Genetic and molecular analysis of trichome and root hair development in *Arabis alpina*

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Table of Contents

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Zusammenfassung

Forschungsansätze der evolutionären Entwicklungsbiologie ('EvoDevo'-Biologie) werden standardmäßig genutzt um homologe Prozesse in nah verwandten Spezies zu untersuchen. Diese Studie beschreibt eine vergleichende Analyse der Trichom- und Wurzelhaarentwicklung der nah verwandten Modellpflanzen der Pflanzenfamilie der Kreuzblütler, *Arabis alpina* und *Arabidopsis thaliana*. Wie zuvor gezeigt werden konnte, unterscheiden sich die Prozesse der Trichom- und Wurzelhaarentwicklung zwischen *A. alpina* und *A. thaliana*. Während in Arabidopsis Trichome regelmäßig auf der Blattoberfläche verteilt sind und Wurzelhaare in Einzelzellreihen ('H-files') vorkommen, die an Nicht-Haarzellreihen ('N-files') angrenzen, sind in Arabis Blätter dicht mit kleinen und großen Trichomen bedeckt und Wurzeln bilden ektopische Wurzelhaare in Nicht-Haarzellreihen. Um die genetische Grundlage der Trichom- und Wurzelhaarentwicklung in *A. alpina* zu verstehen, wurden zwei EMS-mutagenisierte Pflanzenpopulationen untersucht. Mutanten mit Defekten bezüglich Trichom- und Wurzelhaar-Musterbildung und -morphologie wurden ausgewählt. Die Analyse ergab einen ähnlichen Umfang an Mutantenphänotypen, wie sie auch für *A. thaliana* bekannt sind. Mutanten wurden im Anschluss im Hinblick auf betroffene Kandidatengene sequenziert um zugehörige Mutantenallele in Arabis zu lokalisieren. Mutationen in den Arabis-Genen *TTG1* und *TRY* führten zu den gleichen Phänotypen, wie für Arabidopsis erwartet wurde. Arabis *gl3* (*Aagl3*)-mutanten zeigten unbehaarte Blatt- und stark behaarte Wurzelphänotypen, ähnlich wie die gl3 egl3-Mutante in *A. thaliana*. Des Weiteren resultierte Überexpression von Arabis GL3 in *A. alpina* in einer reduzierten Trichomzahl, was auf eine veränderte Funktion von GL3 im Laufe der Evolution hindeutet. Ähnlicherweise änderte sich vermutlich die Funktion von Arabis GL2, da es seine Funktion nur in einer Untergruppe von Trichomen ausübt. Außerdem präsentiere ich die Identifizierung von Mutanten mit

morphologischen Defekten während der Wurzelhaarentwicklung. *Aakjk*, *Aacow1*, *Aascn1*, *Aaark1* und *Aaspi* sind identifizierte Wurzelhaarmutanten, die in der Wurzelhaarbildung von *A. alpina* beeinträchtigt sind. Für mehrere Allele solcher morphologisch beeinträchtigter Mutanten wurde ebenfalls einem Aminosäureaustausch in ihrer Proteinsequenz identifiziert.

Abstract

Evolutionary developmental (Evo-Devo) approaches are commonly used to compare homologous processes in closely related species. In this study, we use *Arabis alpina*, as a second model system of Brassicaceae to study trichome and root hair development and compare it to *Arabidopsis thaliana*. It has been shown previously that trichome and root hair pattern in *A. alpina* is different from *A. thaliana*. In Arabidopsis, trichomes are regularly spaced on the surface of the leaf and root hairs are found in single cell files (H-files) adjacent to the non-hair files (N-files). Arabis leaves are densely covered with small and big trichomes, and roots produce ectopic hairs in N file (Chopra et al. 2014). To understand the genetic basis of trihchome and root hair development in *A. alpina*, two EMS populations were screened. Mutants with defects in trichome (Chopra 2015, PhD thesis) and root hair patterning and morphology were selected. Screen results in *A. alpina* show a similar range of mutant phenotypes as known in *A. thaliana*. Mutants were then sequenced for candidate genes to find the specific mutant alleles in *A. alpina*. Mutations in *Arabis TTG1* (Chopra et al. 2014) and *TRY* (Chopra 2015, PhD thesis) lead to the same phenotypes as expected from *A. thaliana*. However, *Aagl3* mutants showed glabrous leaf and hairy root phenotype similar as the *gl3 egl3* double mutant in *A. thaliana.* Moreover, overexpression of *AaGL3* in *A.alpina* resulted in reduced number of trichomes; indicating an evolutionary change of the *GL3* function. Similarly, *Arabis GL2* function appears to be changed as it functions only in a subset of trichomes. I also present the identification of morphogenesis mutants affecting root hair development. *Aakjk*, *Aacow1*, *Aascn1*, *Aaark1* and *Aaspi* are identified root hair mutants affecting root hair formation in *A. alpina*. Several morphology mutant alleles showing amino acid exchanges have been also identified.

List of abbreviations

µm	micrometer
35S	Cauliflower Mosaic Virus 35S promoter
A	adenine
Aa	Arabis alpina
aa	amino acid
ACT	ACTIN
amiR	artificial microRNA
ANX	ANSUR
ARK	ARMADILLO REPEAT KINESIN
ARP	ACTIN-RELATED PROTEINS
At	Arabidopsis thaliana
AUX	AUXIN
AXR	AUXIN RESISTANT
bHLH	basic helix-loop-helix
BLAST	basic local alignment search tool
bp	base pairs
C	cytosine
C	centigrade
Ca2+	Calcium ions
CAP	Ca2+ ASSOCIATED PROTEIN KINASE
cDNA	complementary DNA
CDS	coding DNA sequence
Cl2	Chlorine gas
cm	Centimetre
Col	Columbia
COW	CAN OF WORMS
CPC	CAPRICE
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CrRLK1L	Catharanthus roseus RECEPTOR-LIKE KINASE 1-LIKE
CSLD	cellulose synthase-like protein
CSLD	cellulose synthase-like d 6
CTR	CONSTITUTIVE TRIPLE RESPONSE
D	Aspartic acid
DIC	Differential interference contrast
DNA	deoxyribonucleic acid
E	Glutamic acid
EGL	ENHANCER OF GLABRA
EIN	ETHYLENE INSENSITIVE
EMBL-EBI	The European Bioinformatics Institute
EMS	Ethyl methanesulfonate
ER	Endoplasmic reticulum
ERH	ECTOPIC ROOT HAIR
ERU	ERULUS
ETC	ENHANCER OF TRY and CPC

ETO	ETHYLENE OVERPRODUCER
ETR	ETHYLEN RESISTANT
evo-devo	evolutionary developmental
F	filamentous
F	Phenylalanine
FAST	fluorescence-accumulating seed technology
FER	FERONIA
FW	forward
G	Glycine
G	guanine
GDP	guanosine diphosphate
GFP	green fluorescent protein
GL	GLABRA
GN	GNOM
GTP	Guanosine-5'-triphosphate
GUS	β-glucuronidase
H	root hair
h	hours
HCl	Hydrochloric acid
I	Isoleucine
K	Lysine
KEU	KEULE
KJK	KOJAK
L	leucine
LD	long day condition
LRR-RLK	leucine-rich-repeat receptor-like kinase
LRX	LEUCINE-RICH REPEAT EXTENSIN
M	molar
M	methionine
MBW	MYB/ bHLH/ WD40 complex
mm	milimeter
MRH	MORPHOGENESIS OF ROOT HAIR
MS	Murashige and Skoog medium
MYB	MYELOBLASTOSIS
N	non root hair
N	Asparagine
NaCl	Sodium chloride
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
Naocl	Sodium hypochlorite
NBT	Nitrobluetetrazolium
Nr	Number
P	proline
pAaGL2	promoter Arabis alpina GLABRA2
Paj	Pajares
PCR	polymerase chain reaction
PEP	PERPETUAL FLOWERING
Pfam	protein families
PFN	profilin
pH	potential hydrogen

PI	Propidium iodide
PI(4)P	phosphatidylinositol-4-phosphate
PP2A	SERINE/THREONINE PROTEIN PHOSPHATASE
qRTPCR	Quantitative real-time PCR
R	maize R gene
R	Arginine
R2R3MYB	R2 and R3 repeat MYB
R3MYB	single R3 repeat MYB
Raf	Rapidly Accelerated Fibrosarcoma
RAN	RAN GTPASE
rbohC	respiratory burst oxidase homolog C
RHD	ROOT HAIR DEFFECTIVE
RHL	ROOT HAIR LESS
Rho	Rho factor protein in RNA transcription termination
RLK	receptor like kinase
RNA	Ribonucleic acid
ROP	RHO-RELATED PROTEIN FROM PLANTS
ROS	reactive oxygen species
RV	Reverse
S	Serine
Sac	SUPPRESSOR OF ACTIN
S-acyl	palmitoyl transferase
SCM	SCRAMBLED
SCN	SUPERCENTIPEDE
SDM	site directed mutagenesis
SEC	SECRETORY
SEM	scanning electron microscope
sgRNA	single guide RNA
SHV	SHAVEN
SNP	single nucleotide polymorphism
SPI	SPIRRIG
SSC	splice site changes
T	thymine
T	threonine
TAIR	The Arabidopsis Information Resource
TCL	TRICHOMELESS
T-DNA	transfer DNA
THE	THESEUS
TIP	TIP GROWTH DEFECTIVE
TRH	TINY ROOT HAIR
TRN	TORNADO
TRY	TRIPTYCHON
TTG	TRANSPARENT TESTA GLABRA
UDP	Uridine diphosphate
UV	Ultraviolet
VLN	VILLIN
WD	tryptophan-aspartic acid
WER	WEREWOLF
wt	wild type
YFP	yellow fluorescent protein

List of figures

List of tables

1. Introduction

The evolutionary developmental biology approach (Evo-Devo) help us to understand how molecular variations lead to phenotypic changes between different species (Rudel and Sommer 2003; Wilkins 2002). It is used to characterize the key players known to be relevant for a given process in model organisms of evolutionary distant related species (Sommer 2009). For a functional comparison, it is necessary to study homologous processes in closely related species. It helps to understand similarities and differences in the regulatory networks at a mechanistic level. Excellent examples are the comparisons of segmentation and appendix formation between *Tribolium castaneum* and *Drosophila melanogaster* (Roth and Hartenstein 2008), and pattern formation and induction comparison of *Pristionchus pacificus* to *Caenorhabditis elegans* (Hong and Sommer 2006).

In this work, I study the evolution of trichome and root hair development. Trichome and root hair developments are best studied in *A. thaliana* and are governed by partially overlapping gene regulatory networks. Characterization of individual mutants in closely related species suggests that trichome and root hair developments have a common basis in crucifer (Chopra et al. 2014; Nayidu et al., 2014; Dolan and Costa 2001). Within the crucifer family, trichomes show a wide range of density and morphology phenotypes (Beilstein et al., 2006) and root hairs exhibit stripes pattern (Dolan and Costa 2001). It is conceivable that the underlying regulatory machinery of trichome/root hair development is conserved in crucifers but it is also expected to show ample variation. A functional comparison of trichome and root hair development of *A. thaliana* with another evolutionary distant crucifer species should enable us to recognize functional differences and diversifications.

1.1. *Arabis alpina* as a model plant

Arabis alpina belongs to the family Brassicaceae and is evolutionarily separated from *A. thaliana* by about 26 million years (Ansel et al. 2008; Koch et al 2006). These two species are close enough to enable us to study homologous processes while they are distant enough to expect differences in the regulation (Ansell et al. 2008; Koch et al. 2006).

A. alpina is already established as a perennial genetic model (Wang et al. 2009): it is diploid, self-fertile and has a small genome consisting of eight chromosomes, the molecular analysis is facilitated by the availability of a fully sequenced genome (Willing et al. 2015), and it can be transformed by *Agrobacterium* mediated floral dip (Wang et al., 2009). These properties make *A. alpina* ideal to study the evolution of gene regulatory networks governing the development of trichomes and root hairs in Brassicaceae. The aim of this study is to do a genetic screen to identify gene functions based on mutant phenotypes and compare the spectrum of phenotypes to that known in *A. thaliana*.

1.2. Trichome patterning in *A. thaliana*

Trichomes are leaf hairs that play different roles in plant protection against UV, irradiation and herbivores. They control leaf temperature by reducing transpiration and increase tolerance against freezing (Johnson 1975; Mauricio and Rausher 1997). In *A. thaliana*, trichomes are regularly spaced on the surface of adult leaves (Hülskamp et al. 1994). Trichome patterning is a *de novo* process such that an individual epidermal cell is selected among others to adopt trichome fate. It's known that positive and negative regulators are involved in trichome development to generate a regular trichome pattern through intercellular interactions (Pesch and Hülskamp, 2009). Genetic screens and

molecular analysis revealed genes which are relevant for trichome patterning (Hülskamp, 2004; Tominaga-Wada et al., 2011).

Mutations in positive regulators result in a reduced number of trichomes. *TRANSPARENT TESTA GLABRA1* (*TTG1*), *GLABRA3* (*GL3*), *ENHANCER OF GLABRA3* (*EGL3*) and *GLABRA1* (*GL1*) are positive regulators (Hülskamp et al., 1994; Morohashi et al., 2007; Oppenheimer et al., 1991; Zhang et al., 2003). *TTG1* encodes a WD40 protein (Walker et al. 1999); *GL3* and *EGL3* encode basic-helix-loop-helix (bHLH) transcription factors (Payne et al., 2000; Zhang et al., 2003) and *GL1* encodes an R2R3 MYB transcription factor (Oppenheimer et al. 1991). These positive regulators act partially redundant and form an activator complex known as MYB-bHLH-WD40 (MWB) complex (Larkin et al., 1997 and 2003; Digiuni *et al.* 2008, Zhao *et al.* 2008).

Mutations in negative regulators result in increased number of trichomes. *TRIPTYCHON* (*TRY*), *CAPRICE* (*CPC*), *ENHANCER OF TRY AND CAPRICE1* (*ETC1*), *ETC2*, *ETC3*, *TRICHOME LESS1* (*TCL1*) and *TCL2* are negative regulators of trichome formation (Yang and Ye 2013, Wang *et al.* 2008, Wang *et al.* 2014, Hülskamp 2004, Wada *et al.*, 1997; Schellmann *et al.*, 2002; Kirik *et al.*, 2004 a & b). These genes are members of a subfamily of small MYB-like transcription factors called the R3MYB. They contain only one DNA binding domain and compete with the positive regulators (R2R3MYB) for binding to bHLH proteins within the MBW complex (Wada et al. 1997; Schellmann et al. 2002; Esch et al. 2003; Kirik et al. 2004a).

