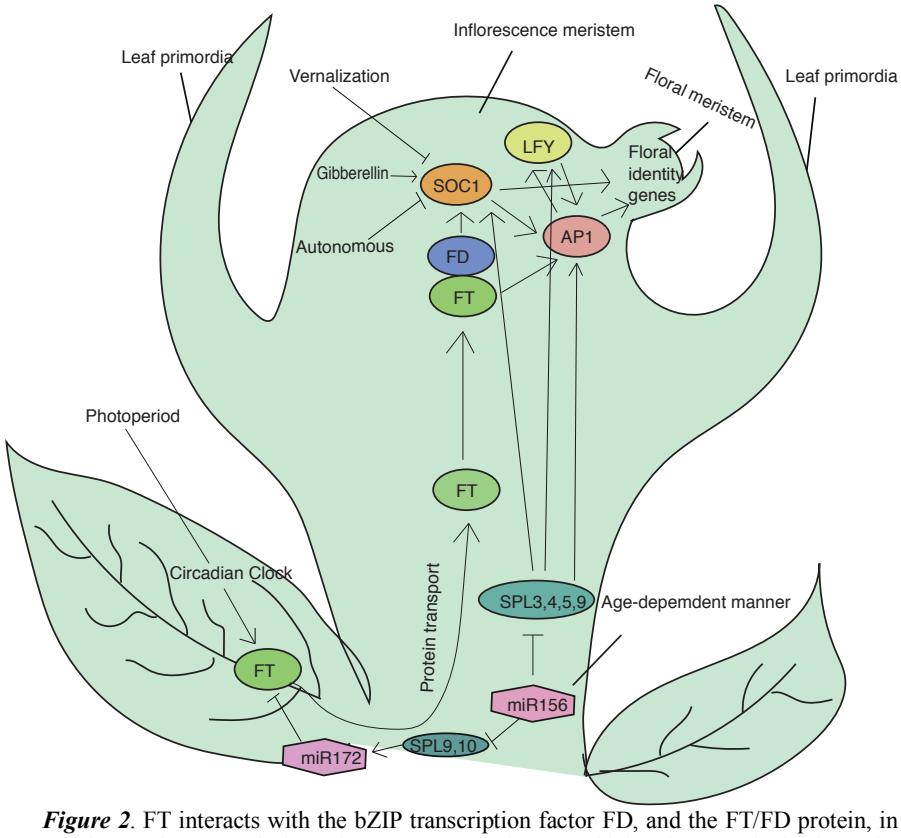

Figure 2. FT interacts with the bZIP transcription factor FD, and the FT/FD protein, in turn, activates the transcription of *SOC1*, *LFY* and *AP1*, which subsequently leads to the

activation of the floral homeotic identity genes. The gibberellin pathway promotes the expression of the *SOC1* gene at the shoot apex, whereas the vernalization and autonomous pathways negatively regulate *SOC1* at the shoot apex. In an age-dependent manner, miRNA156 regulate flowering by targeting *SPL* genes. miRNA156 levels decrease, and *SPL* genes level increase with plant age. miRNA156 repress flowering by targeting *SPL* genes. With increasing levels of *SPL* transcripts during ageing, *SPL* genes promote flowering by activating *SOC1*, *LFY* and *AP1*. Also, miRNA156 repress flowering by activating miRNA172 dependent repression of FT in the leaf. Lines with an arrow indicate activation; lines with a perpendicular bar indicate repression. This figure is a re-drawing inspired from Amaniso (2010), Huijser and Schmidt (2011), Taiz and Zeiger (2010, 5th ed).

During the floral transition in Arabidopsis, the shoot apical meristem (SAM) converts into inflorescence shoot apical meristem (IM). Inflorescences are subtended by fully developed bracts, and floral meristems (FM) are formed in the axil of rudimentary bracts (Kwiatkowska, 2005; 2008). The inflorescence meristem is divided into three different zones with different cell division rates: the central zone (CZ), the peripheral zone (PZ) surrounding the CZ and the rib zone (RZ) underneath the CZ (Bowman & Eshed, 2000; Caboche, 1994). Flower primordia are initiated from the PZ. After initiation, flower primordia expand and divide to generate a concentric group of cells, from which floral tissues are derived (Kwiatkowska, 2005; Bossinger and Smyth, 1996).

The activities of the SAM, IM and FM are controlled by complex genetic networks consisting of positive and negative regulators. The SAM consist of undifferentiated stem cells, including founder cells from which lateral organ arise. Flower organogenesis occurs from actively proliferating meristems. The balance between maintenance and proliferating meristem cells, which is shifted towards floral organogenesis, depends on several regulatory genes. In the SAM, *SHOOT MERISTEMLESS* (*STM*), which is a *KNOTTED1*-like homeobox (*KNOX*) gene, negatively regulates the MYB transcription factors *ASYMMETRIC LEAVES1 (AS1)* and *ASYMMETRIC LEAVES2 (AS2*) to prevent cells in the SAM to enter premature differentiation (Byrne et al., 2000; Byrne et al., 2002). Furthermore, the homeodomain-containing transcription factor *WUSCHEL* (*WUS*) controls the balance between cell proliferation and cell recruitment to the differentiated tissues in the SAM (Sablowski, 2007; Laux et al., 1996). In *wus* mutant background the SAM lacks stem cells (Mayer et al., 1998) suggesting that the WUS protein is needed for the maintenance of stem cells in the SAM. In the stem cells, *CLAVATA (CLV)* genes are active in the SAM and balance the number of cells within the meristem (Fletcher, 1999). The *CLV* repress the activity of *WUS* outside of the stem cell niche, which restricts the accumulation of number of the cells in the SAM (Brand, 2000). Similarly, it has also been reported that *CLV1, CLV2* and *CLV3* controls the number of cells in the floral meristem from which homeotic genes act to initiate and regulate floral organ development (Clark et al., 1993, 1995; Kayes and Clark, 1998).

Apart from the WUS-CLV regulatory loop that maintains meristem size, the TERMINAL FLOWER 1(TFL1) protein maintain IM identity (Alvarez, Guli, Yu, & Smyth, 1992; Ohshima, Murata, Sakamoto, Ogura, & Motoyoshi, 1997; Shannon, 1991; 1993). During the conversion of IM to FM, the IM genes *TERMINAL FLOWER 1*(*TFL1*) and *EMBRYONIC FLOWER 1* and *2* (*EMF1* and *EMF2*) (Aubert, 2001; L. Chen, 1997) are repressed in the FM, whereas the floral meristem identity (FMI) genes, *LEAFY* (*LFY*) (Huala & Sussex, 1992; Weigel, Alvarez, Smyth, Yanofsky, & Meyerowitz, 1992; Waigel et al., 1992) *APETALA1* (*AP1*) (Alejandra Mandel, Gustafson-Brown, Savidge, & Yanofsky, 1992; Huala & Sussex, 1992; Mandel & Yanofsky, 1995a), *APETALA2* (*AP2*) (Huala & Sussex, 1992), and *CAULIFLOWER* (*CAL*) (Kempin, Savidge, & Yanofsky, 1995) are up-regulated. The Flowering locus T (FT) protein is a homolog of TFL1 (Kardailsky, 1999; Koornneef, Hanhart, & van der Veen, 1991), which is produced in the leaf and later transported through the phloem to the SAM where it integrates signals to promote flowering (Corbesier et al., 2007). External factors (vernalization and light) and internal factor (gibberellins) have been reported to influence the action of FT on flowering (reviewed in Andrés & Coupland, 2012; Song, Ito, & Imaizumi, 2013). The FT protein interacts with two bZIP transcription factors FD and FD PARALOUGE (FDP) that are expressed in the SAM before floral induction. The FT/FD complex, in turn, activate the expression of the floral meristem identity gene *AP1* (Abe, 2005; Wigge, 2005).

1.1.3 Gene networks for floral organ identity

MADS-box genes are critical components in the genetic mechanisms regulating floral organ development (Mandel & Yanofsky, 1995b; Bowman et al., 1993). MADS is an acronym derived from the four founder genes of this gene family (MCM1; from Saccharomyces cerevisiae), AGAMOUS (AG; from Arabidopsis thaliana), DEFICIENS (DEF; from Antirrhinum majus) and SERUM RESPONSE FACTOR (SRF; from Homo sapiens). Type II MADS-box genes are plant specific and refers the MIKC-type of MADS-domain proteins (Gramzow & Theissen, 2010); M denotes the MADS-domain that is responsible for DNA binding, I denotes the intervening region which is a variable region and also referred to as the linker, K denotes the Keratin like domain that is responsible for protein-protein interactions, and C stands for the C-terminal region which is a variable region (Schwarz-Sommer, Huijser, Nacken, Saedler, & Sommer, 1990; Litt and Irish, 2003).

Within the floral meristem of an Arabidopsis plant, different circular regions or whorls are established shortly after the floral meristem initiation. In each

whorl, floral organs with distinct identity develop. In the first and outermost whorl sepals are formed, the second whorl initiate petals and the third whorl stamens. In the innermost fourth whorl, two fused carpels are formed that after pollination will develop into a fruit. Distinct sets of genes are involved in the regulation of different floral organ identities.

Key components of the gene regulatory network that specify floral organ identity are commonly referred to as the homeotic ABC genes. In Arabidopsis, the ABC genes are encoded by *AP1*, *AP2*, *AP3*, *PI* and *AG* (Coen & Meyerowitz, 1991; Ng, 2001; Wagner, 1999; Lamb et al., 2002). Apart from *AP2*, which belongs to ERBP gene family; the ABC genes encode transcription factors belonging to the MADS-box gene family. The classic ABC model was based on studies of homeotic mutants in *Arabidopsis thaliana* and *Antirrhinum majus* (Coen & Meyerowitz, 1991). In this model, the different classes of homeotic genes and their overlapping activities are necessary to specify different floral organs, A specifies sepal, A and B specify petal, B and C specify stamen and C specifies carpel (Coen & Meyerowitz, 1991; Bowman et al., 1991). *AP1* and *AP2* are A-function genes, *AP3* and *PI* are B- function genes, and *AG* is C-function gene. However, the homeotic gene model that explains how floral identity is specified is now extended with D- and E-function genes. *SEPALLATA* (*SEP1-4*) are the E- function genes required for proper floral organ specifications in all four whorls (Ditta, Pinyopich, Robles, Pelaz, & Yanofsky, 2004; Pelaz, Ditta, Baumann, Wisman, & Yanofsky, 2000). In *sep1 sep2, sep3* and *spe4* quadruple mutants floral organs are converted into leaf-like organs demonstrating that the *SEP* genes are needed for the formation of all floral organs (Ditta et al., 2004; Pelaz et al., 2000). *SEEDSTICK* (*STK*), *SHATTERPROOF1* (*SHP1*) and *SHP2* are D-function genes that interact in a larger complex with the E-class genes to specify ovule identity (Favaro, 2003; Pinyopich et al., 2003).

In Arabidopsis, the *LFY* gene has been found to activate the expression of floral homeotic genes (Blázquez & Weigel, 2000; Weigel & Meyerowitz, 1993; Nilsson et al., 1998) by direct binding to the promoter regions of *AP1*, *AP3* and *AG* (Busch, 1999; Lohmann et al., 2001; Parcy, Nilsson, Busch, Lee, & Weigel, 1998; Wagner, 1999). In addition, *LFY* cooperates with UNUSUAL FLORAL ORGAN (UFO), an F-box component of the SCF ubiquitin ligase complex to regulate the transcription of the floral organ identity genes *AP1* and *AP3* (Lee et al., 1997; Chae, Tan, Hill, & Irish, 2008). Also, WUSCHEL (WUS), a homeodomain containing protein activate *AG* in conjunction with *LFY* (Lenhard, Bohnert, Jürgens, & Laux, 2001; Lohmann et al., 2001).