Several models have been developed to describe trichome patterning. The most accepted one is the activator-Inhibitor model, in which WD40-bHLH-R2R3MYB proteins form a complex to activate trichome initiation in epidermal cells destined to become a trichome. R3 single repeat genes act as inhibitors by attaching to bHLHs thereby preventing the formation of the trimeric MBW complex. It is known that R3MYB gene expression is activated by this trimeric complex in trichome cells and that the inhibitors

move to the neighboring cells, preventing them from forming trichomes (Figure 1.1) (Pesch and Hülskamp 2009; Pesch and Hülskamp 2004; Ishida et al. 2008; Larkin et al. 1996; Esch 2003; Scheres 2002; Szymanski et al. 2000; Hülskamp & Schnittger 1998; Scheres 2002; Schiefelbein 2003; Larkin et al. 2003). A second model describing trichome patterning is activator-depletion model. In this model, *TTG1* is expressed ubiquitously and moves freely between epidermal cells. When entering trichome precursor cells *TTG1* is trapped by GL3 and accumulates inside the trichome cells (Bouyer et al. 2008). In this scenario, the absence of TTG1 prevents trichome formation around incipient trichomes (Figure 1.1).

In trichome cells, the MBW complex activates the expression of *GLABRA2* (*GL2)* (Morohashi et al., 2007). *GL2* is a homeodomain transcription factor which is necessary for trichome differentiation. It is also involved in regulating endoreduplication, branching, and maturation of the cell wall (Rerie et al., 1994). Another gene involved in the cell fate determination is the WRKY transcription factor called *TRANSPARANT TESTA GLABRA 2* (*TTG2)* (Johnson et al., 2002). Mutations in *GL2* and *TTG2* lead to aborted and underdeveloped trichomes (Rerie *et al.* 1994; Johnson *et al.* 2002; Pesch et al. 2014).

1.3. Root hair patterning in *A. thaliana*

Root hairs have a tubular, long shape that are important for nutrient uptake, anchorage and interactions with other micro-organisms in the soil (Cutter 1978). Root hairs in *A. thaliana* are found in single cell files, named H-files, which develop only over the cleft of two underlying cortical cells. Non-hair files, which develop over one single cortical cell, are named N-files (Dolan *et al*., 1994; Schiefelbein 2000). Epidermal cells in root hair files are called trichoblasts and epidermal cells in non-hair files are called atrichoblasts. They differ from early on in epidermal development prior to hair formation.

Trichoblasts display a higher rate of cell division (Berger *et al*., 1998), reduced cell length (Dolan *et al*., 1994; Masucci *et al*., 1996) and a dense cytoplasm (Dolan *et al*., 1994; Galway *et al*., 1994).
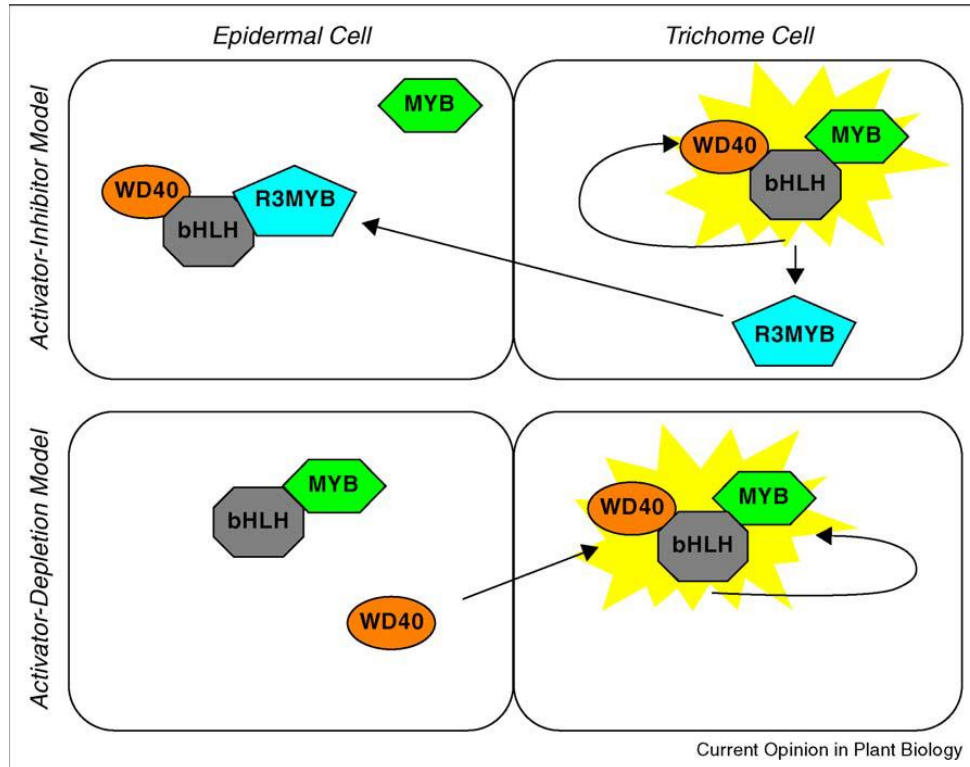
Figure 1.1. Models exhibiting trichome patterning. An active complex is indicated by a yellow star. The top row (activator–inhibitor model) shows that in epidermal cell destined to become trichome: the WD40, bHLH and MYB proteins form an active complex. The R3MYB factors move into neighboring cells, replacing MYB and rendering the complex inactive. The bottom row (activator–depletion model) shows that the WD40 protein moves from neighboring cells into incipient trichomes and trapped by the bHLH factors in trichome initials (modified from Pesch and Hulskamp, 2009).

Several mutants in Arabidopsis have been identified with defects in their root epidermal cell specification. Mutations in *TTG1*, *GL3*, *EGL3*, *WERWOLF* (*WER*) and *GL2* cause root hairs to form in almost all epidermal cells, indicating that the normal role of these genes is to promote non-hair cell specification (Galway et al., 1994; DiCristina et al.,

1996; Masucci et al., 1996; Lee and Schiefelbein, 1999; Bernhardt et al., 2003). *WER* is a MYB transcription factor of the R2R3 class (Lee and Schiefelbein, 1999) and interacts with bHLHs to form a WD40-bHLH-MYB complex. This trimeric transcription factor complex positively regulates the expression of *GL2* which in turn promotes non-hair cell fate. *GL2* is preferentially expressed in non-hair cells (Masucci et al., 1996).

Mutation in *CAPRICE* (*CPC*) results in reduced number of root hairs, indicating that it is a positive regulator of root hair fate (Wada et al., 1997). *CPC* encodes a single R3 MYB repeat which lacks a typical transcriptional activation domain (Wada et al., 1997). Other R3 MYB proteins, shown to act in a partially redundant manner for root hair pattern, are *TRY* and *ETC1* (Schellman et al., 2002; Kirik et al., 2004a; Simon et al., 2007; Wang et al., 2010).

The *SCRAMBLED* (*SCM*) gene encodes a leucine-rich-repeat receptor-like kinase (LRR-RLK). It enables epidermal cells to perceive their relative position (Kwak et al, 2005) which as a result promote a distinct gene expression pattern to adopt appropriate fates. Therefore *scm* mutants alter the distribution of hair and non-hair cells (Kwak et al, 2005).

A simple model describes the control of root epidermal cell fate in Arabidopsis (Lee and Schiefelbein, 2002; Schiefelbein et al., 2009). In this model, the pattern relies on the activity of two competing sets of transcription factors, R2R3MYB (*WER*) vs. R3MYB (*CPC*). These different MYB transcription factors form an active or inactive complex respectively, together with WD40 (*TTG1*)-bHLH (*GL3/EGL3*). Immature epidermal cells in N position highly express and accumulate *WER*, resulting in the formation of active complex, activating *GL2* expression and non-hair fate. In immature epidermal cells of H position, *CPC* is accumulated which results in formation of the inactive complex. *GL2* is not activated and root hair fate is determined. *SCM* provides the positional cues at the early stages, initiating differential accumulation of *WER* and *CPC* (Figure 1.2).

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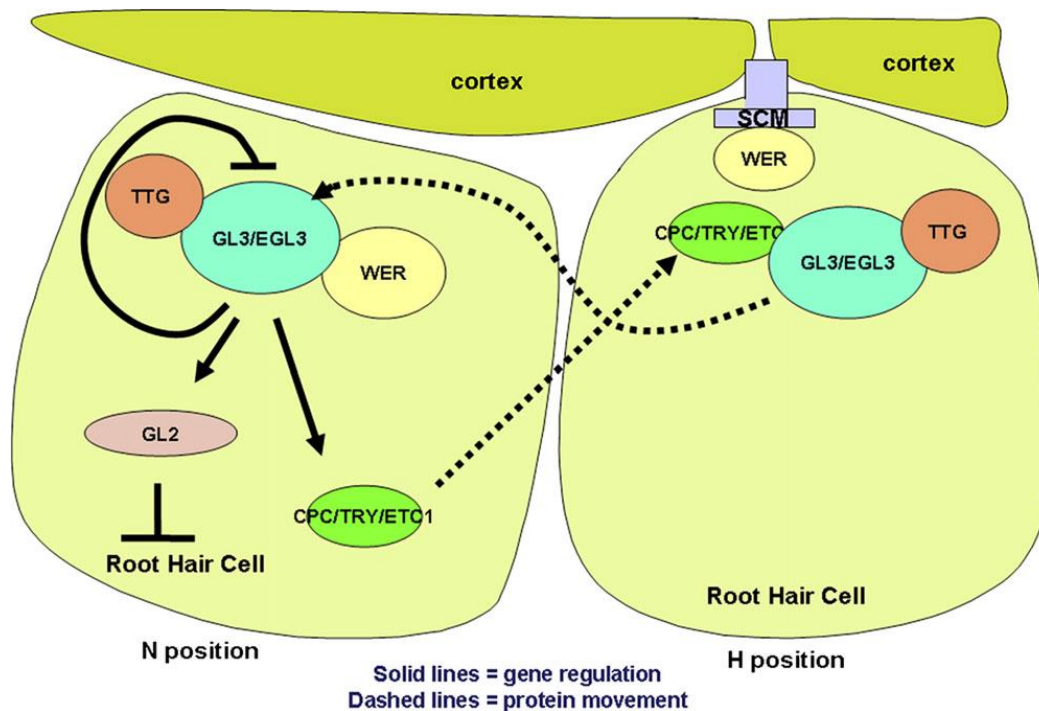
Figure 1.2. A model for Arabidopsis root epidermis patterning. The position-dependent *SCM* signaling influences the accumulation of the WER-GL3/EGL3-TTG transcriptional complex, which directs non-hair cell differentiation via *GL2* as well as the hair cell fate by lateral inhibition via *CPC/TRY/ETC1* (modified from Schiefelbein et al., 2009).

1.4. Root hair cell differentiation in *A. thaliana*

Early differentiation of root epidermal cells is controlled by a number of genes. *ROOT HAIR LESS 1* (*RHL1*)*, RHL2* and *RHL3* produce a nuclear proteins, required for the formation of polarized outgrowth and subsequent root hair formation (Schneider et al. 1998; Sugimoto-Shirasu et al. 2005). *ECTOPIC ROOT HAIR 1* (*ERH1*)*, ERH2* and *ERH3* are genes that act antagonistically to the *ROOT HAIR LESS* genes, by promoting non-hair cell fate (Schneider et al. 1997). *TORNADO1* (*TRN1*) and *TRN2* are required for the position-

dependent cell specification of root hair and non-hair cell differentiation in the epidermis (Cnops et al., 2000).

After the root hair pattern is fixed and trichoblasts are distinguishable from atrichoblasts, plant hormones, namely auxin and ethylene, play their role to promote root hair differentiation. They also affect root hair position in the hair cell. In general, increased auxin or ethylene signaling moves the hair initiation site to a more apical position (i.e. closer to the root tip) and increases hair elongation. Decreased auxin or ethylene signaling has the opposite effect (Grierson et al., 2014). It is known that *ethylene resistant1 (etr1)* mutants display a more basal shift while *ethylene overproducer1 (eto1)* exhibit a more apical shift in their root hair positions (Masucci and Schiefelbein 1994). *AUXIN1* (*AUX1*) and *GNOM* (*GN*) which affect auxin influx carriers also alter the location of root hairs and shift it to a more apical position where the auxin concentration is likely to be highest (Fischer et al., 2006). *AUXIN RESISTANT2* (*AXR2*) *and AUXIN RESISTANT3* (*AXR3*) are transcriptional regulators of auxin-responsive genes that affect root hair initiation (Nagpal et al., 2000; Leyser et al., 1996). *axr2* and *axr3* roots have fewer hairs than the wild type, while trichoblasts are normally formed indicating that they act downstream of pattern formation (Wilson et al., 1990; Leyser et al., 1996). *axr2* root hairs exhibit an overall basal shift in the bulge site (Masucci and Schiefelbein 1994). *AXR1* and *ETHYLENE INSENSITIVE2* (*EIN2*) are required for the full-length root hairs and their mutants produce some root hairs that stop growing after elongation begins (Pitts et al., 1998). *CONSTITUTIVE TRIPLE RESPONSE1* (*CTR1*) encodes a Raf-like protein kinase and acts in the ethylene response pathway. Mutations in this gene affect early initiation of root hairs and makes plants to behave as if they were grown in the presence of ethylene in that they produce ectopic root hairs (Kieber et al. 1993; Dolan et al. 1994).

1.5. Root hair formation in *A. thaliana*

Root epidermal cells that are committed to hair formation become highly specialized and adopt a characteristic shape.

1.5.1. Root hair initiation

In the first phase of root hair formation, the polarity of the cell expansion is restricted. Growth occurs at one point at the apical end of epidermal cells and initiates a bulge. This polarized outgrowth starts with the migration of the nucleus along the cell wall (Ryan et al. 2001). Before hairs begin to grow, small GTP-binding proteins from the RHO-RELATED PROTEIN FROM PLANTS (ROP) family localize at the growth site (Molendijk et al., 2001). These ROP proteins are unique to the plants, but related to Rho small GTPases that control morphogenesis of animal and yeast (Vernoud et al., 2003; Chant, 1999). A cytoplasmic domain of the receptor-like kinase *FERONIA* (*FER*) has been shown to be important for ROP accumulation at the tip of root hairs (Duan et al., 2010). ROP proteins remain at the tip of developing hairs until growth ceases (Molendijk et al., 2001).

Within minutes of ROP localization, the cell wall starts loosening by decreasing the apoplastic pH which is required for the modification of the cell wall polymers (Bibikova et al. 1998). This cell wall loosening may also be organized by the localized enzymatic activity of expansin proteins (Baluska et al. 2000; Monshausen et al., 2007). When the bulge starts growing, the endoplasmic reticulum condenses (Ridge et al., 1999) and F-actin accumulates (Baluska et al., 2000). Microtubules are also involved in regulating the location and size of the bulges (Kost et al. 1999). At the end of the first phase, the bulge length is about 20-40 µm (Dolan et al., 1994; Favery et al. 2001). *ROOT HAIR DEFFECTIVE 1* (*RHD1*) encodes a UDP-glucose 4-epimerase, which is involved in cell wall synthesis and

regulates the degree of the cell wall loosening (Seifert et al., 2002). *rhd1* mutants have root hairs which are swollen at the base (Schiefelbein and Somerville 1990). Mutations in *TIP GROWTH DEFECTIVE1* (*TIP1*) also result in hairs which are swollen at the base. Hairs are shorter and wider than in wild type and are often branched. There is usually more than one hair emerging from each bulge from *tip1* trichoblasts. *TIP1* encodes a palmitoyl (or S-acyl) transferase, which regulates the protein localization and activity at the plasma membrane (Hemsley et al. 2005). This indicates that the *TIP1* protein spatially controls the degree of the cell wall loosening and limits the number of sites of growth initiation early in the hair development. *tip1* mutants are generally small plants and defective in the pollen growth (Schiefelbein et al. 1993, Ryan et al. 1998).

ROOT HAIR DEFFECTIVE 6 (*RHD6*) acts through an auxin/ethylene development pathway and localizes the site of hair initiation in the trichoblast. *rhd6* mutants produce fewer hairs and in more apical position as compared to the wild type (Masucci and Schiefelbein 1994). *TINY ROOT HAIR1* (*TRH1*) encodes a potassium transporter (Rigas et al., 2001). It controls the number of swellings through an unknown mechanism. *trh1* mutants have multiple hairs (Rigas et al., 2001). Mutation in *SUPERCENTIPEDE1* (*SCN1*) results in excessive ROP accumulation and the mislocalization of Reactive Oxygen Species (ROS), which in turn produces multiple swellings from each hair cell (Carol et al., 2005).

1.5.2. Root hair tip growth

The second phase of root hair formation starts when hairs are about 40 μm long (Dolan et al., 1994). It is characterized by the localization of growth to the tip of the hair (Carol et al. 2005). This process is organized by a polarized cytoplasm in the bulge (Favery et al. 2001), which results in localized secretion and cell wall synthesis. This is a rather fast process with about 9000 exocytosis events per minute (Ketelaar et al., 2008). The major

molecular events during tip growth include a tip-focused calcium influx, cytoskeleton remodeling, polarized membrane trafficking and cell wall synthesis.

Calcium influx. Root hair tip growth requires a tip-focused calcium influx to generate a Ca^{2+} gradient (Schiefelbein et al. 1992; Wymer et al. 1997). This ion influx controls the growth direction (Pei et al., 2012). Calcium is provided via calcium channels at the tip of the hairs (Lew, 1996; Very and Davies, 2000). These channels are activated by ROS, which in turn is localized by the activity of *RHO-RELATED PROTEIN FROM PLANTS2* (*ROP2*) and *ROOT HAIR DEFECTIVE2* (*RHD2*) (Foreman et al., 2003; Jones et al., 2007). The tip-focused Ca^{2+} gradient is maintained as long as tip growth proceeds and disappears when hairs stop growing (Wymer et al., 1997).

Cytoskeleton. The cytoskeleton of the root hair is composed of actin filaments, microtubules, and their associated proteins. Actin filaments provide a backbone over which cytoplasmic streaming driven (Grierson et al., 2014). Mutations in the *ACTIN2* (*ACT2*) exhibit defects in root hair initiation and tip growth (Gilliland et al., 2002; Ringli et al., 2002). Actin associated proteins play an important role in this context. *ACTIN-RELATED PROTEINS 2* (*ARP2*) and *ARP3* are important regulators of actin organization, when mutated plants produce sinuous hairs (Mathur et al., 2003a). *CYCLASE-ASSOCIATED PROTEIN 1* (*CAP1*) is a fundamental facilitator of actin dynamics. *cap1* root hairs are short and exhibit disrupted actin filaments and disorganized cytoplasm in the tip growth zone (Deeks et al., 2007; Chaudhry et al., 2007). *VILLIN4* (*VLN4*) encodes a protein that bundles actin filaments. v*ln4* mutants produce short hairs because of the finer actin organization and decreased cytoplasmic streaming (Zhang et al., 2011). Myosin XI motor proteins in plants are involved in actin dependent organelle trafficking and cytoplasmic restructuring (Lee and Liu, 2004). Mutation in *MYOSIN XI K* (*ATXIK*) results in reduced root hair growth because of altered actin organization (Park and Nebenfuhr, 2013).

Microtubules control growth polarity by localizing growth to a small region of the tip. Vesicle transport is directed by microtubule associated motor proteins (Miller et al. 1997; Bibikova et al. 1999; Kirchner et al. 1999). *ARMADILLO-REPEAT KINESIN 1* (*ARK1*) also called *MORPHOGENESIS OF ROOT HAIR 2* (*MRH2*) controls microtubule organization in the root hairs to maintain a straight growth axis. *ark1* mutants exhibit wavy and occasionally branched hairs (Sakai et al., 2008).

Polarized membrane trafficking. During root hair tip growth, newly-synthesized cell wall components are packed into secretory vesicles, emerging from the Golgi network, and are subsequently targeted to the apical plasma membrane. Therefore, control of membrane trafficking plays a critical role in root hair formation (Grierson et al., 2014). *ROOT HAIR DEFFECTIVE 4* (*RHD4*) encodes a Sac1p-like phosphoinositide phosphatase that regulates the accumulation of PI(4)P on membrane compartments at the tip of the hairs. *rhd4* mutants produce short hairs which randomly form bulges along their length (Thole et al., 2008). *CAN OF WORMS1* (*COW1*) is a phosphatidylinositol transfer protein that localizes to the site of the hair growth and plays a key role in the polarized membrane trafficking (Böhme et al. 2004). *cow1* mutants have short and wide hairs and usually two hairs emerge from each bulge (Grierson et al. 1997). *COW1* is therefore important for the maintenance of tip growth and restricts hair initiation sites and hair diameter in the wild type (Ryan et al. 2001). *ROOT HAIR DEFFECTIVE 3* (*RHD3*) encodes a protein with GTP binding motifs (Wang et al. 1997) which is involved in the formation of ER network (Chen et al. 2011) probably by mediating ER fusion events during vacuole enlargement (Zhang et al., 2013). The *RHD3* gene acts after the transition to the tip growth. Mutants are dwarf with short, wavy and sometimes branched hairs (Schiefelbein and Somerville 1990). They also show abnormalities in their trichome shape. This phenotype is correlated with a reduction of vacuole enlargement and asymmetric tip growth suggesting that correct vesicle trafficking is relevant (Galway et al. 1997). *KEULE* (*KEU*) encodes the SEC1 protein that is involved in secretory trafficking at the growing

root hair tip. *keule* mutants have stunted and radially swollen root hairs (Assaad et al., 2001; Karnik et al. 2013).

Cell wall synthesis. Cellulose is required for cell wall synthesis of a growing root hairs (Park et al., 2011). Cell wall synthesis happens at the tip of the root hair through accumulation of secretory vesicles containing proteins and polysaccharide polymers. The cellulose synthase-like protein (AtCSLD3) encoded by the *KOJAK* (*KJK*) gene is required for the synthesis of a non-cellulosic wall polysaccharide. *kjk* mutants have weak cell wall that cannot contain the growing protoplast and burst (Favery et al. 2001). *SHAVEN2* (*SHV2*) and *SHV3* also encode proteins that affect cell wall synthesis. Root hairs in *shv2* are short, wide and burst at the tip (Parker et al. 2000; Jones et al., 2006). Tip growth in *shv3* root hair cells is blocked due to a rupture at the tip (Hayashi etl a. 2008). The *LEUCINE-RICH REPEAT/EXTENSIN 1* (*LRX1*) encodes a cell wall protein with homology to extensins which regulates cell wall expansion. Root hairs in *lrx1* mutants are stunted and branched (Baumberger et al., 2001).

Root hair growth is sustained by auxin. As the root tip grows away from the young root hairs, they receive less auxin until the auxin supply is lost and they stop growing (Jones et al., 2009).

1.6. Trichome patterning in *A. alpina*

Trichome development in *A. alpina* is reminiscent to *A. thaliana* (Chopra et al., 2014). In contrast to *A. thaliana*, adult leaves in *A. alpina* are covered densely with trichomes (Figure 1.4 A). There are two types of trichomes in *A. alpina*: class I trichomes are small and regularly distributed on the surface of the leaf. Class II trichomes are larger and interspersed between small trichomes with regular distances to each other (Chopra

et al., 2014) (Figure 1.4 B). It's been suggested that trichomes in *A. alpina* are initiated at the basis of young leaves and also in distal area between already existing trichomes (Chopra et al., 2014).
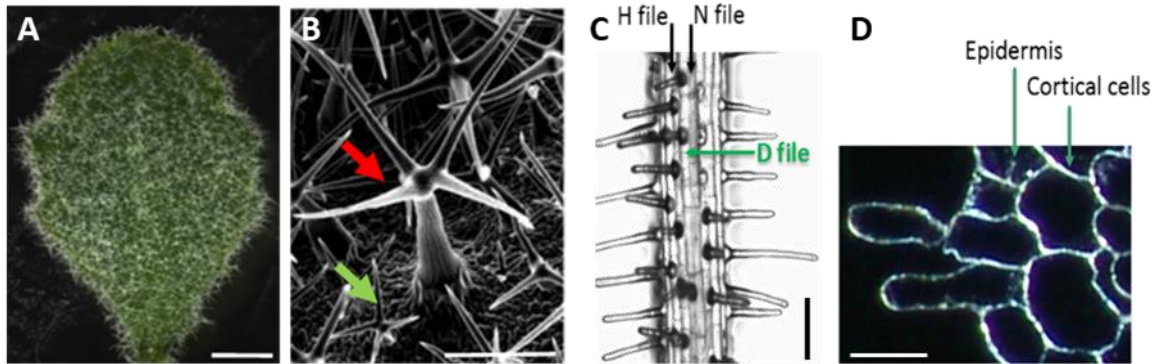
Figure 1.4. Trichome and root hair patterning in *Arabis alpina*. A, *A. alpina* adult leaf **B,** SEM of an adult *A. alpina* leaf showing the large (red arrow) and the small classes of trichomes (green arrow). **C,** Wild type *A. alpina* root. Root hair file (H) and non-hair file (N) indicated by black arrows; non-hair file with root hair stretches (D-file) indicated by green arrow. **D,** Wild type *A. alpina* root cross section depicting one root hair at the H-position over the cleft of two underlying cortex cells and a neighboring root hair in an N-position. Scale bar = 500 µm in A, 100 µm in B and C, 40 µm in D (Modified from Chopra *et al.* 2014).

Trichome patterning in *A. thaliana* is controlled by four major partially redundant activators (*TTG1*, *GL3*, *EGL3* and *GL1*) and seven redundantly acting inhibitors (*TRY*, *CPC*, *ETC1*, *ETC2*, *ETC3*, *TCL1* and *TCL2*) (Figure 1.5 A) (Hülskamp, 2004). From eleven studied genes, nine orthologs of trichome pattern regulators (*AaTTG1*, *AaGL3*, *AaEGL3*, *AaGL1*, *AaTRY*, *AaCPC*, *AaETC1*, *AaETC3*, *AaTCL2*) are present in the Arabis genome as judged by sequence similarity and synteny (Figure 1.5 B) (Willing et al., 2015; Chopra 2015, PhD thesis). Synteny is described by the co-localization of homologous genes on their respective chromosomes. Genes that are syntenic in different species are considered

14

orthologous genes, as they are considered to be derived from common ancestors (Lyons et al., 2008). However, *ETC2* and *TCL1* orthologs were not found based on these criteria, while two *CPC* homologs were found in Arabis genome (Figure 1.5 B) (Willing et al., 2015). In most cases the synteny of homologous genes is maintained either on both sides or up or downstream of the genes (Table S1) (Chopra 2015, PhD thesis). Orthologs of two genes involved in trichome differentiation in *A. thaliana* (*GL2* and *TTG2*) (Morohashi et al., 2007; Johnson et al., 2002) also found in the *A. alpina* genome (Figure 1.5 B) (Willing et al., 2015; Chopra 2015, PhD thesis). Synteny is conserved for these two genes (Table S1) (Chopra 2015, PhD thesis).
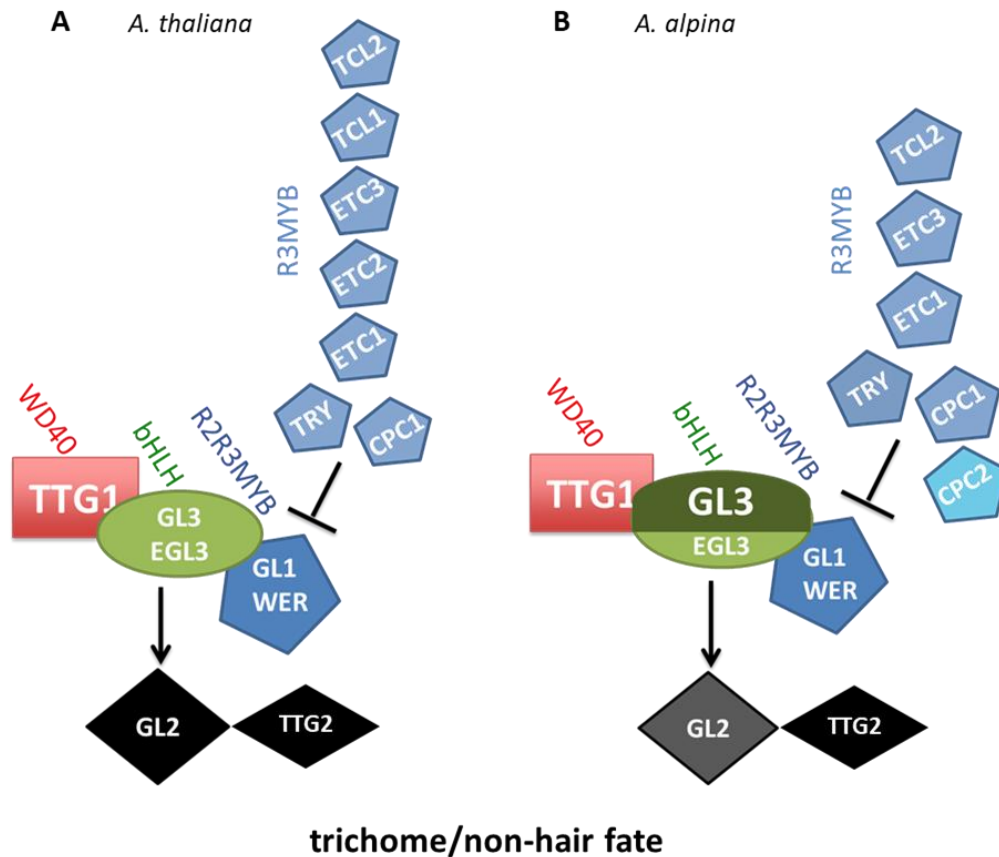
Figure 1.5. Schematic representation of *in silico* comparison of trichome and root hair patterning genes between *A. thaliana* and *A. alpina*. A, Genes involved in trichome and root hair patterning in *A. thaliana* **B**, Genes involved in trichome and root hair patterning in *A. alpina*. The simplified interactions are shown in cells destined to be trichome/non-hair cells. Arrows indicate positive control, blunted lines indicate negative regulation. (Modified from Chopra 2015, PhD thesis)