It has been proposed that the *AP2* has the function in the outer two floral whorls to specify the identities of sepals and petals by repressing the activity of *AG* (Drews, Bowman, & Meyerowitz, 1991). In *ap2* loss of function mutants,

ectopic expression of *AG* in the first two whorls results in the conversion of sepals and petals into carpels and stamens, respectively (Drews et al., 1991; Bowman et al., 1991).Whereas, in *ag* loss of function mutants, stamens are converted into petals in the 3rd whorl, and fourth whorl organs are converted into sepals (Bowman et al., 1991). Also, in *ag* mutants, the floral meristem does not terminate, and another flower is formed within the first flower, repeating the pattern: sepal, petal, petal sepal.

In contrast to the MADS-box genes, expression of the *AP2* gene, which encodes an ERF-transcription factor, is not restricted to one or two floral whorls (Jofuku, 1994). The mRNA of *AP2* is expressed in the vegetative leaf, inflorescence meristems, throughout the floral meristem and in all four types of floral organs (Jofuku, 1994). Hence, the spatial distribution of *AP2* transcription does not reconcile with its proposed function in regulating organ identity in the two outermost floral whorls. However, it has been reported that *AP2* is translationally repressed by microRNA172, which is expressed in the third and fourth whorls of the flower (X. Chen, 2004). Hence, the negative regulation of *AP2* by microRNA172 in the third and fourth whorls restrict *AP2* activity to the first and second whorls, even if *AP2* transcripts are present in all floral organs in Arabidopsis.

1.1.4 Conserved molecular mechanisms underlying reproductive development in gymnosperms and angiosperms

Evolutionary development studies show that the molecular mechanisms underlying reproductive development are at least partially conserved between angiosperms and gymnosperms (Mathews & Kramer, 2012; R. Melzer, Wang, & Theißen, 2010). The floral organ identity homeotic B and C genes orthologs have been identified in several gymnosperms (Gramzow, Weilandt, & Theißen, 2014). Studies of gene expression studies of B and C genes in gymnosperms have shown that the genes are active in developing male cones (Möuradov et al., 1999; Sundstr m et al., 1999; Winter et al., 1999) and that C-gene homologs are active in the ovuliferous scales, which is the ovule-bearing organ of female cones (Rutledge et al., 2002; Tandre, Svenson, Svensson, & Engström, 2002).

Even though proposed orthologs to the angiosperm B and C genes were cloned from different gymnosperm species, already in the early 1990s, a clear candidate for an A-function ortholog was lacking for a long time. This was attributed to the lack of a sterile perianth in gymnosperms. However, recent efforts to sequence the genomes of different conifer species have resulted in the identification of a putative A-class ortholog (Birol et al., 2013; Gramzow et al.,

2014; Nystedt et al., 2013). Since gymnosperms lack sepals and petal, the role of this putative A-class gene may be different from that of the angiosperm A-function genes. It is tempting to speculate that the function of a putative A-class *P. abies* ortholog would possibly be more related to meristem identity, rather than floral organ identity.

Orthologs of the *LFY* gene, which is crucial for reproductive meristem identity in angiosperms, have also been identified in diverse gymnosperm species (Frohlich and Meyerowitz, 1997). In conifers, two *LFY* homologs, *PRFLL* (Mellerowicz et al., 1998) and its paralog *NEEDLY* (*NLY*) (Mouradov et al., 1998) have been identified in *Pinus radiata*. Expression analyses of *LFY* and *NLY* homologs in other gymnosperms have shown that the genes are active both in female and male cones (Shindo, Sakakibara, Sano, Ueda, & Hasebe, 2001; Vázquez-Lobo et al., 2007).

SOC1 and FT are well known floral regulators in angiosperms and putative *SOC1* and *FT* conifer orthologs have been identified in the sequenced genome of *Picea abies* (Nystedt et al., 2013). The heterologous expression of *P. abies FT*-like gene in Arabidopsis delayed flowering and mimicked floral repressor *TFL1* function when overexpressed (Karlgren et al., 2011; Klintenäs, Pin, Benlloch, Ingvarsson, & Nilsson, 2012). On the other hand, *PaFTL2* has been associated with bud set and growth cessation in *P. abies* (Gyllenstrand, Clapham, Kallman, & Lagercrantz, 2007; Karlgren, Gyllenstrand, Clapham, & Lagercrantz, 2013).

It has been proposed that the putative *SOC1 P. abies* ortholog, *DEFICIENS AGAMOUS LIKE 19* (*DAL19*) is associated with an early cone-setting phenotype in the mutant *P. abies* var. *acrocona* (Uddenberg et al., 2013). In addition, the *P. abies DEFICIENS AGAMOUS LIKE 1* (*DAL1*) gene has been suggested to have a possible role in an age-dependent pathway that regulates reproductive development (Carlsbecker, Tandre, Johanson, Englund, & Engström, 2004). Ectopic expression of *DAL1* in transgenic Arabidopsis showed precocious flowering (Carlsbecker et al., 2004). In a phylogenetic study, *DAL1* and other gymnosperms genes formed an orthologous sister clade to the angiosperm AGAMOUS LIKE 6 (AGL6)-clade (Carlsbecker et al., 2013). The *AGL6* sub-family is closely related to the *SEP* (E-class) and *SQUAMOSA* (*SQUA*) sub-families in angiosperms. Interestingly, it has been proposed that *AGL6* gene functions redundantly with the *SEP* genes in *Petunia hybrida* (Rijpkema, Zethof, Gerats, & Vandenbussche, 2009). phenotype of transgenic Arabidopsis plants expressing a *P. abies, AGL6* ortholog is in agreement with the hypothesis that this conifer gene may be associated with cone-setting. In support of this notion, protein-protein and protein-DNA interaction studies among *Gnetum gnemon* MADS-box proteins suggest that AGL6-like proteins,

together with DEF/GLO-like (B-class) and AG-like proteins can form tetramer complexes (Y.-Q. Wang, Melzer, & Theißen, 2010).

1.2 *Picea abies* var. *Acrocona*

Picea abies var. *acrocona* is a naturally occurring mutant of Norway spruce. *Acrocona* is named for its ability to form cones in apical positions of top shoots ("acro" meaning "at the top"). Wild-type *P. abies* never form cones in this position, and vegetative buds always occupy the apical position of leading shoots. As in wild-type, twigs that set cones terminates, which renders homozygous *acrocona* mutants a bushy appearance. The *acrocona* mutant was first reported in the mid 19th century, from vicinities outside of Uppsala, Sweden (Fries, 1890). The *acrocona* mutant sets cones almost every year, even during years when wild-type Norway spruce does not produce cones. However, it also produces shoots with a transition phenotype that has both vegetative and reproductive characters (Carlsbecker et al., 2013). During years when environmental conditions are not favourable for cone production, the *acrocona* mutant produces transition shoots in apical positions on leading branches. The shoot apical meristems of these transition shoot initially form vegetative organ primordia at the base, but during development, the meristem makes a shift and starts to initiate ovuliferous scale-like structures subtended by bracts in the apical part of the shoot (see **Figure1**, presented in manuscript III).

In inbred crosses of two *acrocona* ramets, one-fourth of the resulting segregating sibling population segregated with respect to an early cone-setting phenotype (Uddenberg et al., 2013). Provided that the ramets were heterozygous, this suggested that a mutation in a single locus caused the *acrocona* phenotype. In agreement with this notion the early cone-setting *acrocona* mutant has been mapped to linkage group 6 in *P. abies* (Acheré et al., 2004). However, the gene responsible for this mutation is still unknown.

Due to the long generation time, reverse genetic approaches are difficult to employ in conifer trees. Next-generation sequencing technologies, *e.g.* RNA-*seq* now provide the opportunity to expand our studies of non-model species. Hence, the naturally occurring *acrocona* mutant with its transition shoot phenotype provides us with an opportunity to study the genetic network active during in cone initiation and cone development in *P. abies*.

2 Purpose of the study

From an evolutionary point of view, this thesis is aimed at providing additional genetic data to understand the evolutionary relationship between angiosperms and gymnosperms and how they evolved from a common ancestor; a question that was formulated already by Charles Darwin in his famous "Abominable Mystery"? Apart from the evolutionary context, this thesis is also aimed at improving forest tree breeding, by providing increased knowledge about the genetic mechanism that regulates reproductive phase change and cone-setting in conifers. A knowledge that could be used to facilitate future tree breeding programs.

Specifically, this thesis is focused on understanding the molecular mechanisms underlying reproductive phase change and cone-setting in *P. abies*. In line with this, specific aims have been to:

- Identify genes potentially involved in early cone development in three distinct growth phases in *P. abies*.
- Identify marker genes associated with different bud identities (male, female and vegetative).
- Identify genes potentially active in *acrocona* transition shoot meristems before the morphological shift to ovuliferous scale-like structures.
- Identify marker genes associated with female meristems identity.

3 Results and Discussions

3.1 Early cone development is distinguished by three distinct growth phases (Manuscript I)

In order to study differences in transcriptional regulation between female and vegetative buds, we have performed a morphological study of early bud development in *Picea abies*. This morphological study allows for a more accurate sampling of bud materials from a well-characterized sequence of early developmental stages.

The buds at the apical position of lateral branches in *P. abies* tend to initiate as vegetative or female cones, while, buds at the base become male cones. This pattern of cone initiation in *P. abies* is consistent with early bud development in Douglas fir, as reported by Allen and Owens, 1972. On the other hand, the buds at the shoot apex of apical branches are always vegetative. In our study, we collected vegetative buds from the apical position of leading branches in the same region of the tree as the female cones. This allowed us to compare similar developmental stages between female and vegetative buds since female buds and vegetative shoots at leading positions progress through early bud development at the approximate same time period. In contrast, bud development is delayed, relative to female bud development, in vegetative buds formed on lateral branches. Now, the question arises how female and vegetative buds can be distinguished on lateral branches, especially in early stages when the buds are relatively small in size. When the buds start to develop, female buds are formed with a rounded base and a bullet shape. In contrast, vegetative buds are formed with a narrow base and a pointed shaped tip. Also, during years of ample cone initiation, on cone-setting branches in the upper part of a tree, almost every lateral twig situated 20-30 cm below the leading shoot initiate a female cone. During the year of collection, we identified a candidate tree with ample cone

initiation and collected potential female buds and leading vegetative buds from branches in the cone-setting region of the tree. The tree was later revisited to certify that cone-initiation had occurred.

Using these collection criteria, we collected female buds and vegetative shoots at three collection dates, each corresponding to a characteristic growth phase (phase I to phase III) during early cone and vegetative shoot development.

During Phase I, the buds harboured enlarged meristems. The female buds collected at this phase harboured a rounded dome shaped meristem. In contrast to female buds in Phase I, the vegetative buds harboured meristems with a more pointed shape. In Phase II buds, the growing meristems had starting to initiate lateral organs acropetally forming early cones or vegetative shoots. Phase II female buds consisted of two types of scales: spirally arranged ovuliferous scale primordia each subtended by a sterile bract. Similarly, needles had initiated in Phase II vegetative shoots. Lateral organs had initiated along half portion of the bud meristem in Phase II. During Phase III, cellular differentiation occurred in the developing lateral organs. Almost all ovuliferous scales and needles had initiated in this phase. For this paragraph, see **Figure1**, presented in the manuscript I.

The morphological characterisation of early female and vegetative bud development provided a foundation for further molecular studies of the sequential changes in gene activities that occurred during early female cone development.

Using the collected buds, we aimed to study genes active during the three defined growth phases and compare expression levels between both growth phases and bud identity. With this aim, we performed massively parallel sequencing of mRNA from the collected female bud samples of three distinct growth phases and compared the transcriptomes in those samples with mRNA samples extracted from the corresponding vegetative buds.

3.1.1 RNA-seq analysis of early cone development phases reveal distinct gene expression pattern in vegetative and female buds, reflect important markers for female and vegetative bud identity (Manuscript I)

In order to identify genes active during early phases of cone development, we sequenced the transcriptomes of female and vegetative bud samples collected at the meristematic phase (Phase I; collection date August 1st), the lateral organ initiation phase (Phase II; collection date August 19th) and during the phase when lateral organs undergo cell differentiation (Phase III; collection date September 16. In a principal component analysis (PCA) analysis the samples

were separated both based on bud identity, *i.e.* vegetative or female identity, and developmental phase, suggesting that different transcriptional programmes are active during early female and vegetative bud development.

By performing a differential expression analysis on normalised read counts we identified genes that were significantly differentially expressed between female and vegetative buds at the three defined growth phases. The analysis showed different numbers of differentially expressed genes between female and vegetative buds in each phase. In order to identify genes that were commonly associated with distinct phases of early female cone development, we applied a hierarchical cluster analysis of all differentially expressed genes. The analysis showed that the differentially expressed genes could be grouped into ten major clusters based on their gene expression profiles. In Cluster 1 to Cluster 4, genes were up-regulated in female buds, whereas Cluster 5 to Cluster 10 harboured genes that were up-regulated in vegetative buds. Cluster 1 could be further sub-divided into Cluster 1a and Cluster 1b. Cluster 1a harboured genes that predominantly expressed in female buds during the first meristematic phase as compared to vegetative, whereas expression was elevated in female buds in all three developmental phases in Cluster 1b. Cluster 2 harbored genes that predominantly expressed in female buds during the lateral organ initiating phase II. In Cluster 3a and 3b, we identified genes which expressed at high levels in female buds as compared to vegetative buds in the first two phases, however, at the third phase, those genes expressed at similar levels in both female and vegetative buds. Genes in these clusters, therefore, may be associated with similar processes in female and vegetative buds, during the third growth phase. Cluster 4 harbuored genes that predominantly expressed in cell differentiating female buds during phase III. Here, a schematic representation of gene expression patterns between female and vegetative buds in three distinct growth phases is given in **Figure 3**.

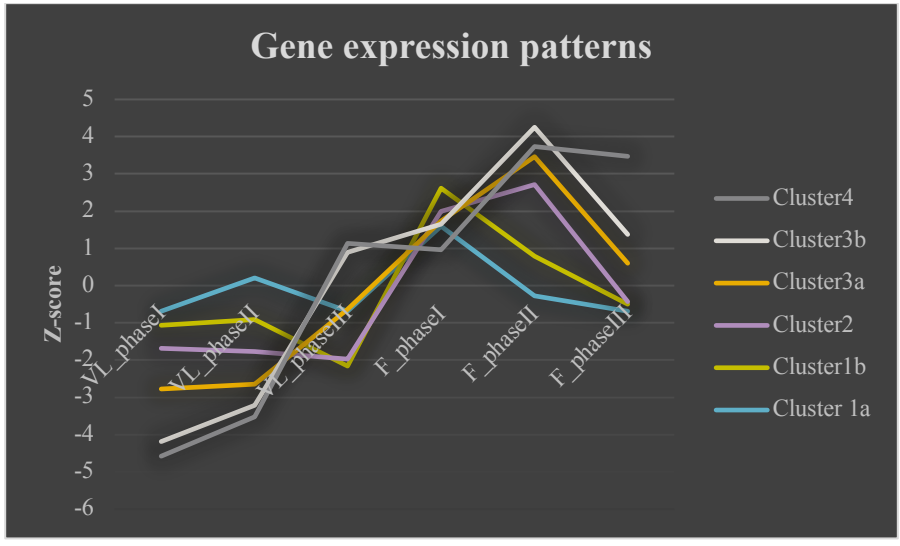

Figure 3. Schematic figure of gene expression patterns between vegetative and female buds in three distinct growth phases. This graph is based on Z- scores from converted expression count data of one example gene in each cluster. The X-axis represents different Clusters (1a, 1b, 2, 3a, 3b & 4); the y-axis represents Z-scores.

In order to identify important biological, molecular and cellular processes associated with these genes, we performed a Gene Ontology (GO) analysis on the differentially expressed genes. The analysis showed that DNA binding and protein dimerisation were enriched GO terms in Cluster 1 (a, b), 3 (a, b) and 4. Other processes such as metabolic and catalytic processes were also enriched in Cluster 1, 3 and 4. In Cluster 2, extracellular region, chromatin binding and photosynthesis were enriched GO terms.

We identified transcription factors associated with the enriched GO terms "DNA-binding" and "protein dimerisation" in the different clusters. In Clusters, 1a and 1b, genes encoding MADS-domain proteins, bZIP transcription factors, SBP-proteins, *LFY*- orthologs, AP2-domain, MYB-domain, B3, and AT-hook transcription factors were identified. No transcription factors were found in Cluster 2. In Cluster 3a and 3b, we identified MADS-box genes and bHLH transcription factors. In Cluster 4, NAC and AP2 transcription factors were identified.

Based on the transcriptome study, a schematic representation of the association of transcription factor gene families in different clusters is given in **Figure 4.**

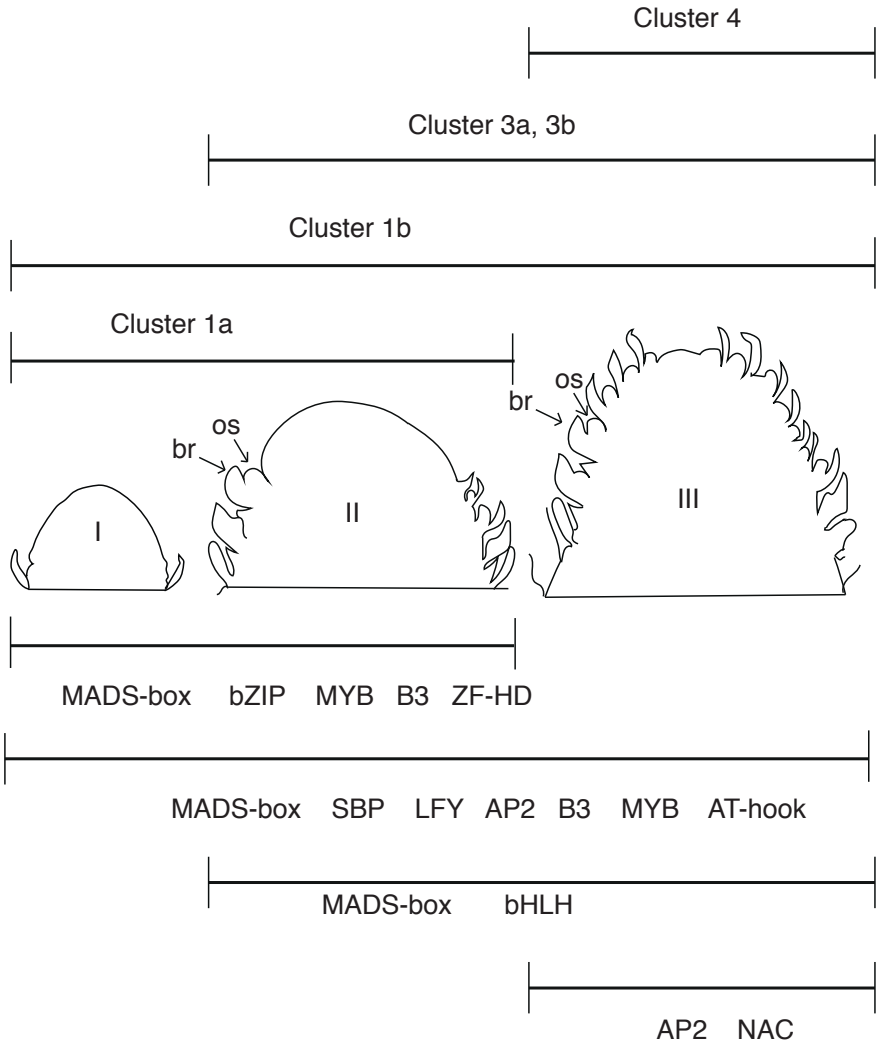

Figure 4. Schematic figure of the association of transcription factor gene families in different clusters during the three distinct growth phases of female bud development. Roman numbers denote growth phases; large shoot apical meristem containing female bud during phase I (I); lateral organ initiating female bud during phase II (II); cell differentiating female buds in phase III (III). This figure is a drawing of longitudinal sections of female buds at three distinct growth phases. Small letters with arrow towards bud sections indicate lateral organs; bract (br) and ovuliferous scale (os). Straight lines above the longitudinal bud

sections representing association of clusters (Cluster 1a, 1b, 3a, 3b & 4) with three distinct growth phases buds; Straight lines below the longitudinal bud sections represent transcription factors (MADS-box, bZIP, MYB, B3, ZF-HD, SBP, LFY, AP2, AT-hook, bHLH, NAC) associated with different clusters and bud growth phases.

Among the MADS-box genes identified in our study as being up-regulated in female meristems, several have previously been characterized. Some of the genes have suggested functions during later stages of cone development and some have been suggested to promote reproductive shoot identity and cone-setting. The reported in activity in early meristems corroborate these previous findings.

In order to increase our understanding of the evolutionary relationships between MADS-box genes associated with the early phases of female cone development, we reconstructed a phylogeny with MADS-box gene sequences from both selected angiosperms and gymnosperms. The phylogenetic reconstruction showed, that several of the MADS-box genes in our data, that had not been characterized previously formed gymnosperm specific sub-clades in the tree. These MADS-box genes were predominantly expressed in female meristems during phase I as compare to the other two phases. Hence, our results identify several conifer specific MADS-box genes expressed preferentially in female meristems. It is tempting to speculate that these genes may have roles in defining reproductive identity to the female meristems, analogous to the function of MADS-box genes that determine inflorescence meristem identify in angiosperms.

It has been shown that SOC1 is a multifunctional protein, which regulates not only flowering time but also floral patterning and floral meristem identity (C. Liu, Xi, Shen, Tan, & Yu, 2009; S. Melzer et al., 2008).

Our phylogenetic reconstructions indicated that several of the previously uncharacterized MADS-box genes identified in this study grouped within a large sub-clade of gymnosperm genes. This sub-clade, termed the DAL19-clade, have in previous studies been proposed to be a gymnosperm specific sister-clade to the angiosperm SOC1/TM3-like clade (Gramzow et al., 2014; Uddenberg et al., 2013). In the differential expression analysis genes in this clade were expressed at a high level in either female or vegetative buds. Hence, our data suggest that members of the *SOC1/TM3*-like genes play diverse roles in *P. abies* buds and may be of importance for the development of both vegetative and reproductive shoots.

In the analysis, we identified the *LFY* ortholog *PaLFY* as being specifically up-regulated in phase III female buds. The up-regulation of *PaLFY* is consistent with previous mRNA *in-situ* hybridization experiments, which have

demonstrated that *PaLFY* is specifically expressed in developing ovuliferous scales in autumn cones (Carlsbecker et al., 2013).

3.2 Mutually exclusive exons of MADS-box gene *DAL19* isoforms distinctly associated with male, female and vegetative bud identity in *Picea abies* (Manuscript II)

In previous studies, two *DAL19* mRNA transcripts (Acr42124 and KC347015) have been identified (Carlsbecker et al., 2013; Uddenberg et al., 2013). In order to identify potential differences between the two transcripts, we aligned the sequences to each other and looked for sequence differences. In the alignment, nucleotides that differed were predominantly situated in the MADS-domain, which commonly is encoded by the first exon in many MADS-box genes, see, *e.g*. (Sundstr m et al., 1999). Mapping of the two published transcripts of *DAL19* to the published *P. abies* genome (*Picea abies* V 1.0) showed that the sequence corresponding to the MADS-domain (M) in Acr42124 mapped to one genomic scaffold (MA_329880), whereas the sequence corresponding to the MADS-domain in KC347015 mapped to another (MA_16120). The remaining parts of the *DAL19* nucleotide sequence in both Acr42124 and KC347015 mapped to the genomic scaffolds MA_54911, MA_844703 and MA_166116. Hence, the different first exons of *DAL19* were named as *DAL19_α* and *DAL19_β* respectively.