Genes involved in trichome development in *Arabis alpina* have been identified using a forward genetic mutagenesis screen (Chopra 2015, PhD thesis). Two glabrous lines with mutations in the *AaTTG1* gene were identified. It was shown that all five traits governed by *AtTTG1* in *A. thaliana*, including glabrous leaves, ectopic root hairs, yellow seeds and no anthocyanin and seed coat mucilage production (Ramsay and Glover 2005; Balkunde et al., 2010), are affected in *Aattg1* alleles (Chopra et al., 2014). Two other

16

glabrous mutants were discovered with relevant mutations in the *AaGL3* gene (Chopra 2015, PhD thesis). This was unexpected, as *gl3* mutants in *A. thaliana* have trichomes. Double mutants of *Atgl3 Ategl3* are completely glabrous indicating that *GL3* and *EGL3* act in a partially redundant manner in *A. thaliana* (Zhang et al., 2003). Thus in *A. alpina*, *AaGL3* takes over the full bHLH function in trichome patterning and *AaEGL3* appear not to be functionally relevant in this context. However, AaEGL3 protein could rescue the *Atgl3 Ategl3* Arabidopsis double mutant efficiently, when expressed under the 35S promoter (Chopra 2015, PhD thesis). This indicates that the protein is fully functional and the functional change is due to differences in the regulation of the expression. None of the glabrous mutants in *A. alpina* displayed mutations in the *AaGL1* gene (Chopra 2015, PhD thesis). Two mutants exhibiting trichome clusters, reminiscent to *try* mutants in *A. thaliana*, were identified as *Aatry* alleles (Chopra 2015, PhD thesis). *Aagl2* mutant displayed two classes of underdeveloped trichomes. The population of small trichomes shares similarities with *gl2* trichomes in *A. thaliana* (Rerie *et al.* 1994). However, large trichomes have no clear counterpart in *A. thaliana* (Chopra 2015, PhD thesis).

1.7. Root hair patterning in *A. alpina*

Root hair development in *A. alpina* is reminiscent to *A. thaliana* (Chopra et al., 2014). Hairs are formed in H-files adjacent to non-hair files named N-files (Figure 1.4 C). In contrast to *A. thaliana*, 30 to 40 percent of cells in N-file positions also form root hairs in *A. alpina*. Hairs in these files are always discontinuous and are named D-files (Figure 1.4 C). Wild type *A. alpina* root cross section depicted that hairs are seen in H and N positions (Figure 1.4 D). However cells in N-position in *A. alpina* share other characteristics with N-position cells in *A. thaliana*. It was shown that cell length in N-position is doubled as compared to H-position in *A. alpina* (Chopra et al., 2014).

WER is a specific root hair pattern regulator in *A. thaliana* promoting non-hair cell fate (Lee and Schiefelbein, 1999). An ortholog of *WER* has been identified in *A. alpina* genome using sequence similarity and synteny (Figure 1.5 B; Table S1) (Chopra 2015, PhD thesis).

Mutations reducing trichome number in *A. thaliana* lead to the production of additional root hairs; because the MBW complex activates trichomes and non-hair fates (Tominaga-Wada et al. 2011). As mentioned before, *Aattg1* mutants produce ectopic root hairs compared to the wild type (Chopra et al., 2014). Excessive root hair production was reported also for *Aagl3* mutants (Chopra 2015, PhD thesis). This is in contrast to *A. thaliana* where extra hairs are only found in *Atgl3 Ategl3* double mutants (Bernhardt *et al.* 2003). In *A. thaliana GL2* is required for position-dependent cell differentiation in the root epidermis and is only expressed in N-files, indicating a role in promoting the non-hair cell fate (Masucci *et al.* 1996). Similarly to the *gl2* mutant in *A. thaliana*, *Aagl2* produced ectopic root hairs in *A. alpina* (Chopra 2015, PhD thesis).

1.8. Aim of this work

Aim of this project is to study the evolution of two developmental processes in the shoot and root, namely trichome and root hair development, which are genetically related to each other. For this, we use Evolutionary Developmental Biology Approach so called Evo-Devo; and we aim to do a functional evolutionary comparison. For that, it is necessary to study clearly homologous processes in closely related species. *Arabis alpina* which is 26 million years apart from *A. thaliana* has been selected for this purpose. We took a forward genetic approach to identify genes which are involved in trichome and root hair development in *A. alpina*. The beauty of using *Arabis alpina* as an evolutionary second model system for *Arabidopsis thaliana*, is that they are closely related enough to study clearly homologous processes and are distant enough to expect phenotypic differences.

Using these approaches we designed our experiments in a way aiming to answer different questions. We first wanted to analyze how stable the regulatory genes have been during evolution. Where we may find gene duplications or gene losts within the network? Are these changes in the pathway explaining phenotypic differences? We would have then wanted to know how the function of genes have evolved within trichome and root hair regulatory pathways. Finding mutants together with producing overexpression, knock down and/or knock out lines in different positions of the gene regulatory cascade would shed light to answer these questions. We would also wanted to point out where new genes might have been recruited into the pathways during evolution. And finally, to what extend the functional overlap between the two pathways is evolutionarily conserved.

2. Results

2.1. Analysis of trichome and root hair patterning in *Arabis alpina*

2.1.1. Identification of a new *Aattg1* allele

Two Arabis alpina *ttg1* alleles have been previously identified in an EMS screen for trichome mutants (Chopra et al. 2014). Similarly as in Arabidopsis, both alleles show five defects including no trichomes, ectopic root hairs, yellow seeds and no anthocyanin and seed coat mucilage production.

In my screen for root hair mutants, I identified one additional *Aattg1* allele based on ectopic root hair formation (Figure 2.1 B) (Table S17). This mutant showed glabrous leaves, yellow seeds and lacked anthocyanin and seed coat mucilage (Figure 2.1 C-J). Sanger sequencing revealed a point mutation leading to a Glycine (G) to Aspartic acid (D) exchange at position 45 (Figure 2.1 K). This mutation along with the phenotypic spectrum suggests that this mutant is a *ttg1* allele that is termed *Aattg1-3* in the following.

2.1.2. Identification of new *Aagl3* alleles

Two *Arabis alpina gl3* alleles have been previously identified in an EMS screen for trichome mutants (Chopra 2015, PhD thesis). They have been reported to show glabrous leaves and hairy roots (Table S17). This indicates a functional divergence of *GL3* between

Arabidopsis and Arabis, since *Atgl3* mutants only show a weak trichome phenotype (Koornneef et al. 1982; Payne et al. 2000).
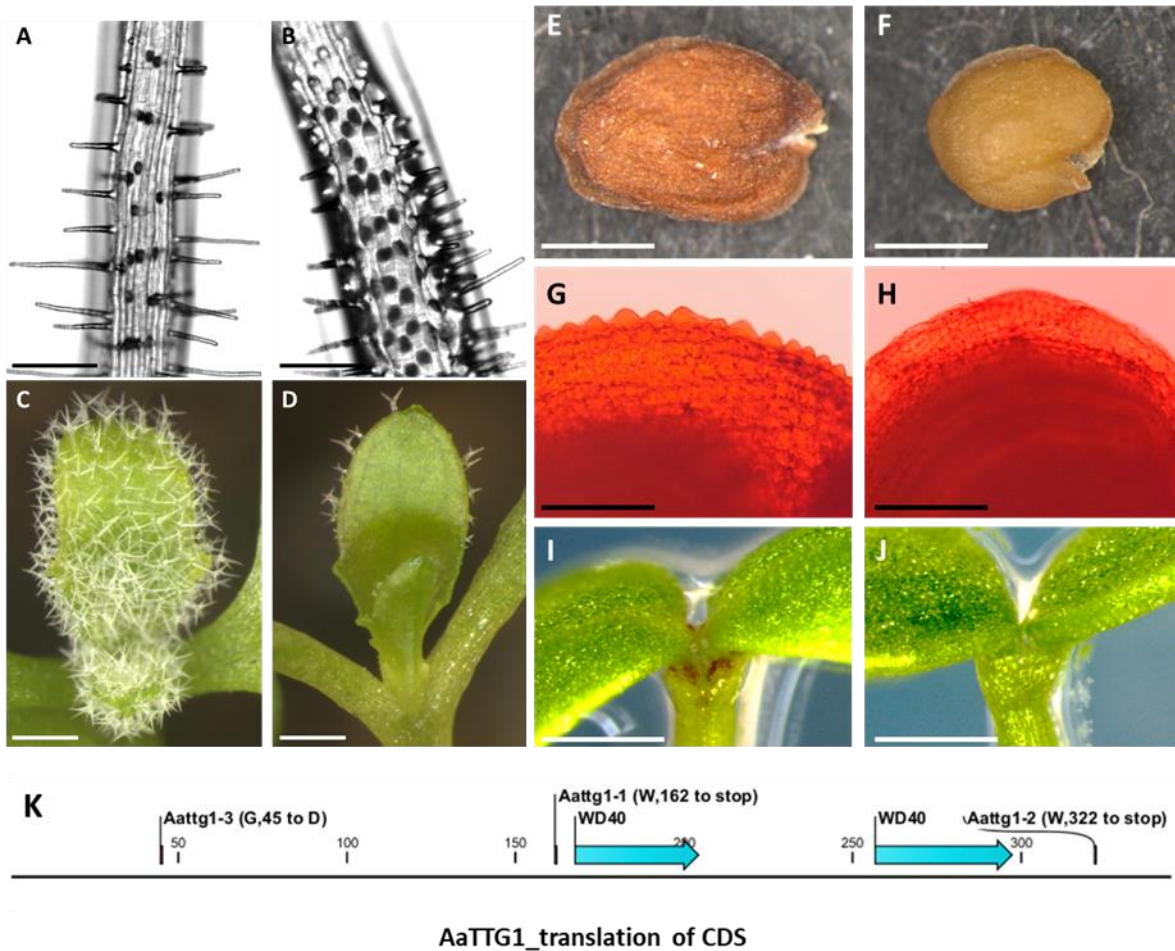
Figure 2.1. *TTG1* regulating phenotypes in wild type and *Aattg1-3*. Respective wild type for *Aattg1-3* is *pep1-1* .**A,** *pep1-1* root. **B**, Ectopic root hairs in *Aattg1-3* **C,** *pep1-1* leaves. **D,** Glabrous leaves of *Aattg1-3*. **E,** *pep1-1* seed. **F,** Yellow seed of *Aattg1-3*. **G,** *pep1-1* seed coat mucilage production. **H,** Lack of mucilage in *Aattg1-3*. In G and H, seeds were stained with Ruthenium Red and pictures were taken using light microscope. In *pep1-1*, the columella is seen as large domes. **I,** Anthocyanidin accumulation in *pep1-1* hypocotyl. **J,** Lack of anthocyanidin accumulation in *Aattg1-3*. I and J show 7 day-old seedlings grown on MS medium with 1% sucrose at constant light. **K,** Schematic representation of *TTG1* amino acid sequence in *A. alpina*. The position of the WD40 domains (indicated by blue arrows) were determined using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* are marked. Scale bar = 250 μm in A and B, 500 μm in C-F, I and J, 200 μm in G and H.

I identified an additional *Aagl3* allele in my root hair screen based on its hairy root characteristic (Figure 2.2 B) (Table S17). This mutant showed glabrous leaves (Figure 2.2 D). The presence of trichomes on the edge of the leaf is excluded from the phenotyping. Sanger sequencing revealed a G to A exchange at position 157, resulting in a stop codon at amino acid position 27 (Figure 2.2 E). Presence of an early stop codon along with the full phenotypic spectrum known for *Aagl3*, suggests that this is a *gl3* allele named *Aagl3-3* hereafter.
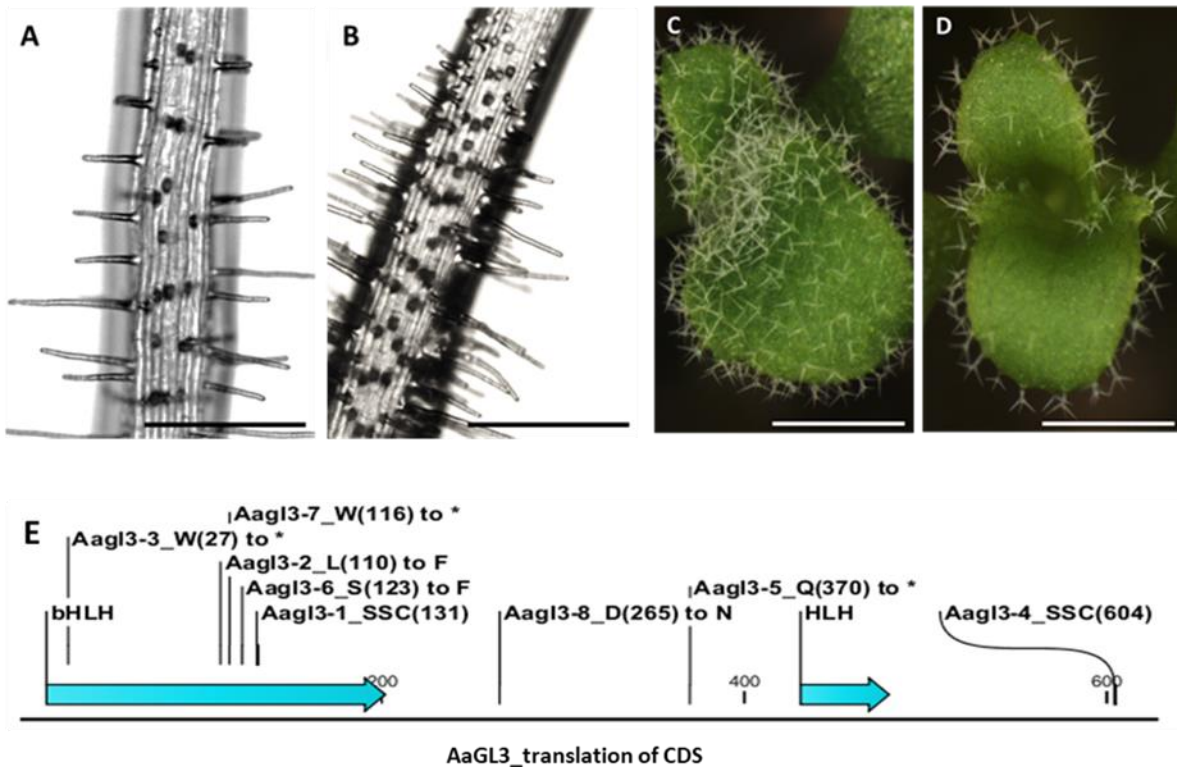
Figure 2.2. *GL3* regulating phenotypes in wild type and *Aattg1-3*. Respective wild type for *Aagl3-3* is *pep1-1*. **A,** *pep1-1* root. **B,** Ectopic root hairs in *Aagl3-3*. **C,** *pep1-1* leaves. **D,** Glabrous leaves of *Aagl3-3*. **E,** Schematic representation of *GL3* amino acid sequence in *A. alpina*. The position of the bHLH and HLH domains (indicated by blue arrows) were determined using Pfam (Finn et al., 2016). The amino acid position changed in mutant alleles in *A. alpina* are marked. Scale bar = 250 µm in A and B, 1 mm in C and D.