In order to verify the presence *DAL19* transcripts that harboured alternate first exons, we performed 3' and 5'RACE to clone and Sanger sequence the different *DAL19* isoforms. The 3' RACE resulted in a version of *DAL19* with an alternate C-terminal domain as compared to previously reported 3′ end associated with the genomic scaffold MA_16120. This alternate C-terminal domain of *DAL19* mapped to the genomic scaffold MA_16120 but in a different position than that of the previously reported 3′ end. Hence, the different C-terminal isoforms of *DAL19* were named *DAL19_δ* and *DAL19_γ* respectively. *DAL19_δ* mapped to the position 4650–4944, whereas *DAL19_γ* mapped to the position 3239–3505 at the genomic scaffold MA_16120 (see **Figure 1**, presented in manuscript II). The mapping of alternative C-terminal exons to the same genomic scaffold suggested that the identified variants of *DAL19* were transcribed from a single large genomic locus, although the two N-terminal exons, *DAL19_α* and *DAL19_β* mapped to two different genomic scaffolds. In support of this notion all *DAL19* exons, including *DAL19_α* and *DAL19_β* mapped to same genomic contig in the *Picea glauca* genome (PG29-V4.0), with which *P. abies* shares substantial sequence similarity (Sundell et al., 2015).

On the *P. glauca* scaffold, *DAL19_α* and *DAL19_β* mapped approximately 16 kb apart and they were in turn separated from the next exon in *DAL19* by an intron of approximately 100 kb. The introns separating *DAL19* exons that encode the I- and K -domains are relatively short and consist only of approximately one hundred bases each. The exons encoding the I- and K-domains were common in all *DAL19* transcripts and the region was in this work defined as a core region (*ψ*). The core region was separated from the two variable C-terminal exons by a 28 kb intron. Apart from the full-length MIKC variants of *DAL19* two short variants of *DAL19* that lacked MADS-domain were also identified. Taken together, our data revealed six variants of *DAL19*: four long variants with alternate first and last exons (*DAL19_αψδ, DAL19_αψγ, DAL19_βψδ*, and *DAL19_βψγ*), and two short variants (*DAL19_ψδ* and *DAL19_ψγ*). *DAL19_αψγ* and *DAL19_βψγ* variants are relatively shorter than *DAL19_αψδ* and *DAL19_βψδ* and harbor premature stop codon at the C terminal end. Taken together, the association of all *DAL19* variants on the same genomic scaffold in *P. glauca,* and alternate C-terminal exons on the same genomic scaffold in *P. abies* suggest that the mature mRNA *DAL19* variants are indeed isoforms and transcribed from a single large genomic locus.

In order to provide independent evidence of the cloned *DAL19* mRNA isoforms, we developed a novel approach to assemble transcripts from short-read Illumina sequences. As data-source, we used mRNA samples derived from *P. abies* buds. Starting from a defined sequence this approach generates transcripts for every possible 5′ and 3′ path. The assembly showed consistency with cloned and Sanger sequenced *DAL19* isoforms. Also, our transcripts assembly approach identified one putative additional *DAL19* isoform, which instead of *DAL19_α* or DAL19-*β* harboured a third alternate MADS-domain. We named this isoform *DAL19_ η*. The method of our novel assembly approach is presented in manuscript II.

Apart from Sanger sequencing, and short-read assembly of different *DAL19* isoforms, we also found support for the presence of the *DAL19* isoforms among Pacbio isoseq circular consensus sequence derived from a pool of 33 *P. abies* sample (Akther et al., 2018). The Pacbio isoseq sequences of *P. abies* sequences are the result of a community-based effort to which our group has contributed with reproductive samples.

In order to examine if the *DAL19* isoforms were differentially expressed in different tissue samples, we performed isoform-specific expression analysis using quantitative Real-Time PCR (qRT-PCR), normalized read count data from the RNA-*seq* experiments, and mRNA *in-situ* hybridization. The expression analyses showed that the *DAL19* isoforms with alternate first exons, encoding the MADS-domain, expressed in a bud specific manner. *DAL19_α* was

preferentially up-regulated in male buds, whereas *DAL19_ η* expressed predominantly in female buds, DAL19-*β* was in turn up-regulated in vegetative buds. On the other hand, the alternate C-terminal ends of *DAL19* expressed in a cell-specific manner within a single bud meristem. Hence, it suggests that cell specific splicing can occurs within a single bud meristem.

Next, we applied the assembly method to other known MADS-box genes in order to examine if they also were expressed as isoforms. The assembly identified usage of alternate first exons in the MADS-box genes: *DAL3, DAL3_like, DAL4, DAL32,* and *DAL33*. Using our method, we did not find any evidence of isoforms in exons encoding the intervening region (I) or the K-domain in any of the MADS-box genes analysed.

In order to analyse the evolutionary relationship between the *P. abies* MADS-box genes, and known angiosperms MADS-box genes, we performed a phylogenetic parsimony analysis. Interestingly in our phylogenetic analysis all *P. abies* genes harbouring alternate MADS-domain isoforms, e.g., *DAL3, DAL4, DAL19, DAL32*, and *DAL33*, grouped into one common clade. Whereas *P. abies* MADS-box genes that express only as single isoforms were distributed evenly in the gene phylogeny. The clade which harboured *DAL3, DAL4, DAL19, DAL32*, and *DAL33* have in previous (Gramzow et al., 2014; Uddenberg et al., 2013) analyses been proposed to be a gymnosperm specific sister-clade to the angiosperm TM3/SOC1-clade, and has been termed the DAL19-clade. The position of the DAL19-clade in our phylogenetic tree supports this notion.

Here, a schematic representation of *DAL19* gene clade phylogeny is given in **Figure 5**.

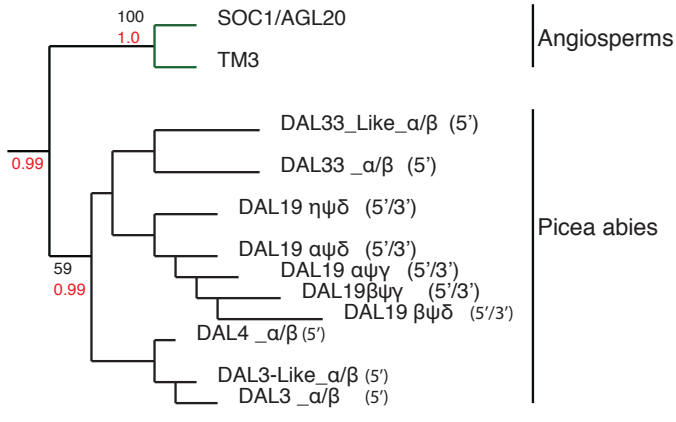

Figure 5. Schematic figure of the phylogenetic relationship between MADS-box genes present in the DAL19-clade. This figure is a drawing from a phylogeny established with

maximum parsimony analysis. Greek alphabets indicating mutually exclusive first and last exons isoforms; α, and β representing first exon isoforms; δ and γ representing last exon isoforms. Values in black represent bootstrap support and red represent posterior probability support derived from a MrBayes-analysis.

The expansion of the members of genes in the DAL19-clade has been observed in previous analyses the MADS-box gene family (F. Chen, Zhang, Liu, & Zhang, 2017; Gramzow et al., 2014). From our study, we can conclude that the complexity of the DAL19-clade and the observed expansion in the number of genes, may in part be due to frequent usage of mutually exclusive first exons.

Taken together, our results suggest that *DAL19* isoforms that use mutually exclusive first exons were expressed in a bud-identity specific manner. The first exon encodes the DNA-binding MADS-domain. Hence, it is possible that the different isoforms of *DAL19* bind to the different sets of target genes and that the *DAL19* isoforms in this manner may regulate different sets of downstream targets genes. *DAL19* has been proposed to have similar roles as a floral integrator as *SOC1/TM3* (Uddenberg et al., 2013). Our findings on the differential regulation of *DAL19* isoforms support the notion that alternative splicing of *DAL19* isoforms may influence the formation of buds with different identities.

3.3 Transcript profiling of *acrocona* transition shoots identifies candidate genes of importance for female bud development in *Picea abies* (Manuscript III)

In contrast to wild-type *P. abies*, the mutant *P. abies* var. *acrocona* produce ovuliferous scale-like structures on the apical shoot of leading branches. The leading branches in wild-type trees are always initiated as vegetative buds. The bud meristem on apical branches produce vegetative needles at the base and later in the growth season they make a morphological shift and start to produce ovuliferous scales and bracts instead of needles. Due to the morphological shift from needle to ovuliferous scale-like structures subtended by bracts, *acrocona* leading shoots are often described as transition shoots. During years of ample cone induction, buds on lateral shoots produce normal female cones in a similar manner as wild-type *P. abies*. Here, we address the hypothesis that the *acrocona* transition shoot meristems express markers for female reproductive development already before they make the morphological shift.

In our study, we aimed to identify genes active in *acrocona* transition shoots before and after the morphological shift. To meet this end, we sequenced transcriptomes of collected *acrocona* transition shoots before and after morphological shift using massively parallel sequencing (RNA-*seq*). In the samples collected before the morphological shift, the buds consisted of enlarged

shoot apical meristems with only a few or no lateral organs initiated. In the samples collected after morphological shift, needles had been formed in the basal part of the shoot, and bracts and ovuliferous scale-like structures had been formed in the apical part of the shoot. We collected ordinary female meristems from *acrocona* as a comparator. In addition, we included samples of wild-type vegetative and female buds as comparators with an aim to identify commonly expressed genes in *acrocona* transition shoots, female *acrocona* and female wild-type buds. In order to identify the changes of gene expression patterns across collected tissues, we performed differential gene expression analyses on pairwise comparisons of the different tissues. The analyses showed different numbers of differentially expressed genes in paired tissue comparisons.

In order to identify genes that are distinctly and commonly up-regulated across different samples, we implemented a hierarchical clustering method on all differentially expressed genes. The analysis identified eleven major clusters based on differential gene expression profiles across all samples used in comparisons. Based on the gene expression profiles across different clusters, we focused on Cluster 1 (A, B), 4 (A, B), 8A & 11 (A, B). Genes in Cluster 1A were predominantly expressed in vegetative buds at late developmental phase but down-regulated in transition shoot meristems and female meristems from both wild-type and *acrocona* samples. Genes in Cluster 1B were commonly up-regulated in vegetative buds at the late developmental phase, transition shoot meristems and female meristems from both wild-type and *acrocona*.

In the Clusters 4A and 4B, genes were up-regulated in transition shoot- and *acrocona* female meristems but down-regulated in wild-type female meristems collected at similar stages. We identified transcription factors in Cluster 4A & 4B, including members of the MADS-box gene family, SBP-family and the AP2 family, which have been reported to be involved in regulating flower development in angiosperms (Jofuku, 1994; Kim et al., 2012; Mandel & Yanofsky, 1995a; Bowman et al., 1993).

In the Cluster 8A, genes were commonly expressed in transition meristems and female meristems from both *acrocona* and wild-type. We identified several members of transcription factor gene families in this cluster, including MADS-box genes, bZIP, SBP, AP2, Homeobox, GRAS, GATA, MYB and AT-hook containing transcription factors.

In Cluster 8A, we also identified the gymnosperm specific MADS-box genes *DAL10* and *DAL21,* which both have been associated with reproductive shoot identity (Carlsbecker et al., 2013; 2003), and members of the DAL19-clade. Hence, it suggests with an implication that these gene members in this cluster associated with cone-setting competence active early in developing *acrocona*

transition shoots, even before it is possible to discriminate the transition shoot from an ordinary vegetative shoot, based on morphology.

We found a gene in this cluster associated with the GO-term "regulation of flower development" which encoded a phosphatidylethanolamine-binding protein. The PEBP-protein family harbours the angiosperm genes *FLOWERING LOCUS T (FT) and TERMINAL FLOWER1 (TFL1)* (Xiaohong Zhang et al., 2016). In *P. abies*, *FT-like* genes have been associated with bud set and growth cessation (Gyllenstrand et al., 2007; Karlgren et al., 2013). Therefore, the observed up-regulation of a *FT*-like gene in transition shoots and female meristems may be related to the cessation of growth of last season's annual shoot.

In this cluster, we also identified a *SPL3*-like gene. In Arabidopsis, *SPL3* has been shown to be an important factor regulating the activity of *FT* (Kim et al., 2012). Also, it has been proposed that miRNA156 targeted degradation of *SPL3* is important for regulating flowering in an FT/FD-independent manner (J.-W. Wang, Czech, & Weigel, 2009). At the shoot apex, *SPL3* act as a positive regulator of *SOC1*, which in turn activate the floral meristem identity genes *LFY* and *AP1*.(J. Lee, Oh, Park, & Lee, 2008).

In our data, the *FT*-like gene, together with bZIP transcription factors and gymnosperm *SOC1*-like genes are up-regulated in transition shoot meristems and female meristems both in wild type and *acrocona*. Even though we at this point cannot determine the molecular function of the conifer genes they provide us with interesting candidates for further studies. The joint expression of these genes in reproductive meristems allows us to speculate about a possible interaction between one of the bZIP transcription factors and the FT-like protein, analogous to the interaction between FD and FT in Arabidopsis, and possible activation of the *P. abies SOC1*-like gene that is expressed in the same tissues.

Using the same reasoning, the *SPL3*-like gene expressed in the transition shoot meristems and female meristems both in wild-type and *acrocona* could be a putative candidate for a gene involved cone-setting pathway in an age-dependent manner, analogous to the interaction between *SPL3* and *FT* or *SPL3* and *SOC1* in Arabidopsis.

The up-regulation of distinct floral integrator like genes in the transition shoot meristems, together with the up-regulation of gymnosperm specific MADS-box genes that previously have been associated with reproductive shoot identity, provide joint evidence that these shoots have reproductive characters even when they still initiate needles. Hence, the genes identified as differentially expressed between the vegetative shoots and the reproductive shoots in our comparison likely do not reflect differences in morphology but rather shoot identity.

Based on our transcriptomes study, a schematic representation of putative candidate genes in transition and female meristems is given in **Figure 6**.

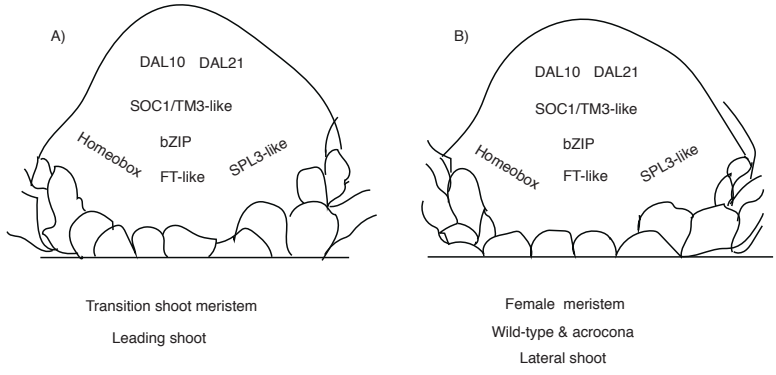

Figure 6. Schematic figure of the association of candidate genes and gene families (Homeobox, *FT*-like, bZIP, *SPL3*-like, *SOC*/*TM3*-like, *DAL10* and *DAL21*) in transition shoot meristem (A) and female meristem from both *acrocona* and wild type (B) *P. abies*.

3.4 GA signalling may be involved in the transition of vegetative to female organ in *acrocona* (Manuscript III)

In contrast to ordinary female meristems, the initiation of ovuliferous scales occurs later in the growth season in the transition shoots. However, the question arise what kind of genetic mechanisms that are involved in the transition from vegetative needles to ovuliferous scales in the transition shoots. To find a possible answer to this question, we studied genes up-regulated in *acrocona* transition shoots. We focused on Cluster 11 (A & B). In Cluster 11A, relative to vegetative samples, genes were commonly up-regulated in the transition shoot meristems and transition shoots after the morphological shift. While, in the Cluster 11B, genes were only up-regulated in the transition shoots after morphological shift. We identified transcription factors, including MADS-box, AP2, bZIP, GATA, HSF and MYB gene families in these clusters.

It has been known since the mid-1970s that the plant hormone gibberellin (GA) induce cone setting in *P. abies* (Chalupka, 1978) and GA treatments are regularly used to promote cone-setting in seed orchards (Högberg K-A and Eriksson U, 1994). Interestingly, GA treatments occasionally lead to the formation of transition shoots also in wild-type *P. abies* (C.Almqvist personal communication). In our analysis DELLA proteins, which are negative regulators

of GA-responsive genes, are expressed at a high level in early *acrocona* samples. Whereas genes encoding F-box proteins and ubiquitin are expressed at high levels in the late *acrocona* samples. F-box and ubiquitin proteins play diverse roles in plant development as they are part of several plant hormonal signalling pathways, including the GA-signalling pathway (Dill, 2004). It is tempting to speculate that *acrocona* transition shoots, which express reproductive identity genes, have the competence to respond to a late autumn GA-signal and produce ovuliferous scale-like structures, and that this response is reflected in the observed up-regulation of F-box and ubiquitin proteins. Here, an illustration of transition shoots after morphological shift and the association of F-box and ubiquitin proteins and other gene families are given in **Figure 7.**

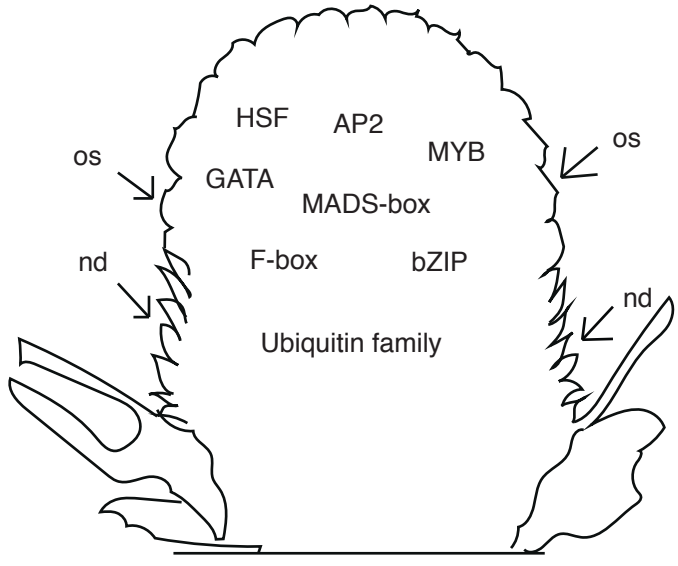

Figure 7. Schematic figure of the association of genes encoding F-box protein and Ubiquitin and other gene families; (MADS-box, bZIP, AP2, MYB, GATA, HSF) in the transition shoots after morphological shift. Small letters with arrow towards transition shoot indicates needle (nd) formation at the base, and ovuliferous scale formation at the top (os).

4 Conclusion

These are the main conclusions from the works presented in this thesis:

Differential expression analysis and hierarchical cluster analysis identified sets of genes that express sequentially in the early phases of female cone development.

Genes mainly involved in transcriptional regulation are up-regulated in the meristematic phase of female bud development.

Genes predominantly expressed in female buds during lateral organ initiation encode proteins involved in diverse cellular activities such as cell division, chromatin organisation and protein synthesis.

Cell differentiation in lateral organs of female buds is associated with genes involved in photosynthesis, carbon metabolism and transcriptional regulation.

Expression of different isoforms of the MADS-box gene *DAL19* are associated with different bud identities in *P. abies*.

Genes associated with reproductive development are active in transition shoot meristems, even before it is possible to discriminate transition meristems from vegetative meristem.

Members of Homeobox, MADS-box, PEBP, bZIP and SBP gene family co-expressed in transition meristems and female meristems both from *acrocona* and wildtype.

Genes encoding F-box proteins and ubiquitin are expressed at high levels in late *acrocona* transition shoots, which suggest that hormonal signaling may be associated with the *acrocona* transition shoot phenotype.

5 Relevance of the research for forest tree breeding

Forests are important sources of biodiversity and also have significant economic uses. Forest trees serve great economic value, mainly by providing the stock of woods all over the world and tree breeding has become an integral part of the management of forest, to increase the quality of trees to meet diverse needs and values. Conifer trees are good sources of softwood and highly demanded in the world because of timber, paper, and pulp production. In Europe, Norway spruce (*Picea abies* (L.) Karst) and Scots pine (*Pinus sylvestris* L.) are the most economic conifer tree species. The worldwide demand for wood production is increasing, and therefore, the increase in demand will have to be met by increased production of quality of trees. An efficient tree breeding strategy can fulfil these needs by increasing forest production. *P. abies* and *P. sylvestris* are the most important commercial tree species in the Swedish forestry with continuous breeding programs. The forest research institute of Sweden (Skogforsk) has been running the breeding operations of *P. abies* and *P. sylvestris* and some other Swedish tree species since the mid 20th century, and *P. abies* is now in its third breeding cycle. In Sweden, seed orchards are the primary source for the production of improved genetic materials; still, only 70 percent of the annual planting of Norway spruce plants originates from the Swedish seed orchards (www.skogforsk.se). In a report from skogforsk by Haapanen et al. 2015, it was stated that the demand for spruce seed will not be met until 2030 due to a shortage of seed production and the limited supply of improved seed (www.skogforsk.se). The long juvenile period of *P. abies*, around 20-25 years before developing cones is one of the main reasons for limited seed supply because the long juvenile period slows breeding operations. Moreover, irregular cone setting between trees even after achieving reproductive competence add another obstacle in breeding operations. Pests and pathogens are also reducing the number of seeds produced. In traditional breeding methods, controlled crosses are applied between selected plus-trees with desired characteristics such as phenology and growth. After the controlled crosses, progeny testing is applied to select plus-trees. Under southern Swedish

condition, plus-trees selection is carried out 6-10 years after planting, while in northern Swedish condition, the progeny testing is done 10-20 years after planting. Hence, tree breeders need to wait at least a decade before they can do the next round of controlled crosses.

The implementation of modern biotechnological tools in advanced breeding methods, including hybridization, mutational breeding, marker-assisted selection, and gene technology has become increasingly popular in many crop plants. However, due to the long generation time, forest biotechnology applications are difficult to perform in conifer trees. The short generation time of many angiosperm species, makes it possible to perform functional studies of stable transformants over several generations. Due to the long generation time, similar studies are currently not possible to perform in conifers. Hence, control of the long generation-time is of particular interest in conifer research. Understanding the molecular and genetic mechanisms behind reproductive development phase changes in spruce is vital to control the length of the juvenile time-period. Controlling this important trait will, in turn, facilitate studies of gene functions and thus has the potential considerably speed up forest tree breeding.

6 Future perspectives

Future research includes the following ideas, possibilities and goals:

The findings of genes that express at high levels in early reproductive meristems of *Picea abies* provide us with candidates for future functional studies of genes potentially involved in early cone development. Off course the long generation time of *P. abies* poses a significant problem for functional studies of genes involved in reproductive development. However, the early cone-setting phenotype of the *acrocona* mutant could facilitate such studies.

Establishment of embryogenic cultures of the *acrocona* mutant would allow us to produce *P. abies* plants that would initiate cones within a reasonable time-frame, *i.e*. one to two years.

Provided that transformation protocols can be established for the *acrocona* cell-lines, these could then be used to perform knock-down or over-expression studies of genes with putative functions in reproductive structures.

Candidate genes that would be interesting to study the function of are the *FT/TFL*-like genes, the *SOC1*-like MADS-box genes, and *SBP*-like gene that are expressed at high levels in *acrocona* transition shoot meristems.

To further characterise the selected candidate genes, green fluorescent protein (GFP)/ Red fluorescent protein (RFP) tagged reporter lines could be used to study their localised expression.

Potentially this could lead to the identification of a gene that controls cone-setting also in wild-type *P. abies*.

On another note, it would be interesting to study further the effect of external applications of GA or GA-inhibitors on both cone-setting in the *acrocona* mutant, and the expression of the candidate genes identified in this PhD-project.

Potentially this could contribute to an increased understanding of the role of GA in promoting cone-setting in *P. abies*. This knowledge could then be used to increase the precision in the GA treatments that are used to enhance cone-setting in seed orchards.