Five different unidentified glabrous mutants were left from trichome screen, reported to show glabrous leaves and hairy roots (Chopra 2015, PhD thesis) (Table S17). Using whole genome sequencing approach, I could show that all these unknown mutants are different alleles of *AaGL3*; which will be named *Aagl3-4* to *Aagl3-8* in the following (Figure 2.2 E). *Aagl3-4* has a splicing site change at the end of exon 6, results in a truncated protein. *Aagl3-5* and *Aagl3-7* alleles have stop codons at positions 370 and 116 which result in deletion of approximately one and two third of the protein, respectively. Finally, *Aagl3-6* and *Aagl3-8* have non-synonymous amino acid exchanges. *Aagl3-6* has a Serine (S) to Phenylalanine (F) exchange at position 123; while, *Aagl3-8* has a substitution of Aspartic Acid (D) to Asparagine (N) at position 265.

2.1.3. Overexpression of *AaGL3* in *A. alpina*

In *A. thaliana*, overexpression of *GL3* in wild-type results in an increased number of trichomes (Payne et al. 2000) and reduced number of root hairs (Bernhardt et al. 2003). This is consistent with the idea that *GL3* promotes trichome and non-hair cells. To analyze the effect of *AaGL3* overexpression in wild type *Arabis alpina*, I transformed the 35S::*AaGL3* construct (kindly provided by Dr. Chopra, Chopra 2015, PhD thesis) into *pep1-1*. I recovered three transgenic T1 plants. qRT-PCR revealed increased *GL3* expression in two of them (Figure 2.3 A) (Table S2). In plant No. 1, expression was 30 fold higher than in wild type. Plant No. 2 exhibited 10 fold higher expression. The higher expression of GL3 was confirmed by qRT_PCR in four individual T2 plants (Figure 2.3 B) (Table S3 and S4).

In the next generation, 30 single plants were analyzed with respect to their root hair and trichome numbers (Table S5). In the T2 generation it is expected to have homozygous, heterozygous and wild type seedlings. Root hairs were counted in the root hair zone equivalent to that described in Arabidopsis (Grierson and Schiefelbein, 2002) in

23

a one millimetre long section. In line No. 1, I found reduced root hair formation in 22 plants (Figure 2.4) (Table S5). In line No. 2, the root hair numbers were indistinguishable from wild type in all plants (Table S5). This finding supports the idea that *GL3* promotes a non-root hair fate in *Arabis alpina*, though the level of its expression needs to be high enough that the root hair phenotype can be seen clearly. Trichome density was determined on the same plants analyzed for the root hair pattern before. The third leaf was assessed when it had reached a length of 0.6 to 1 cm (Trichome counting was performed by Prof. Martin Hulskamp). In both lines, 72.5 % of the T2 plants displayed a reduction in trichome number down to 10% of that in wild type (Table S5). This is consistent with the expectation that 75% of the population are either heterozygous or homozygous for the construct. In line No.1, nearly all individual T2 plants with reduced root hair formation, also showed a reduced number of trichomes (Table S5). A reduction in trichome number is in sharp contrast to what is described in *A. thaliana*, where overexpression of *GL3* results in increased number of trichomes (Payne et al. 2000). When inspecting older leaves, I realized that trichomes are regularly distributed on the surface of the leaf and fairly similar in size (Figure 2.5).
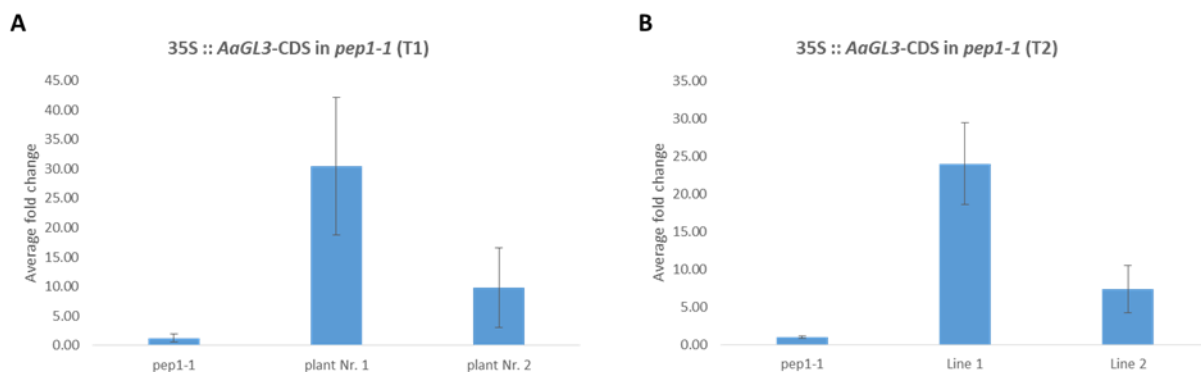
Figure 2.3. qRT-PCR result of 35S::AaGL3 in *pep1-1*. A, T1, only one individual per line was analyzed. **B**, T2, four individuals were analyzed for each line.
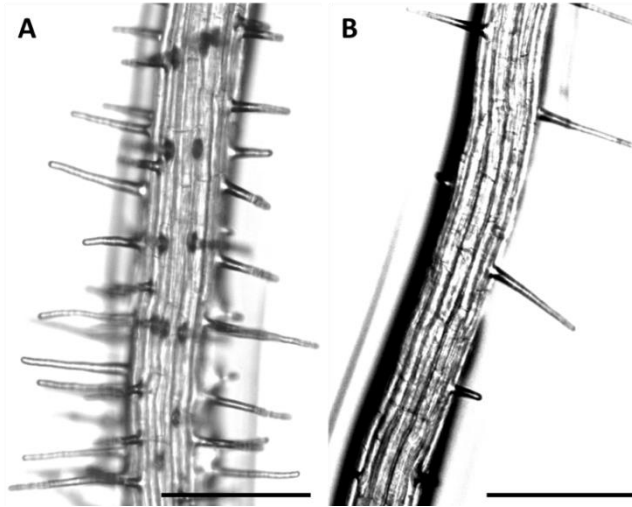
Figure 2.4. 35S::*AaGL3* root hair phenotype. The respective wild type background is *pep1-1*. **A,** *pep1-1* root. **B**, 35S::*AaGL3* root with less root hairs. Scale bar = 250 µm.
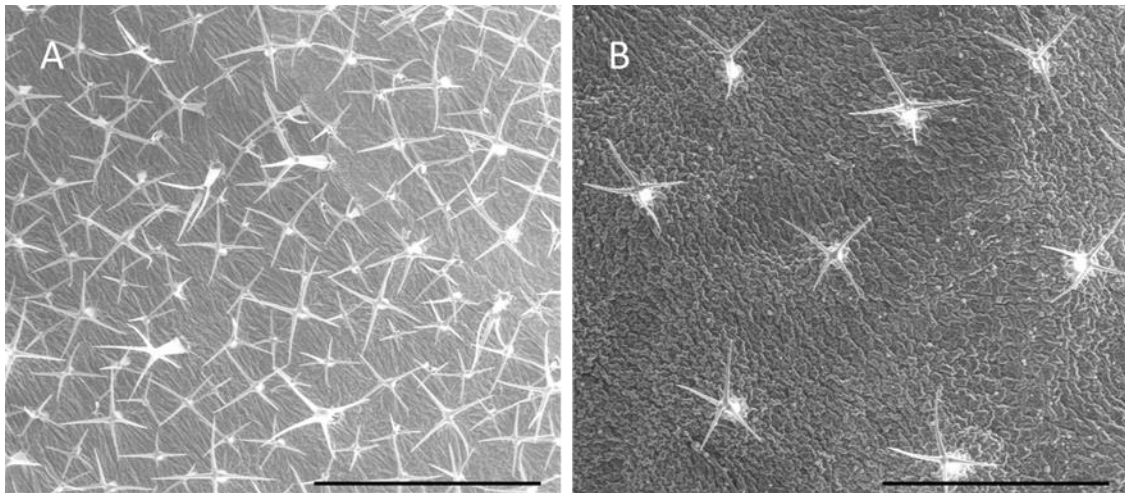
Figure 2.5. SEM of 35S::*AaGL3* trichome phenotype. The respective wild type background is *pep1-1*. **A,** *pep1-1* leaf with large and small trichomes. **B**, 35S::*AaGL3* leaf with the same sized trichomes. Scale bar = 1 mm.

2.1.4. Overexpression of *AaGL3* in *Aattg1*

It is known that overexpression of the R gene from maize, which is a homolog of *GL3*, can rescue epidermal-specific defects of *ttg1* mutants in *A. thaliana* (Lloyd et al. 1992; Galway et al., 1994). A rescue of the *Atttg1* trichome phenotype was also obtained upon *AtGL3* overexpression, though not to wild type levels. It has been discussed that the *GL3* protein is less efficient than the maize R gene in inducing trichome formation (Payne et al. 2000). Overexpression of *AtGL3* in *Atttg1* reduces the number of root hairs to about wild-type level (Bernhardt et al. 2003).

Since *AaGL3* appears to take over the role of *EGL3* in *A. alpina* I tested if *AaGL3* overexpression can rescue the epidermal defects of *Aattg1* mutants. Towards this end, I overexpressed *AaGL3* in the *Aattg1* mutant under the 35S promoter (provided by Dr. Chopra, Chopra 2015, PhD thesis). I recovered one transgenic plant that showed no rescue in the T1 and the T2 generations. qRT_PCR experiment revealed that this transgenic line has a 8 fold expression of *AaGL3* compared to the *Aattg1* or wild type background (Fig 2.6) (Table S13 and S14).
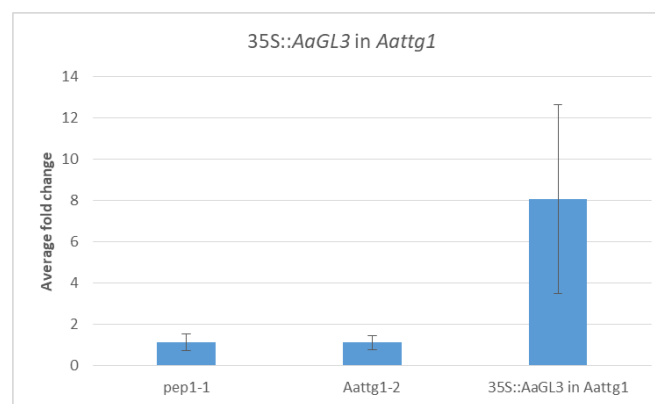
Figure 2.6. qRT-PCR result of 35S::*AaGL3* in *Aattg1*. Line expressing *AaGL3* in *Aattg1* shows 8 fold more *GL3* expression compared to *Aattg1-2* (Chopra et al., 2014) and *pep1-1* (Table S13 and S14).

2.1.5.   Analysis of *AaGL2* in *A. alpina*

One null allele of *Aagl2*, with aborted and underdeveloped trichomes, was previously identified (Chopra 2015, PhD thesis). To further analyze the role of *GL2* in trichome development of *A. alpina*, I analyzed the trichome development of *Aagl2* and the wild type in young stages (Figure 2.7). As described before (Chopra 2015, PhD thesis), in *Aagl2*, trichome growth is arrested from the initiation stages. However, two classes of big and small underdeveloped trichomes are clearly distinguishable (Figure 2.8). The small trichomes are mostly unbranched and spike like. The big trichomes are regularly distributed on the leaf surface with typically one or two small trichome cells in between. This phenotype suggests the presence of two types of genetically distinct trichomes.

In *A. thaliana*, aborted trichomes of *gl2* mutants are formed mostly at the leaf margin and the density is significantly reduced (Hülskamp et al. 1994). SEM pictures (Figure 2.7 E-H) show more developed trichomes at the edge of *gl2* mutant in *A. alpina* and a reduction of trichome density (Figure 2.8 B).