References

Abe, M. (2005). FD, a bZIP Protein Mediating Signals from the Floral Pathway Integrator FT at the Shoot Apex. *Science*, *309*(5737), 1052–1056. http://doi.org/10.1126/science.1115983

Acheré, V., Faivre-Rampant, P., Jeandroz, S., Besnard, G., Markussen, T., Aragones, A., et al. (2004). A full saturated linkage map of Picea abies including AFLP, SSR, ESTP, 5S rDNA and morphological markers. *Theoretical and Applied Genetics*, *108*(8), 1602–1613. http://doi.org/10.1007/s00122-004-1587-y

Akhter, S., Kretzschmar, W. W., Nordal, V., Delhomme, N., Street, N. R., Nilsson, O., et al. (2018). Integrative Analysis of Three RNA Sequencing Methods Identifies Mutually Exclusive Exons of MADS-Box Isoforms During Early Bud Development in Picea abies. *Frontiers in Plant Science*, *9*, 1625. http://doi.org/10.3389/fpls.2018.01625

Alejandra Mandel, M., Gustafson-Brown, C., Savidge, B., & Yanofsky, M. F. (1992). Molecular characterization of the Arabidopsis floral homeotic gene APETALA1. *Nature*, *360*(6401), 273–277. http://doi.org/10.1038/360273a0

Alvarez, J., Guli, C. L., Yu, X.-H., & Smyth, D. R. (1992). terminal flower: a gene affecting inflorescence development in Arabidopsis thaliana. *The Plant Journal*, *2*(1), 103–116. http://doi.org/10.1111/j.1365-313X.1992.00103.x

Amasino, R. (2010). Seasonal and Developmental Timing of Flowering. The Plant journal: for cell and molecular biology, 61(6), 1001–1013

Andrés, F., & Coupland, G. (2012). The genetic basis of flowering responses to seasonal cues. *Nature Reviews Genetics*, *13*(9), 627–639. http://doi.org/10.1038/nrg3291

Aubert, D. (2001). EMF1, A Novel Protein Involved in the Control of Shoot Architecture and Flowering in Arabidopsis. *The Plant Cell Online*, *13*(8), 1865–1875. http://doi.org/10.1105/tpc.13.8.1865

Bell, C. D., Soltis, D. E., & Soltis, P. S. (2010). The age and diversification of the angiosperms re-revisited. *American Journal of Botany*, *97*(8), 1296–1303. http://doi.org/10.3732/ajb.0900346

Birol, I., Raymond, A., Jackman, S. D., Pleasance, S., Coope, R., Taylor, G. A., et al. (2013). Assembling the 20 Gb white spruce (Picea glauca) genome from whole-genome shotgun sequencing data. *Bioinformatics*, *29*(12), 1492–1497. http://doi.org/10.1093/bioinformatics/btt178

Byrne M.E., Simorowski J., Martienssen R.A. (2002). *ASYMMETRIC LEAVES1* reveals *knox* gene redundancy in Arabidopsis. Development, 1298(1), 1957–1965.

Blázquez, M. A., & Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. *Nature*, *404*(6780), 889–892. http://doi.org/10.1038/35009125

Bossinger G., Smyth D.R. (1996). Initiation patterns of flower and floral organ development in *Arabidopsis thaliana*. *Development*, 1228(1), 1093–1102.

Bowman J.L., Smyth D.R., Meyerowitz E.M. (1991). Genetic interactions among floral homeotic genes of Arabidopsis. *Development*, 1128(1), 1–20.

Bowman, J. L., & Eshed, Y. (2000). Formation and maintenance of the shoot apical meristem. *Trends in Plant Science*, *5*(3), 110–115. http://doi.org/10.1016/S1360-1385(00)01569-7

Bowman J.L., Alvarez J., Weigel D., Meyerowitz E.M., Smyth D.R. Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. (1993). *Development*, 1198(1), 721–743.

Brand, U. (2000). Dependence of Stem Cell Fate in Arabidopsis on a Feedback Loop Regulated by CLV3 Activity. *Science*, *289*(5479), 617–619. http://doi.org/10.1126/science.289.5479.617

Busch, M. A. (1999). Activation of a Floral Homeotic Gene in Arabidopsis. *Science*, *285*(5427), 585–587. http://doi.org/10.1126/science.285.5427.585

Byrne, M. E., Barley, R., Curtis, M., Arroyo, J. M., Dunham, M., Hudson, A., & Martienssen, R. A. (2000). Asymmetric leaves1 mediates leaf patterning and stem cell function in Arabidopsis. *Nature*, *408*(6815), 967–971. http://doi.org/10.1038/35050091

Caboche, M. (1994). Arabidopsis, an atlas of morphology and development: John Bowman, Springer Verlag, 167 figures, 450 pp. 148 D. Mark. *Plant Science*, *102*(1), 118. http://doi.org/10.1016/0168-9452(94)90026-4

Carlsbecker, A., Sundstrom, J., Tandre, K., Englund, M., Kvarnheden, A., Johanson, U., & Engström, P. (2003). The DAL10 gene from Norway spruce (Picea abies) belongs to a potentially gymnosperm-specific subclass of MADS-box genes and is specifically active in seed cones and pollen cones. *Evolution & Development*, *5*(6), 551–561. http://doi.org/10.1046/j.1525-142X.2003.03060.x

Carlsbecker, A., Sundström, J. F., Englund, M., Uddenberg, D., Izquierdo, L., Kvarnheden, A., et al. (2013). Molecular control of normal and acroconamutant seed cone development in Norway spruce (Picea abies) and the evolution of conifer ovule-bearing organs. *New Phytologist*, *200*(1), 261–275. http://doi.org/10.1111/nph.12360

Carlsbecker, A., Tandre, K., Johanson, U., Englund, M., & Engström, P. (2004). The MADS-box gene DAL1 is a potential mediator of the juvenile-to-adult transition in Norway spruce (Picea abies). *The Plant Journal*, *40*(4), 546–557. http://doi.org/10.1111/j.1365-313X.2004.02226.x

Chae, E., Tan, Q. K.-G., Hill, T. A., & Irish, V. F. (2008). An Arabidopsis F-box protein acts as a transcriptional co-factor to regulate floral development. *Development*, *135*(7), 1235–1245. http://doi.org/10.1242/dev.015842

Chalupka W. (1978). Effect of growth regulators on flowering of Norway spruce (Picea abies(L.) Karst.) grafts. *Institute of Dendrology*, 63- 220.

Chandler, L. M., & Owens, J. N. (2004). The pollination mechanism of Abies amabilis. *Canadian Journal of Forest Research*, *34*(5), 1071–1080. http://doi.org/10.1139/x03-255

Chen, F., Zhang, X., Liu, X., & Zhang, L. (2017). Evolutionary Analysis of MIKCc-Type MADS-Box Genes in Gymnosperms and Angiosperms. *Frontiers in Plant Science*, *8*, 403. http://doi.org/10.3389/fpls.2017.00895

Chen, L. (1997). EMF Genes Regulate Arabidopsis Inflorescence Development. *The Plant Cell Online*, *9*(11), 2011–2024. http://doi.org/10.1105/tpc.9.11.2011

Chen, X. (2004). A MicroRNA as a Translational Repressor of APETALA2 in Arabidopsis Flower Development. *Science*, *303*(5666), 2022–2025. http://doi.org/10.1126/science.1088060

Clark S.E., Running M.P., Meyerowitz E.M. CLAVATA1, a regulator of meristem and flower development in Arabidopsis. (1993). *Development*, 1198(1), 397–418.

Clark S.E., Running M.P., Meyerowitz E.M. CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. (1995). *Development*, 1218(1), 2057–2067.

Coen, E. S., & Meyerowitz, E. M. (1991). The war of the whorls: genetic interactions controlling flower development. *Nature*, *353*(6339), 31–37. http://doi.org/10.1038/353031a0

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., et al. (2007). FT Protein Movement Contributes to Long-Distance Signaling in Floral Induction of Arabidopsis. *Science*, *316*(5827), 1030–1033. http://doi.org/10.1126/science.1141752

Darwin, C. (1859). On the Origin of Species (London).

Dill, A. (2004). The Arabidopsis F-Box Protein SLEEPY1 Targets Gibberellin Signaling Repressors for Gibberellin-Induced Degradation. *The Plant Cell Online*, *16*(6), 1392–1405. http://doi.org/10.1105/tpc.020958

Ditta, G., Pinyopich, A., Robles, P., Pelaz, S., & Yanofsky, M. F. (2004). The SEP4 Gene of Arabidopsis thaliana Functions in Floral Organ and Meristem Identity. *Current Biology*, *14*(21), 1935–1940. http://doi.org/10.1016/j.cub.2004.10.028

Doyle, J. A. (1978). Origin of Angiosperms. *Annual Review of Ecology and*

Systematics, *9*(1), 365–392. http://doi.org/10.1146/annurev.es.09.110178.002053
Doyle, J. A. (2012). Molecular and Fossil Evidence on the Origin of Angiosperms. *Dx.Doi.org*, *40*(1), 301–326. http://doi.org/10.1146/annurev-earth-042711-105313
Drews, G. N., Bowman, J. L., & Meyerowitz, E. M. (1991). Negative regulation of the Arabidopsis homeotic gene AGAMOUS by the APETALA2 product. *Cell*, *65*(6), 991–1002. http://doi.org/10.1016/0092-8674(91)90551-9
Eis, S. (1967). CONE CROPS OF WHITE AND BLACK SPRUCE ARE PREDICTABLE. *The Forestry Chronicle*, *43*(3), 247–252. http://doi.org/10.5558/tfc43247-3
Eis, S. (1973). Cone Production of Douglas-fir and Grand Fir and its Climatic Requirements. *Canadian Journal of Forest Research*, *3*(1), 61–70. http://doi.org/10.1139/x73-009
Endress, P. K. (2011). Angiosperm ovules: diversity, development, evolution. *Annals of Botany*, *107*(9), 1465–1489. http://doi.org/10.1093/aob/mcr120
Favaro, R. (2003). MADS-Box Protein Complexes Control Carpel and Ovule Development in Arabidopsis. *The Plant Cell Online*, *15*(11), 2603–2611. http://doi.org/10.1105/tpc.015123
Fletcher, J. C. (1999). Signaling of Cell Fate Decisions by CLAVATA3 in Arabidopsis Shoot Meristems. *Science*, *283*(5409), 1911–1914. http://doi.org/10.1126/science.283.5409.1911
Florin, R. (1951). Evolution in cordaites and conifers. *Acta Horti Bergiani,* 15, 285–388.
Fraser, D. A. (2011). VEGETATIVE AND REPRODUCTIVE GROWTH OF BLACK SPRUCE (PICEA MARIANA (MILL.) BSP.) AT CHALK RIVER, ONTARIO, CANADA. *Canadian Journal of Botany*, *44*(5), 567–580. http://doi.org/10.1139/b66-069
Friis, E. M., Crane, P. R., & Pedersen, K. R. (2011). Early Flowers and Angiosperm Evolution. Cambridge: Cambridge University Press. http://doi.org/10.1017/CBO9780511980206
Fries, T. M. (1890). Strödda Bidrag Till Kännedom Om Skandinaviens Barrträd. Bot Not, 1, 250– 260.
Frohlich, M. W. & Meyerowitz, E. M. (1997). JSTOR: International Journal of Plant Sciences, Vol. 158, No. 6 (Nov., 1997), Pp. S131-S142. International Journal of Plant Sciences.
Gelbart, G., & Aderkas, von, P. (2002). Ovular secretions as part of pollination mechanisms in conifers. *Annals of Forest Science*, *59*(4), 345–357. http://doi.org/10.1051/forest:2002011
Gramzow, L., & Theissen, G. (2010). A hitchhiker's guide to the MADS world of plants. *Genome Biology*, *11*(6), 214. http://doi.org/10.1186/gb-2010-11-6-214
Gramzow, L., Weilandt, L., & Theißen, G. (2014). MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in

seed plants. *Annals of Botany*, *114*(7), 1407–1429. http://doi.org/10.1093/aob/mcu066
G.S. Allen & J.N. Owens. (1972) The Life History of Douglas-Fir. Forestry Service, Environment Canada, Ottawa, 139.
Gyllenstrand, N., Clapham, D., Kallman, T., & Lagercrantz, U. (2007). A Norway Spruce FLOWERING LOCUS T Homolog Is Implicated in Control of Growth Rhythm in Conifers. *Plant Physiology*, *144*(1), 248–257. http://doi.org/10.1104/pp.107.095802
Herendeen, P. S., Friis, E. M., Pedersen, K. R., & Crane, P. R. (2017). Palaeobotanical redux: revisiting the age of the angiosperms. *Nature Plants*, *3*(3), 284. http://doi.org/10.1038/nplants.2017.15
Högberg K-A & Eriksson U (1994) Effects of Root Pruning and Stem Injections with Gibberellin A 4/7 on Flowering and Cone Harvest in Three Picea Abies Seed Orchards. *Scandinavian Journal of Forest Research,* 9, 323-328.
Huala, E., & Sussex, I. M. (1992). LEAFY Interacts with Floral Homeotic Genes to Regulate Arabidopsis Floral Development. *The Plant Cell*, *4*(8), 901–913. http://doi.org/10.1105/tpc.4.8.901
Huijser, P. & Schmid, M. (2011). The Control of Developmental Phase Transitions in Plants. Development, 138(19), 4117–4129.
Jofuku, K. D. (1994). Control of Arabidopsis Flower and Seed Development by the Homeotic Gene APETALA2. *The Plant Cell Online*, *6*(9), 1211–1225. http://doi.org/10.1105/tpc.6.9.1211
Kardailsky, I. (1999). Activation Tagging of the Floral Inducer FT. *Science*, *286*(5446), 1962–1965. http://doi.org/10.1126/science.286.5446.1962
Karlgren, A., Gyllenstrand, N., Clapham, D., & Lagercrantz, U. (2013). FLOWERING LOCUS T/TERMINAL FLOWER1-Like Genes Affect Growth Rhythm and Bud Set in Norway Spruce. *Plant Physiology*, *163*(2), 792–803. http://doi.org/10.1104/pp.113.224139
Karlgren, A., Gyllenstrand, N., Källman, T., Sundström, J. F., Moore, D., Lascoux, M., & Lagercrantz, U. (2011). Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. *Plant Physiology*, *156*(4), 1967–1977. http://doi.org/10.1104/pp.111.176206
Kayes J. M., Clark S. E. CLAVATA2, a regulator of meristem and organ development in Arabidopsis. (1998). *Development*, 1258(1), 3843–3851.
Kempin, S., Savidge, B., & Yanofsky, M. (1995). Molecular basis of the cauliflower phenotype in Arabidopsis. *Science*, *267*(5197), 522–525. http://doi.org/10.1126/science.7824951
Kim, J. J., Lee, J. H., Kim, W., Jung, H. S., Huijser, P., & Ahn, J. H. (2012). The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 Module Regulates Ambient Temperature-Responsive Flowering via FLOWERING LOCUS T in Arabidopsis. *Plant Physiology*, *159*(1), 461–478. http://doi.org/10.1104/pp.111.192369
Klintenäs, M., Pin, P. A., Benlloch, R., Ingvarsson, P. K., & Nilsson, O. (2012). Analysis of conifer FLOWERING LOCUS T/ TERMINAL

FLOWER1-like genes provides evidence for dramatic biochemical evolution in the angiosperm FTlineage. *New Phytologist*, *196*(4), 1260–1273. http://doi.org/10.1111/j.1469-8137.2012.04332.x
Koornneef, M., Hanhart, C. J., & van der Veen, J. H. (1991). A genetic and physiological analysis of late flowering mutants in Arabidopsis thaliana. *Molecular and General Genetics MGG*, *229*(1), 57–66. http://doi.org/10.1007/BF00264213
Kwiatkowska, D. (2005). Flower primordium formation at the Arabidopsis shoot apex: quantitative analysis of surface geometry and growth. *Journal of Experimental Botany*, *57*(3), 571–580. http://doi.org/10.1093/jxb/erj042
Kwiatkowska, D. (2008). Flowering and apical meristem growth dynamics. *Journal of Experimental Botany*, *59*(2), 187–201. http://doi.org/10.1093/jxb/erm290
Lamb R.S., Hill T.A., Tan Q.K., Irish V.F. (2002). Regulation of *APETALA3* floral homeotic gene expression by meristem identity genes. *Development*, 1298(1), 2079–2086.
Laux T., Mayer K.F., Berger J., Jürgens G. (1996) The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis. *Development*, 1228(1), 87–96.
Lee I., Wolfe D.S., Nilsson O., Weigel D. A LEAFY co-regulator encoded by *UNUSUAL FLORAL ORGANS.* Curr. Biol. 1997;78(1):95–104.
Lee, J., Oh, M., Park, H., & Lee, I. (2008). SOC1 translocated to the nucleus by interaction with AGL24 directly regulates LEAFY. *The Plant Journal*, *55*(5), 832–843. http://doi.org/10.1111/j.1365-313X.2008.03552.x
Lenhard, M., Bohnert, A., Jürgens, G., & Laux, T. (2001). Termination of Stem Cell Maintenance in Arabidopsis Floral Meristems by Interactions between WUSCHEL and AGAMOUS. *Cell*, *105*(6), 805–814. http://doi.org/10.1016/S0092-8674(01)00390-7
Leslie, A. B. (2010). Flotation preferentially selects saccate pollen during conifer pollination. *New Phytologist*, *188*(1), 273–279. http://doi.org/10.1111/j.1469-8137.2010.03356.x
Leslie, A. B., & Kevin Boyce, C. (2012). Ovule Function and the Evolution of Angiosperm Reproductive Innovations. *International Journal of Plant Sciences*, *173*(6), 640–648. http://doi.org/10.1086/665818
Linkies, A., Graeber, K., Knight, C., & Leubner-Metzger, G. (2010). The evolution of seeds. *New Phytologist*, *186*(4), 817–831. http://doi.org/10.1111/j.1469-8137.2010.03249.x
Litt ,A. & Irish VF. (2003). Duplication and diversification in the APETALA1/FRUITFULL floral homeotic gene lineage: implications for the evolution of floral development.*Genetics*, 165(2), 821-33.
Liu, C., Xi, W., Shen, L., Tan, C., & Yu, H. (2009). Regulation of Floral Patterning by Flowering Time Genes. *Developmental Cell*, *16*(5), 711–722. http://doi.org/10.1016/j.devcel.2009.03.011
Lohmann, J. U., Hong, R. L., Hobe, M., Busch, M. A., Parcy, F., Simon, R., & Weigel, D. (2001). A Molecular Link between Stem Cell Regulation and

Floral Patterning in Arabidopsis. *Cell*, *105*(6), 793–803. http://doi.org/10.1016/S0092-8674(01)00384-1
Mandel M.A., Gustafson-Brown C., Savidge B., Yanofsky M.F. (1992) Molecular characterization of the Arabidopsis floral homeotic gene *APETALA1*. *Nature*, 3608(1), 273–277.
Mandel, M. A., & Yanofsky, M. F. (1995a). A gene triggering flower formation in Arabidopsis. *Nature*, *377*(6549), 522–524. http://doi.org/10.1038/377522a0
Mandel, M. A., & Yanofsky, M. F. (1995b). The Arabidopsis AGL8 MADS Box Gene Is Expressed in Inflorescence Meristems and Is Negatively Regulated by APETALA1. *The Plant Cell*, *7*(11), 1763. http://doi.org/10.2307/3870185
Mathews, S., & Kramer, E. M. (2012). The evolution of reproductive structures in seed plants: a re-examination based on insights from developmental genetics. *New Phytologist*, *194*(4), 910–923. http://doi.org/10.1111/j.1469-8137.2012.04091.x
Mayer, K. F. X., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G., & Laux, T. (1998). Role of WUSCHEL in Regulating Stem Cell Fate in the Arabidopsis Shoot Meristem. *Cell*, *95*(6), 805–815. http://doi.org/10.1016/S0092-8674(00)81703-1
Mellerowicz, E. J., Horgan, K., Walden, A., Coker, A., & Walter, C. (1998). PRFLL - a Pinus radiata homologue of FLORICAULA and LEAFY is expressed in buds containing vegetative shoot and undifferentiated male cone primordia. *Planta*, *206*(4), 619–629. http://doi.org/10.1007/s004250050440
Melzer, R., Wang, Y.-Q., & Theißen, G. (2010). The naked and the dead: The ABCs of gymnosperm reproduction and the origin of the angiosperm flower. *Seminars in Cell & Developmental Biology*, *21*(1), 118–128. http://doi.org/10.1016/j.semcdb.2009.11.015
Melzer, S., Lens, F., Gennen, J., Vanneste, S., Rohde, A., & Beeckman, T. (2008). Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. *Nature Genetics*, *40*(12), 1489–1492. http://doi.org/10.1038/ng.253
Mouradov, A., Glassick, T., Hamdorf, B., Murphy, L., Fowler, B., Marla, S., & Teasdale, R. D. (1998). NEEDLY, a Pinus radiata ortholog of FLORICAULA/LEAFY genes, expressed in both reproductive and vegetative meristems. *Proceedings of the National Academy of Sciences*, *95*(11), 6537–6542. http://doi.org/10.1073/pnas.95.11.6537
Mouradov, A., Hamdorf, B., Teasdale, R. D., Kim, J. T., Winter, K.-U., & Thei en, G. N. (1999). ADEF/GLO-like MADS-box gene from a gymnosperm:Pinus radiata contains an ortholog of angiosperm B class floral homeotic genes. *Developmental Genetics*, *25*(3), 245–252. http://doi.org/10.1002/(SICI)1520-6408(1999)25:3<245:AID-DVG7>3.0.CO;2-N
Ng, M. (2001). Activation of the Arabidopsis B Class Homeotic Genes by

APETALA1. *The Plant Cell Online*, *13*(4), 739–754. http://doi.org/10.1105/tpc.13.4.739
Nilsson O., Lee I., Blazquez M.A., Waigel D. (1998). Flowering time genes modulate the response to LEAFY activity. Genetics, 1508(1), 403–410.
Nystedt, B., Street, N. R., Wetterbom, A., Zuccolo, A., Lin, Y.-C., Scofield, D. G., et al. (2013). The Norway spruce genome sequence and conifer genome evolution. *Nature*, *497*(7451), 579–584. http://doi.org/10.1038/nature12211
Ohshima, S., Murata, M., Sakamoto, W., Ogura, Y., & Motoyoshi, F. (1997). Cloning and molecular analysis of the Arabidopsis gene Terminal Flower 1. *Molecular and General Genetics MGG*, *254*(2), 186–194. http://doi.org/10.1007/s004380050407
Owens, J. N. (1969). The relative importance of initiation and early development on cone production in Douglas fir. *Canadian Journal of Botany*, *47*(7), 1039–1049. http://doi.org/10.1139/b69-148
Owens, J.N., and Molder, M. (1984). The reproductive cycle of interior spruce [*Picea glauca* and *P.engelmannii*]. Ministry of Forests, Inform. Serv. Bra., Victoria, BC. 30 pp.
Owens, J. N., & Smith, F. H. (1964). THE INITIATION AND EARLY DEVELOPMENT OF THE SEED CONE OF DOUGLAS FIR. *Canadian Journal of Botany*, *42*(8), 1031–1047. http://doi.org/10.1139/b64-096
Ó'Maoiléidigh, D. S., Graciet, E., & Wellmer, F. (2013). Gene networks controlling Arabidopsis thalianaflower development. *New Phytologist*, *201*(1), 16–30. http://doi.org/10.1111/nph.12444
Parcy, F., Nilsson, O., Busch, M. A., Lee, I., & Weigel, D. (1998). A genetic framework for floral patterning. *Nature*, *395*(6702), 561–566. http://doi.org/10.1038/26903
Pelaz, S., Ditta, G. S., Baumann, E., Wisman, E., & Yanofsky, M. F. (2000). B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature*, *405*(6783), 200–203. http://doi.org/10.1038/35012103
Pinyopich, A., Ditta, G. S., Savidge, B., Liljegren, S. J., Baumann, E., Wisman, E., & Yanofsky, M. F. (2003). Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature*, *424*(6944), 85–88. http://doi.org/10.1038/nature01741
Rijpkema, A. S., Zethof, J., Gerats, T., & Vandenbussche, M. (2009). The petunia AGL6gene has a SEPALLATA-like function in floral patterning. *The Plant Journal*, *60*(1), 1–9. http://doi.org/10.1111/j.1365-313X.2009.03917.x
Rutledge, R., Regan, S., Nicolas, O., Fobert, P., Côté, C., Bosnich, W., et al. (2002). Characterization of anAGAMOUShomologue from the conifer black spruce (Picea mariana) that produces floral homeotic conversions when expressed inArabidopsis. *The Plant Journal*, *15*(5), 625–634. http://doi.org/10.1046/j.1365-313x.1998.00250.x
Sablowski, R. (2007). Flowering and determinacy in Arabidopsis. *Journal of Experimental Botany*, *58*(5), 899–907. http://doi.org/10.1093/jxb/erm002