2.1.5.1.   Analysis of *AaGL2* expression

In addition to defects in trichome development, the *Aagl2* mutant is defective in root hair (Table S17) and seed coat mucilage production (Chopra 2015, PhD thesis). Therefore the spectrum of phenotype is similar as reported for *gl2* mutants in *A. thaliana* (Rerie *et al.* 1994; Masucci *et al.* 1996). *GL2* is expressed in trichome and non-hair cells in *A. thaliana* (Szymanski et al. 1998; Masucci et al. 1996). Dr. Chopra decided to analyze the *GL2* expression in *A. alpina* epidermis (Chopra 2015, PhD thesis). Therefore, she transformed *A. alpina pep1-1* plants with two constructs of proAaGL2::GFP and proAaGL2::GUS. I analyzed T2 plants, but no fluorescence signal was observed for either

of the transformed progenies. I further analyzed the structure of transformed constructs and found confusions (The analysis was done by my Bachelor student, Julian Schiffner). Therefore I decided to clone a new construct of proAaGL2::GFP (2116 bp upstream of the start of *GL2* in *A. alpina* was previously cloned by Dr. Chopra) and transformed *pep1-1* plants.
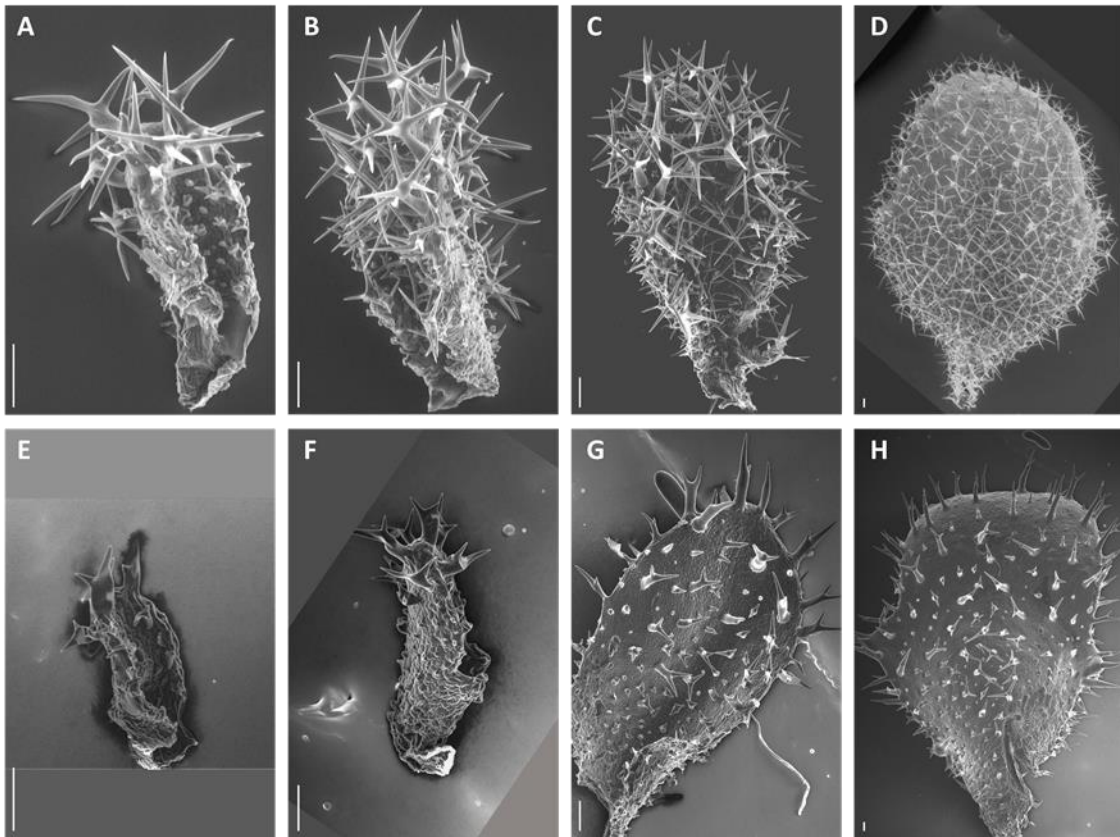
Figure 2.7. SEM pictures presenting trichome development in the wild type and *AaGL2*. A-D, wild type (*pep1-1*) trichome development from young stage (A) to a mature leaf (D). **E-H,** *Aagl2* trichome development from young stage (E) to the mature leaf (H). Scale bar = 100 µm.
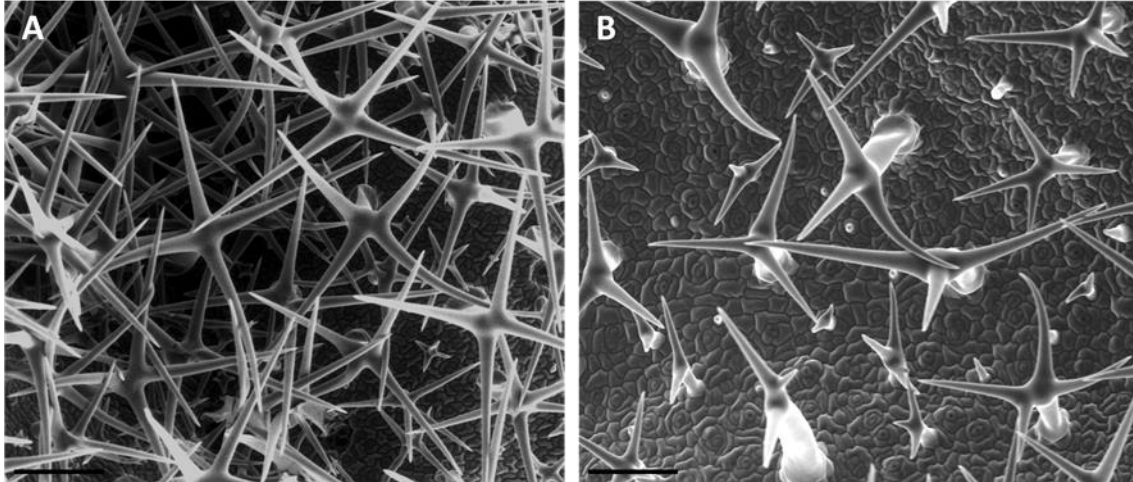
Figure 2.8. SEM pictures of the wild type and *AaGL2* leaves. Big and small trichomes are clearly seen in wild type and Aagl2 leaves. **A,** wild type (*pep1-1*) leaf. **B,** *Aagl2* leaf. Scale bar = 100 µm.

2.1.5.2.   Confirm the role of *AaGL2* in *A. alpina*

In order to confirm that the phenotype observed is caused by the mutation in *AaGL2*, it is necessary to either analyze a second allele or to show rescue by a wild type copy. I therefore created lines to knock down *AaGL2* in *pep1-1* plants by microRNA expression. No T1 plant was recovered in this experiment (see 2.1.6.1).

Overexpression of *GL2* in the *A. thaliana* wild type using the 35S promoter appears to be unfavorable for plants. Progenies with confirmed overexpression showed a *gl2*-like phenotype. Others were hardly viable or wild-type like (Ohashi et al., 2002). 35S::*GL2* did not complement defects in the *gl2* mutant in *A. thaliana* (Ohashi et al., 2002). I wanted to analyze the effect of *AaGL2* overexpression in *A. alpina.* Towards this end, I transformed *AaGL2* into the *Aagl2* mutant under the control of the 35S promoter. One plant was recovered in T1 and showed a higher number of trichomes (Figure 2.9). However in T2, it was indistinguishable from *Aagl2* when analyzed for trichome and mucilage production (Data not shown). Expression analysis by qRT-PCR in the T2

generation revealed that *GL2* is 38 fold higher expressed in 35S::*AaGL2* compared to the *Aagl2* mutant and wild type (*pep1-1*) background (Figure 2.10) (Table S 15 and S 16).
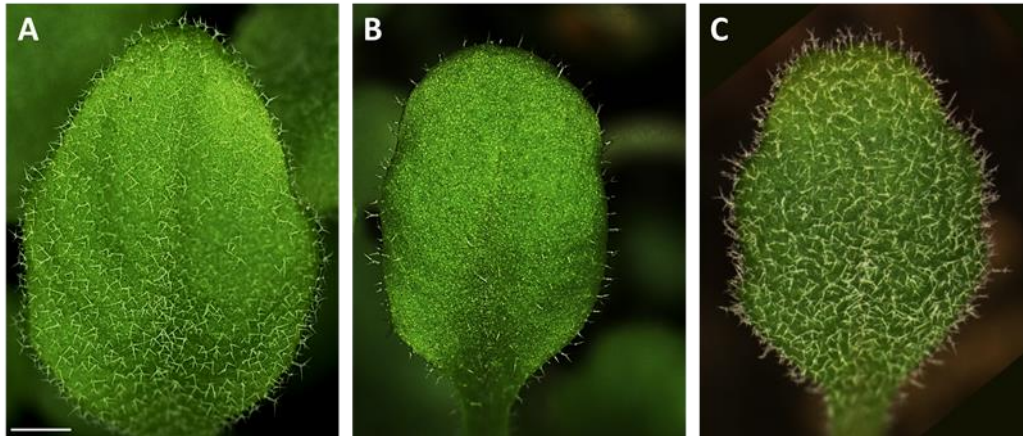
Figure 2.9. Trichome phenotype of 35S::*AaGL2* in *Aagl2* background in T1. The respective wild type background is *pep1-1*. **A,** *pep1-1* leaf. **B**, Aagl2 leaf. **C,** 35S::*AaGL2* in Aagl2 background. Scale bar = 1 mm.
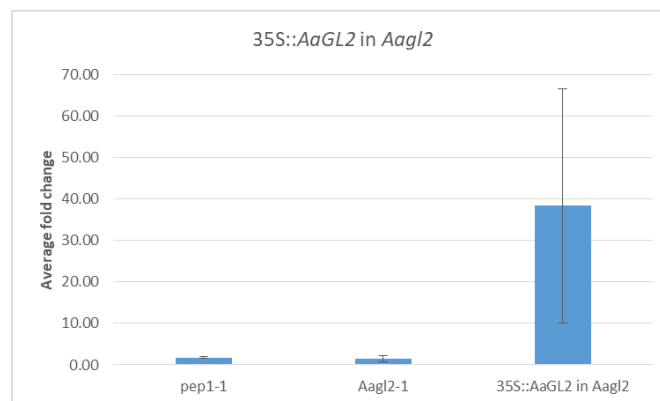
Figure 2.10. qRT-PCR result of 35S::*AaGL2* in *Aagl2-1*. The line expressing *AaGL2* in *Aagl2* shows 8 fold more expression compared to *Aagl2-1* and *pep1-1* (Table S15 and S16).

2.1.6. Attempts to produce transgenic lines in *A. alpina*

2.1.6.1. Failed in recovering transgenic plants

Based on the low rate of transformation efficiency in *Arabis alpina*, approximately 200 individual plants are needed to be transformed per construct (personal communication with Prof. Maria Albani). To analyze the role of different genes in trichome/root hair patterning in *A. alpina*, I planned different transformations (Table S6). Because of space constraints I compromised and transformed 90 plants per construct reasoning that I should get at least few transformants for most of the approaches.

In the T1 generation, I recovered zero to 17 plants per construct, resulting in 110 individuals. In the molecular analysis, only five plants from three transformation events were confirmed to contain an insert (Table S6). For the remaining plants, I could not confirm the presence of the insert during PCR analysis. Consequently, their progeny did not survive Basta in T2. Therefore, I did not proceed.

2.1.6.2. Analysis of the role of *AaEGL3* in *A. alpina*

EGL3 is a bHLH factor that acts redundantly with *GL3* to initiate trichomes. Single mutants in *A. thaliana* show 20% reduction in trichome numbers and fewer branches. Mutant root hairs are indistinguishable from the wild type (Zhang et al., 2003; Bernhardt et al. 2003). Overexpression of *EGL3* results in an increased number of trichomes and reduced number of root hairs (Zhang et al. 2003; Payne et al. 2000 and Bernhardt et al. 2003). Since none of the glabrous and/or hairy root mutants in *A. alpina* were identified as *Aaegl3*, I decided to knock it down using an artificial microRNA, and knock it out using the CRISPR-Cas method (Hyun et al. 2015). The relative constructs targeting *AaEGL3* were

designed and transformed to *pep1-1*. In addition, I transformed the 35S::*AaEGL3*-CDS construct (provided by Dr. Chopra; Chopra 2015, PhD thesis) in *pep1-1* plants to further analyze the role of *EGL3* in *A. alpina*. Among 6 microRNA and 1 overexpression plants in T1, none of them survived selection process in T2 generation indicating that the construct was not transformed. No T1 plant was recovered from CRISPR-Cas transformation.

2.1.6.3.   Analysis of the role of *AaGl1* in *A. alpina*

GL1 is a crucial trichome specific activator which acts at the same level of *TTG1* to initiate trichomes. *Atgl1* null mutants are glabrous on leaves, stems, petioles and sepals (Koorneef et al. 1989; Marks and Feldmann, 1989; Esch et al., 1994; Oppenheimer et al., 1991). No root hair phenotype has been reported for any of *Atgl1* alleles (Larkin et al. 1994b). Unexpectedly, overexpression of *GL1* in wild type *A. thaliana* results in a reduction of trichome numbers (Oppenheimer et al. 1991; Larkin et al. 1994b; Payne et al. 2000). It's been argued that high GL1 protein concentration interferes with trichome formation machinery, resulting in less trichomes (Payne et al. 2000). Since none of our glabrous mutants have been identified as *Aagl1* alleles, I wanted to examine whether *GL1* is important for trichome formation in Arabis. For this purpose, I designed artificial microRNA and CRISPR-Cas constructs targeting *AaGL1* and transformed them to *pep1-1* plants. Additionally, a construct of 35S::*AaGL1*-CDS (CDS was previously cloned by Dr. Chopra) was transformed to *pep1-1* plants to see if the converse phenotype of *GL1* overexpression would be observed in *A. alpina*. Among 26 microRNA and 11 overexpression plants in T1, I could not recover any in T2 after basta selection. No T1 plant was recovered from CRISPR-Cas transformation.

Moreover, overexpression of *GL1* in *try* background results in increased trichome clusters and endoreduplication (Schnittger et al. 1999; Schellmann et al. 2002). To further

analyze the role of *AaGL1* in trichome patterning, I also transformed Arabis try mutants with the 35S::*AaGL1*-CDS construct. Only one plant was recovered in T1 generation, however it was negative in PCR analysis for carrying the insert.

2.1.6.4.   Analysis of the overexpression of *AaTTG1* in *A. alpina*

Overexpression of *AtTTG1* in wild type *A. thaliana* background doesn't have any trichome phenotype (Payne et al. 2000). In order to analyze *AaTTG1* overexpression in wild type *Arabis alpina*, a construct expressing *AaTTG1*-CDS driven by the 35S promoter (provided by Dr. Chopra; Chopra 2015, PhD thesis) was transformed into *pep1-1* plants. Among 11 plants in T1, I could not recover any in T2 generation.

2.1.6.5.   Analysis of the overexpression of *AaTRY* in *A. alpina*

Overexpression of the major trichome inhibitor (*AtTRY*) in *A. thaliana* wild type, results in glabrous leaves and ectopic root hairs (Schellmann et al. 2002). As *TRY* appeared to be functional in *A. alpina*, by showing clustered trichomes when mutated (Chopra 2015, PhD thesis), I wanted to test if its overexpression shows a glabrous phenotype. Therefore, the 35S::*AaTRY*-CDS (CDS was previously cloned by Dr. Chopra) was transformed into *pep1-1* plants. From 11 plants in T1, none survived basta selection in T2 generation.

2.1.6.6.   Analysis of the overexpression of *AaWER* in *A. alpina*

WER is a MYB transcription factor, which plays a role in root hair patterning through promoting non-hair cell fate. *wer* mutants in *A. thaliana* have ectopic root hairs.

Overexpression of *WER* in wild type *A. thaliana* does not change the pattern of root epidermal cells significantly. However, it induces *GL2* expression in H-position (Lee and Schiefelbein, 1999). To analyze the role of *WER* in *A. alpina*, I overexpressed *AaWER*-CDS (CDS was previously cloned by Dr. Chopra) under the 35S promoter in *pep1-1* plants. Among 10 plants in T1, I could not recover any in T2 generation.

2.1.6.7. Analysis of the role of *CPC* in *A. alpina*

CPC is an R3 single repeat MYB transcription factor, which promotes root hair formation by inhibiting non hair cell fate. In *A. thaliana*, *cpc* mutants show a significant reduction of root hair numbers (Wada et al. 1997) and a two fold increase in trichome number (Schellmann et al. 2002). Two *CPC* homologs have been identified in *A. alpina* genome (Willing et al. 2015). In my root hair screen, none of the candidates with reduced number of hairs showed any deviations in their genomic sequence for *AaCPC1* or *AaCPC2*. In order to examine if *CPC* plays a role in root hair patterning in *A. alpina*, I designed artificial microRNA and CRISPR-Cas constructs targeting *AaCPC1* and *AaCPC2* simultaneously. These constructs were transformed to *pep1-1* plants. Among 3 microRNA plants in T1, I could not recover any in T2 generation. No T1 plant was recovered from CRISPR-Cas transformation.

2.2. Analysis of root hair development in *A. alpina*

2.2.1. Root hair development in wild type *A. alpina*

2.2.1.1. Root hair morphogenesis in *A. alpina*

Root hairs in *Arabidopsis thaliana* are defined as long, tubular-shaped outgrowths from root epidermal cells. They grow approximately 10 μm in diameter and 1 mm in length. Root hair formation has two distinct phases: Initiation and tip growth. In the initiation phase, root epidermal cells which are committed to hair formation, change their shape by cell wall modification. Hairs initiate at the apical end of epidermal cells by localized cell expansion at the outer-facing wall of the cell, leading to a local swelling and bulge formation. This swelling will then continue to grow by a specific type of growth, called tip growth, in which cell expansion is highly localized at the tip of the hair (Grierson and Schiefelbein 2002; Ryan et al. 2001).

The root hair morphology of *Arabis alpina* and *Arabidopsis thaliana* is highly similar. Root hairs are initiated at the apical end of root epidermal cells followed by extensive tip growth (Figure 2.11).

2.2.1.2. Root hair cell specification in *A. alpina*

Root hair files in *A. thaliana* are interspersed with the non-hair files. This pattern is formed in a position dependent manner (Dolan et al. 1994, Galway et al. 1994). Root hair cells (trichoblasts) are located at the cleft of two underlying cortical cells, whereas

non-hair cells (atrichoblasts) are found over cortical cells. Trichoblasts are distinguishable from atrichoblasts prior to the outgrowth of the hair, by a variety of characteristics (Dolan et al. 1993, Galway et al. 1994). Epidermal cells destined to become hair cells have dense cytoplasm (Dolan et al, 1994, Galway et al. 1994) and higher rates of cell division (Berger et al. 1998). They delay in vacuole formation (Galway et al. 1994) and have reduced cell length (Dolan et al. 1994, Masucci et al. 1996). This indicates that pattern information is provided at an early stage of epidermal development. Therefore cell length measurements of cells in individual cell files, can be used to distinguish between trichoblasts and atrichoblasts prior to hair formation.

Chopra et al., 2014 showed that the cells in N-position are twice as long compared to cells in H-position in *A. alpina*. To test if this cellular dimorphism between the files is distinguishable prior to hair formation in *A. alpina*, I measured the length of four cells in N and H files, immediately below the cell displaying the formation of a root hair bulge. The position of the hair files were confirmed by determining that they are located over the cleft of two underlying cortical cells (Figure 2.12 A, B). Atrichoblasts and trichoblasts located close to the root tip show no length difference whereas more distant trichoblasts are about 30% shorter than atrichoblast (Figure 2.12 C).

In *A. thaliana, TTG1* acts at an early stage in epidermal cell differentiation as indicated by the finding that in *ttg1* mutants epidermal cells in N files exhibit the characteristics of root hair cells (Galway et al., 1994). By contrast, *GL2* acts later in hair cell differentiation as *gl2* mutants still form trichoblasts and atrichoblasts files at early developmental stages (Masucci et al., 1996).

To analyze at what developmental stage *TTG1* and *GL2* regulate cell differentiation in *A. alpina*, I measured the cell length in H and N files in *Aattg1* and *Aagl2* mutants. In *Aattg1*, cells exhibited the same size in all files prior to hair formation (Figure 2.13 B). The size of these cells correspond to that of H files in wild type. This suggests, that *AaTTG1*

acts at early developmental stages similarly as described for *A. thaliana*. In *Aagl2* mutants, N and H files clearly differ in length (Figure 2.13 C). As *Aagl2* mutants exhibit extra root hairs at later stages of root development I conclude that AaGL2 acts at later steps of trichome differentiation than *AaTTG1*.
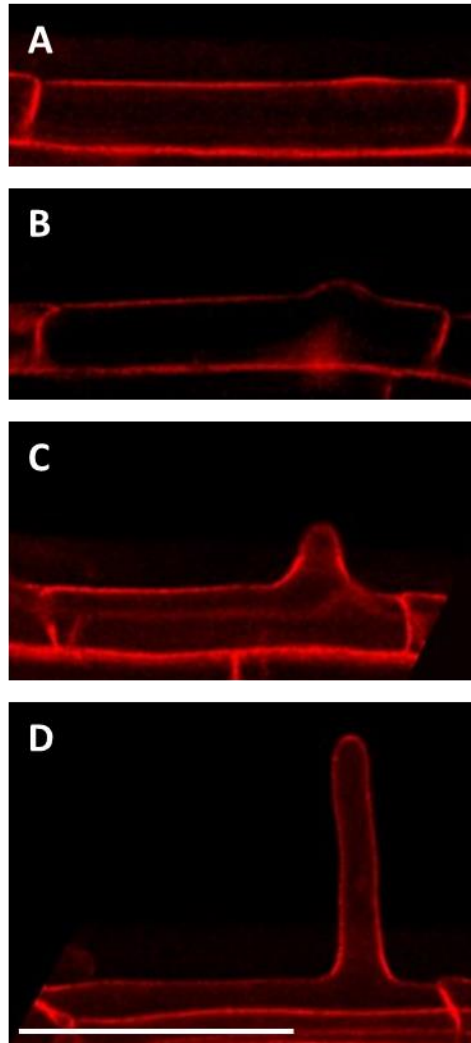
Figure 2.11. Root hair development on *A. alpina*. Propidium iodide staining of wild type root hairs in *Pajares* background .**A,** Root hair site selection (at the apical end of the trichoblast, close to the tip of the root) and initiation. **B**, Bulge formation. **C,** Beginning of tip growth. **D,** Tip growth. Scale bar = 50 µm.
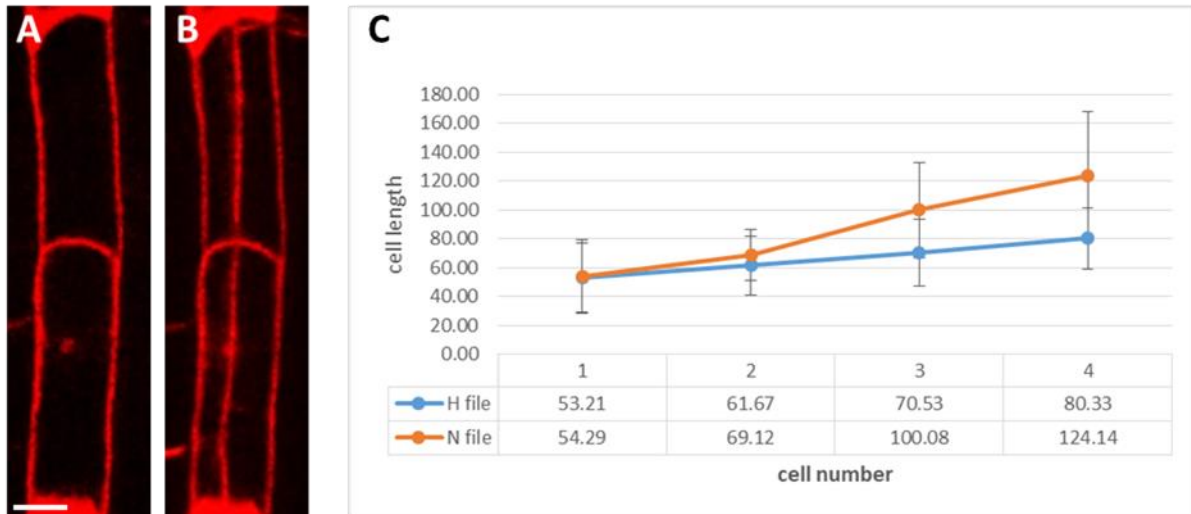
Figure 2.12. Cells in H files are at the cleft of underlying cortical cells and are shorter than cells in N files in _A. alpina_. A, Propidium iodide staining of epidermal cells in H file of the _Pajares_ wild type. **B**, The stack image shows that the epidermal cells in the H-file lie over the junction of two cortical cells. **C,** Cell length is shown for four cells with the first cell representing the closest to the tip and the fourth cell being immediately below the first root hair cell showing a bulge. The upper curve (red) corresponds to the non-hair files presenting longer cells (atrichoblasts) and the lower curve (blue) corresponds to the hair files presenting shorter cells (trichoblasts). The curves end where the first sign of hair formation becomes visible. Data points represent the mean of 15 independent cell files (Data is provided in the supplement file 1). Scale bar = 20 μm.
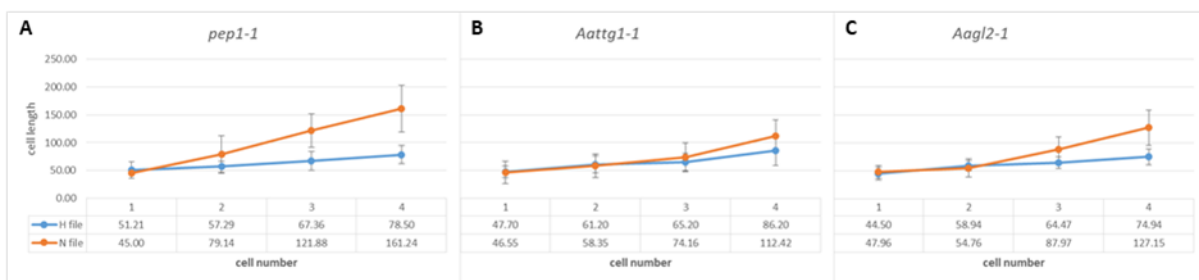
Figure 2.13. Diagrams comparing cell length in N and H files of _Aattg1_, _Aagl2_ and wild type. The corresponding wild type background is _pep1-1_. The upper curves (red) correspond to the non-hair files and the lower curves (blue) correspond to the hair files. The curves end where the first sign of hair formation becomes visible. N and H files were determined in relation to the underlying cortical cells. The length of four cells in each file was measured, starting immediately where the bulge becomes visible towards the root tip. Data points represent the mean of at least 10 independent cell files (Data is provided in the supplement file 1). **A,** _pep1-1_. **B,** _Aattg1-2_. **C,** _Aagl2-1_.

2.2.2. Isolation of root hair mutants in *A. alpina*

We used a forward genetic approach to define different functional steps during root hair development in *Arabis alpina*. Screens were performed in two EMS populations: one in *Pajares* (Wang et al. 2009) and one in the *pep1-1* background (Albani et al. 2012; Bergonzi et al. 2013). Both EMS populations were provided by Prof. Maria Albani and Prof. Goerge Coupland. The *A. alpina Pajares* ecotype needs twelve weeks of vernalization before flowering. *pep1-1* is in *Pajares* background carrying a mutation in *PEP1* gene and does not need vernalization. It enters the reproducing phase after 90 days in long day conditions (Albani et al. 2012; Bergonzi et al. 2013).

In both EMS populations, five M1 plants were pooled. Around 50 M2 seedlings from each pool were screened for root hair phenotypes. 841 pools from the *Pajares* population representing 4,205 M1 plants had been previously screened by Dr. Chopra. She selected 58 candidates in M2 (Chopra 2015, PhD thesis). I confirmed 12 root hair mutants in the M3 generation. I additionally screened 1,360 pools of *pep1-1* population representing 6,800 M1 individuals. 216 candidates were selected in M2 (Table S7). The re-screen in the M3 generation revealed 33 mutants.

I distinguished mutants in which either root hair pattern or morphology is disturbed (Table 2.1). Regarding pattern defects, seven mutants show hairy roots, and 6 establish less root hairs as compared to the wild type. Morphology mutants are divided in five classes. Three mutants show problems in hair initiation, producing only small bulges. Six mutants produce big bulbs at the base of the hair; they are called swollen. Root hairs of three mutants burst; and one mutant produces multiple hairs from each trichoblast. The two last mutant classes initiate hairs normally but show defects in tip growth. Sixteen mutants produce short hairs and three have problems in growth

directionality, producing branched or wavy hairs. Generally, I could identify the same range of phenotypes as described in *A. thaliana* (Schiefelbein 2000; Parker et al. 2000).

	Root hair phenotype	*Pajares*	*pep1-1*	
				Total
Patterning	more hairs	2	5	7
	less hairs	3	3	6
Morphology	only bulge	1	2	3
	swollen	1	5	6
	burst	1	2	3
	multiple hairs	1	0	1
	short	2	14	16
	branch/wavy	1	2	3
	Total	12	33	45

Table 2.1. Root hair mutants in *A. alpina*.

2.2.2.1. Mutants affecting root hair patterning

I identified 13 mutants defective in their root hair pattern. Seven appeared hairy as they produced more root hairs. As root hairs are clearly seen in several neighboring cell files, it is conceivable that plants produce root hairs in N files (Figure 2.14 B).