Sauquet, H., Balthazar, von, M., Magallón, S., Doyle, J. A., Endress, P. K., Bailes, E. J., et al. (2017). The ancestral flower of angiosperms and its early diversification. *Nature Communications*, *8*, 16047. http://doi.org/10.1038/ncomms16047

Schulz, C., Klaus, K. V., Knopf, P., Mundry, M., Dörken, V., & Stützel, T. (2014). Male Cone Evolution in Conifers: Not All That Simple. *American Journal of Plant Sciences*, *05*(18), 2842–2857. http://doi.org/10.4236/ajps.2014.518300

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H., & Sommer, H. (1990). Genetic Control of Flower Development by Homeotic Genes in Antirrhinum majus. *Science*, *250*(4983), 931–936. http://doi.org/10.1126/science.250.4983.931

Shannon, S. (1991). A Mutation in the Arabidopsis TFL1 Gene Affects Inflorescence Meristem Development. *The Plant Cell Online*, *3*(9), 877–892. http://doi.org/10.1105/tpc.3.9.877

Shannon, S. (1993). Genetic Interactions That Regulate Inflorescence Development in Arabidopsis. *The Plant Cell Online*, *5*(6), 639–655. http://doi.org/10.1105/tpc.5.6.639

Shindo, S., Sakakibara, K., Sano, R., Ueda, K., & Hasebe, M. (2001). Characterization of a FLORICAULA/ LEAFYHomologue of Gnetum parvifoliumand Its Implications for the Evolution of Reproductive Organs in Seed Plants. *International Journal of Plant Sciences*, *162*(6), 1199–1209. http://doi.org/10.1086/323417

Silen, Roy R. (1967). How early can Douglas-fir cone crops be predicted? Proc. West. Refor. Coord. Comm., West. For. and Conserv. Assoc.,12-17.

Simpson, G. G. (2002). Arabidopsis, the Rosetta Stone of Flowering Time? *Science*, *296*(5566), 285–289. http://doi.org/10.1126/science.296.5566.285

Soltis, P. S. (2005). Ancient and recent polyploidy in angiosperms. *New Phytologist*, *166*(1), 5–8. http://doi.org/10.1111/j.1469-8137.2005.01379.x

Song, Y. H., Ito, S., & Imaizumi, T. (2013). Flowering time regulation: photoperiod- and temperature-sensing in leaves. *Trends in Plant Science*, *18*(10), 575–583. http://doi.org/10.1016/j.tplants.2013.05.003

Steemans, P., Herisse, A. L., Melvin, J., Miller, M. A., Paris, F., Verniers, J., & Wellman, C. H. (2009). Origin and Radiation of the Earliest Vascular Land Plants. *Science*, *324*(5925), 353–353. http://doi.org/10.1126/science.1169659

Sundell, D., Mannapperuma, C., Netotea, S., Delhomme, N., Lin, Y.-C., Sjödin, A., et al. (2015). The Plant Genome Integrative Explorer Resource: PlantGenIE.org. *New Phytologist*, *208*(4), 1149–1156. http://doi.org/10.1111/nph.13557

Sundstr m, J., Carlsbecker, A., Svensson, M. E., Svenson, M., Johanson, U., Thei en, G. N., & Engstr m, P. (1999). MADS-box genes active in developing pollen cones of Norway spruce (Picea abies) are homologous to the B-class floral homeotic genes in angiosperms. *Developmental*

Genetics, *25*(3), 253–266. http://doi.org/10.1002/(SICI)1520-6408(1999)25:3<253::AID-DVG8>3.0.CO;2-P
Takaso, T., & Bouman, F. (1986). Ovule and seed ontogeny inGnetum gnemon L. *The Botanical Magazine Tokyo*, *99*(3), 241–266. http://doi.org/10.1007/BF02489542
Tandre, K., Svenson, M., Svensson, M. E., & Engström, P. (2002). Conservation of gene structure and activity in the regulation of reproductive organ development of conifers and angiosperms. *The Plant Journal*, *15*(5), 615–623. http://doi.org/10.1046/j.1365-313x.1998.00236.x
Tiren, L. (1935). On the fruit setting of spruce, its periodicity and relation to temperature and precipitation. In reports of the Swedish Institute of Experimental Forestry (Stockholm, Statens Skogs försöksanstalt), pp. 413-524.
Tomlinson, P. B., Braggins, J. E., & Rattenbury, J. A. (1991). Pollination Drop in Relation to Cone Morphology in Podocarpaceae: A Novel Reproductive Mechanism. *American Journal of Botany*, *78*(9), 1289–1303. http://doi.org/10.2307/2444932
Tsai, W.-C., Pan, Z.-J., Su, Y.-Y., & Liu, Z.-J. (2014). New Insight into the Regulation of Floral Morphogenesis (Vol. 311, pp. 157–182). Elsevier. http://doi.org/10.1016/B978-0-12-800179-0.00003-9
Uddenberg, D., Reimegard, J., Clapham, D., Almqvist, C., Arnold, von, S., Emanuelsson, O., & Sundstrom, J. F. (2013). Early Cone Setting in Picea abies acrocona Is Associated with Increased Transcriptional Activity of a MADS Box Transcription Factor. *Plant Physiology*, *161*(2), 813–823. http://doi.org/10.1104/pp.112.207746
van der Niet, T., & Johnson, S. D. (2012). Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution*, *27*(6), 353–361. http://doi.org/10.1016/j.tree.2012.02.002
Vázquez-Lobo, A., Carlsbecker, A., Vergara-Silva, F., Alvarez-Buylla, E. R., Piñero, D., & Engström, P. (2007). Characterization of the expression patterns of LEAFY/FLORICAULA and NEEDLY orthologs in female and male cones of the conifer genera Picea, Podocarpus, and Taxus: implications for current evo-devo hypotheses for gymnosperms. *Evolution & Development*, *9*(5), 446–459. http://doi.org/10.1111/j.1525-142X.2007.00182.x
Wagner, D. (1999). Transcriptional Activation of APETALA1 by LEAFY. *Science*, *285*(5427), 582–584. http://doi.org/10.1126/science.285.5427.582
Wang, J.-W., Czech, B., & Weigel, D. (2009). miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. *Cell*, *138*(4), 738–749. http://doi.org/10.1016/j.cell.2009.06.014
Wang, Y.-Q., Melzer, R., & Theißen, G. (2010). Molecular interactions of orthologues of floral homeotic proteins from the gymnosperm Gnetum gnemon provide a clue to the evolutionary origin of “floral quartets.” *The Plant Journal*, *64*(2), 177–190. http://doi.org/10.1111/j.1365-

313X.2010.04325.x
Weigel, D., & Meyerowitz, E. M. (1993). Activation of floral homeotic genes in Arabidopsis. *Science*, *261*(5129), 1723–1726. http://doi.org/10.1126/science.261.5129.1723
Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F., & Meyerowitz, E. M. (1992). LEAFY controls floral meristem identity in Arabidopsis. *Cell*, *69*(5), 843–859. http://doi.org/10.1016/0092-8674(92)90295-N
Westerman, J. M., & Lawrence, M. J. (1970). Genotype-environment interaction and developmental regulation in Arabidopsis thaliana I. Inbred lines; description. *Heredity*, *25*(4), 609–627. http://doi.org/10.1038/hdy.1970.66
Wickett, N. J., Mirarab, S., Nguyen, N., Warnow, T., Carpenter, E., Matasci, N., et al. (2014). Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(45), E4859–68. http://doi.org/10.1073/pnas.1323926111
Wigge, P. A. (2005). Integration of Spatial and Temporal Information During Floral Induction in Arabidopsis. *Science*, *309*(5737), 1056–1059. http://doi.org/10.1126/science.1114358
Winter, K. U., Becker, A., Munster, T., Kim, J. T., Saedler, H., & Theissen, G. (1999). MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. *Proceedings of the National Academy of Sciences*, *96*(13), 7342–7347. http://doi.org/10.1073/pnas.96.13.7342
Zhang, Xiaohong, Wang, C., Pang, C., Wei, H., Wang, H., Song, M., et al. (2016). Characterization and Functional Analysis of PEBP Family Genes in Upland Cotton (Gossypium hirsutum L.). *Plos One*, *11*(8), e0161080. http://doi.org/10.1371/journal.pone.0161080

Acknowledgements

I would like to express my sincere gratitude to my principal supervisor Jens Sundström for assigning me on this interesting project. Your continuous support in my PhD study with patience and knowledge helped me in scientific thinking and writing this thesis. I enjoyed most of the discussions we had together about the results in different projects. You had listened to me with the intend to understand and smartly discussed the matter.

I would like to express my sincere thanks to my co-supervisor Malin Elfstrand for giving me the bright ideas on my transcriptomes project. Your logical and brainy comments in my thesis helped to improve it. You have always been supportive in my PhD study. You are a positive person, and your positive attitude helped me to feel control in difficult moments. I am fortunate that you accepted to be my co-supervisor.

My sincere thanks also go to my co-supervisor Olof Emanuelsson for his all support in my project. You had found time to read my manuscripts and provided valuable comments even you have other essential works.

I thank my previous co-supervisor Sara Von Arnold for her all ideas and support in my project at the starting time of my PhD.

I thank to Veronika and Warren to give support in this project. I Would like to thank Nico for teaching me the different parts of RNA-*seq* analysis and providing support when I had problems. Your all inputs to my transcriptomes project helped me to sort issues and to get excellent output.

I would like to thank Ove Nilsson, Nathaniel Robert Street for their feedback on manuscripts. Thanks to our collaborator Curt, Mats, and Eva from Skogforsk.

Thanks to Daniel Uddenberg for giving me useful information on Somatic embryogenesis of spruce at the beginning of my PhD. To Kanita and Gunnar for their help to maintain our spruce embryonic culture and Marie for giving me useful information on *in-situ* hybridization technique.

I would like to thank Eva Sundberg, Lotta, Qing, Monika and Mattias for their important support. Thanks to Björn to solve all computer errors. Urban and per for their help in the phytotron. Anna and Anneli for making all the products ordered in time.

Thanks to Anders, par and Sofia to evaluate my PhD study.

I would like to thank my previous project supervisors Sadhna and Björn for supervising me in a scientific project during my master's thesis.

Many thanks for being excellent to me at the working place: Cecilia, Katarina, Georgios, Jose, Pruthbi, Pascal, Nathan Zivi, Kai, Anders, Suayb, Kristian, Anna Åsman, Reza, Chandra, Vikash, Lejon, Miyuki, Stefan, Minerva, Xinyi, Rami, Anna, Luz, Shamik, Laurene. Thanks, Anis for creating make-up night club and had fun with all club members. Thanks to Prashanth for being an excellent colleague in the department. I always get useful information's from you. Thanks to Margherita for having our beautiful moments during my starting time in the lab. Thanks also to all past and present members that I haven't written name here.

Last but not least, I would like to thank my all family members, relatives and friends for their support. Special thanks to "My Mother" for being my best friend.