Six mutants produce less root hairs as compared to wild type. They can be divided in three different sub-classes. Two produce almost no hairs in the root hair zone (Figure 2.15 B). In two others, some hairs initiate, though they are not able to grow further. In these two mutants roots are also twisted (Figure 2.15 C). In the two last mutants root hair morphology is fairly normal, and the root hair numbers are reduced (Figure 2.15 D).
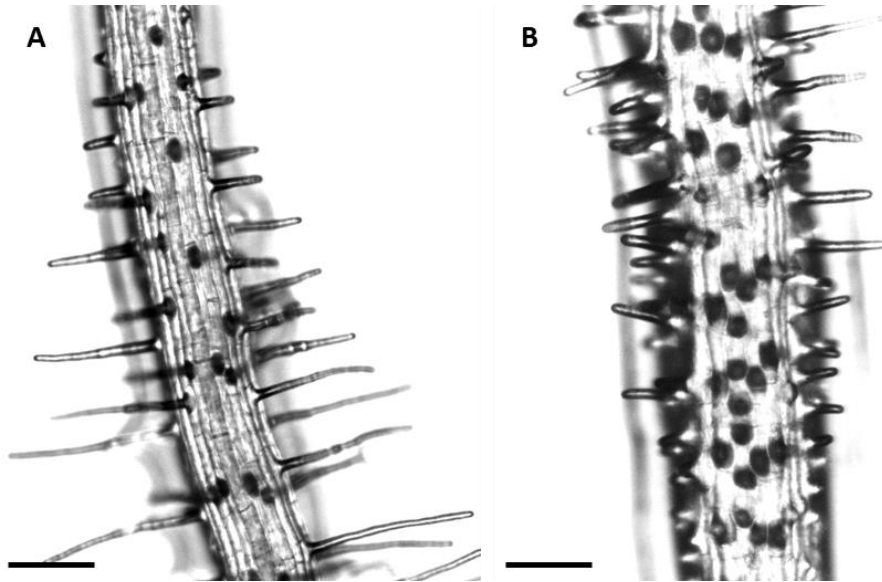
Figure 2.14. Root hair patterning phenotypes. A, Wild type. **B,** Excessive root hair production (line 1264; see table S10). Scale bar = 100 μm.
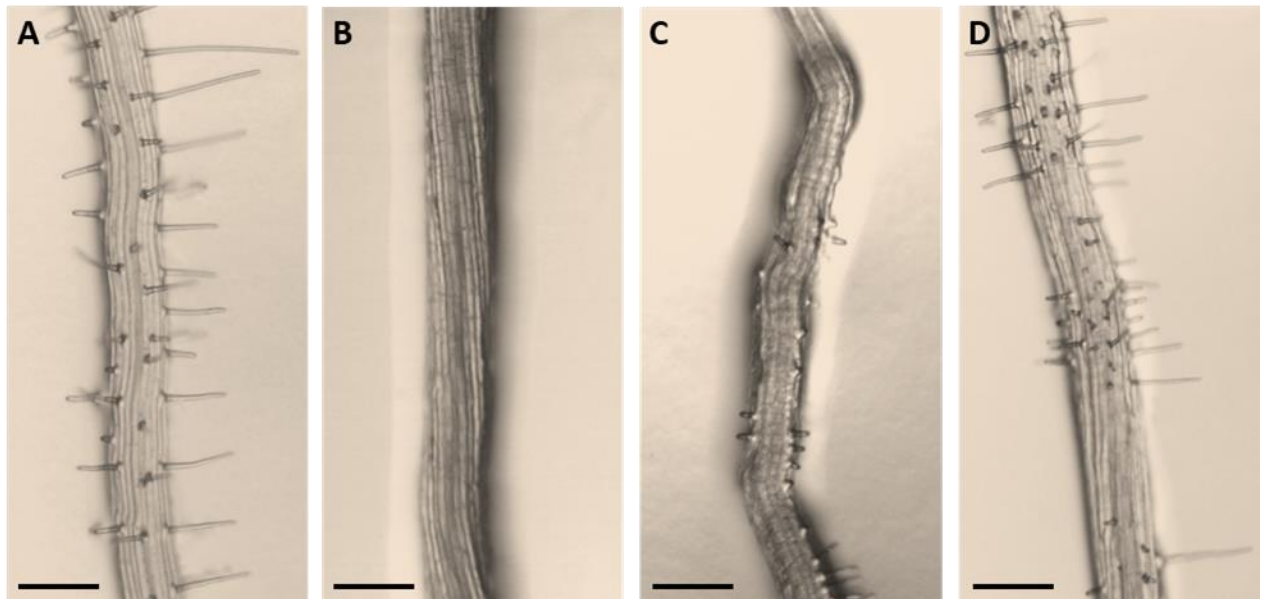
Figure 2.15. Root hair patterning phenotypes. A, Wild type. **B,** No root hair production (line 1101; see table S10). **C,** Root hairs are reduced and short (line 616). **D,** Reduced root hair production (line 1304). Scale bar = 250 μm.

2.2.2.2. Mutants affecting root hair initiation

Root hair initiation was affected in 13 mutant lines showing either defects in root hair formation or the number of hairs emerging from each trichoblast. I found three lines exhibiting only bulges (Figure 2.16 B). In these mutants, cell expansion is disturbed. In seven mutants, hairs initiate from an excessive swelling on the epidermal cell and produce a bulbous base (Figure 2.16 C). Three lines show a bursting phenotype (Figure 2.16 D). Typically, in this class root hairs rupture at the tip soon after initiation. One line produced multiple sites of growth from each epidermal cell (Figure 2.16 E).

2.2.2.3. Mutants affecting root hair tip growth

I identified 19 mutant lines, in which tip growth is affected. In sixteen lines I observed short hairs (Figure 2.16 F). Three lines displayed branched and/or wavy hairs (Figure 2.16 G, H).
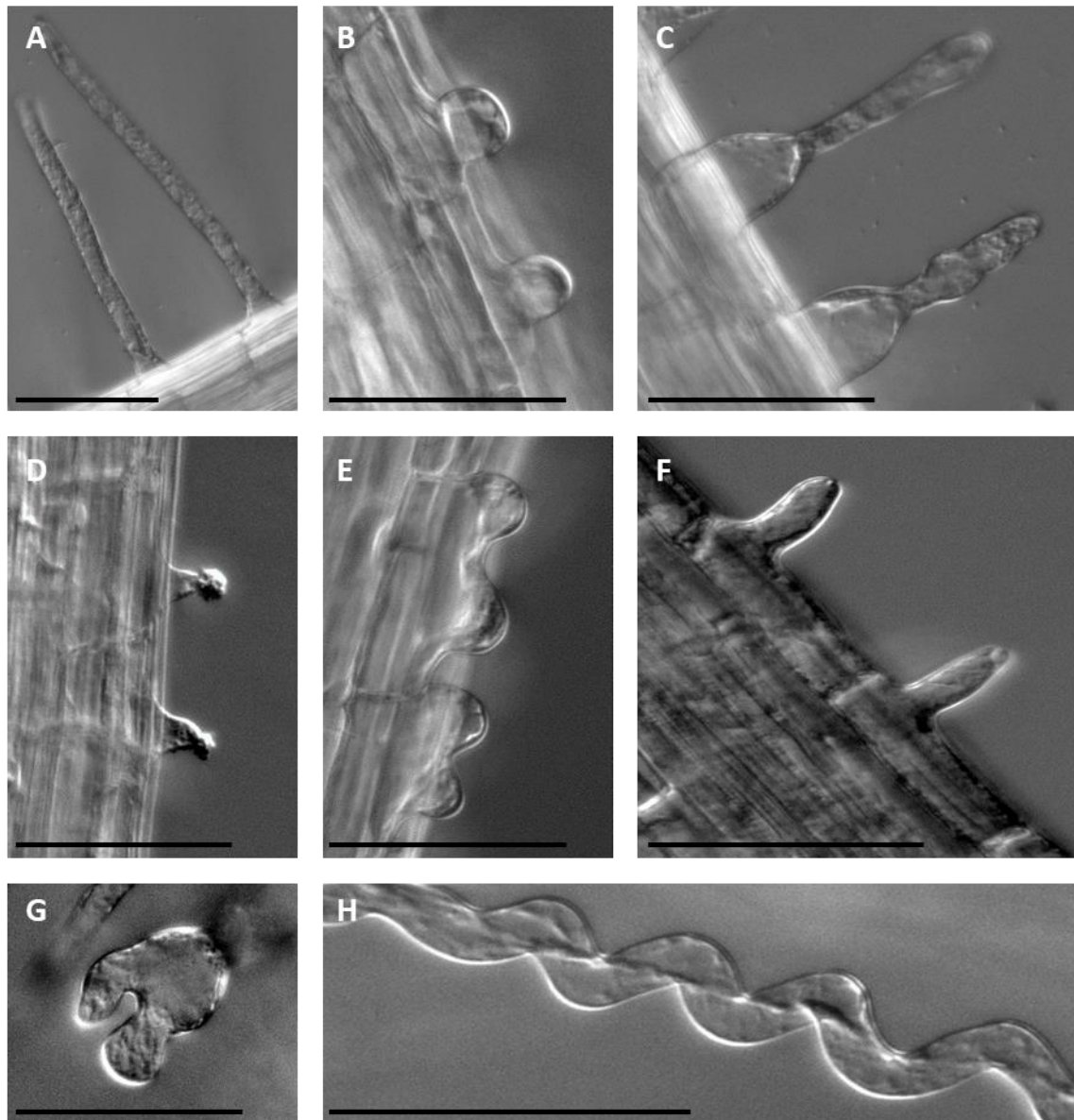
Figure 2.16. DIC pictures of root hair morphogenesis phenotypes. A, Wild type root hair. **B,** Only bulge formation (line 366; see table S10). **C,** Root hairs are swollen at the base (line 1221). **D,** Bursting root hairs (line 1185). **E,** Multiple outgrowths from each epidermal cell (line 1384). **F,** Short root hairs (line 1091). **G,** Branched root hairs (line 216). **D,** Wavy root hairs (line 216). Scale bar = 75 µm.

2.3. Identification of mutant alleles in *A. alpina*

Both *Arabis alpina* and *Arabidopsis thaliana* are crucifers, and evolutionarily separated for about 26 million years (Koch et al., 2006; Beilstein et al., 2010). I assumed that the majority of mutant phenotypes found in the *A. alpina* root hair screen are caused by the mutations in genes that are orthologous to the respective *A. thaliana* genes. Initially, I searched for a selection of genes involved in root hair development of *A. thaliana* (Table S8). Root hair and trichome developments share many regulatory genes involved in both processes, and root hair tip growth is highly similar to tip growth in pollen tubes. Therefore, genes involved in trichome and pollen tube development were also added to the list of root hair candidate genes, leading to a total number of 178 genes. According to the *A. alpina* genome assembly and annotation (Willing et al. 2015), 374 *A. alpina* genes were identified as paralogs or orthologs of the 178 *A. thaliana* genes and thus considered for further analysis (Table S8) (This part of analysis was performed by Hequan Sun and Korbinian Schneeberger from MPIPZ).

Following the assumption that mutations in *A. alpina* root hair genes lead to the same phenotype as in *A. thaliana*, one would expect to find relevant mutations in the respective genes in the root hair mutants. I therefore sequenced candidate genes in selected root hair mutants similarly as done before for finding trichome genes in *A. alpina* (Chopra et al., 2014; Chopra 2015, PhD thesis). Using this approach I identified three mutant-specific alleles (Table S9).

Precise root hair phenotyping in *Arabis alpina* is difficult because of high variability in the wild type. In addition, the high number of candidate genes rendered gene by gene sequencing inefficient. I therefore decided to continue the work using whole genome

sequencing. I sent genomic DNA from different mutants for whole genome sequencing aiming for an average of 15X fold coverage. The results were preliminary analyzed by Hequan Sun from Korbinian Schneeberger's lab to find SNPs located in the genes of interest. For each SNP, the position in the chromosome, the reference allele, the mutant allele, the location of exchange regarding the gene model, mutant allele coverage and mutant allele frequency are provided (Supplemental file 2). The mutant allele coverage describes the number of reads for that specific allele. The mutant allele frequency describes the frequency of the mutant allele related to the number of reads. Annotated SNPs, stringently selected for each mutant with a minimum mutant allele frequency of 0.85 and minimum mutant allele coverage of 3, were chosen for further analysis. Next I correlated SNPs to the phenotype of the respective mutants. As summarized in Table S10 I could identify 28 mutant-specific alleles. I considered stop codons as mutant specific alleles, while amino acid exchanges were only considered when leads to a non-synonymous amino acid exchange.

2.3.1. Analysis of burst root hair mutants in *A. alpina*

2.3.1.1. Analysis of *Aakjk* mutants

KJK encodes a cellulose synthase-like protein (AtCSLD3), which localizes to the endoplasmic reticulum. It is required for the synthesis of a non-cellulosic wall polysaccharide required for root hair elongation (Favery et al. 2000). It is notable that the

protein produced by the *KJK* gene in *A. alpina* shows 95% sequence similarity to *A. thaliana* and synteny is also maintained between these two species (Table S11).

A

Aakjk-2, G(248) to R
cellulose synthase-like protein
Aakjk-3, W(843) to *
Aakjk-1, T(854) to M
500
1.000

*AaKJK*_translation of CDS

B

Atkjk-2, Q(121) to *
cellulose synthase-like protein
Atkjk-3, E(829) to *
Atkjk-1, R(889) to *
500
1.000

*AtKJK*_translation of CDS
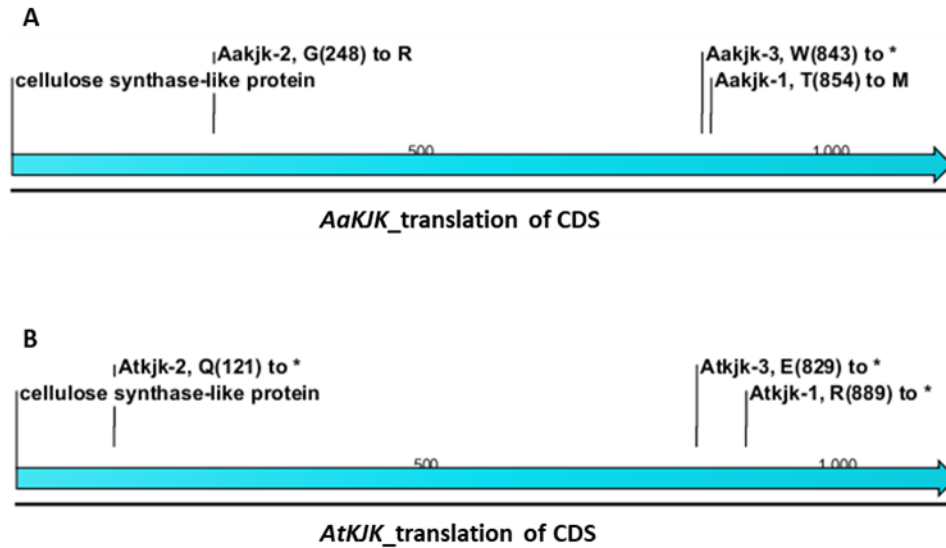
Figure 2.17. Schematic representation of *KJK* amino acid sequence in *A. alpina* and *A. thaliana*. A, *AaKJK*. **B**, *AtKJK*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* and *A. thaliana* are marked.

In *kjk* mutants an initial bulge is formed at the correct position on the trichoblasts. The incipient bulge starts to swell spherically until it ruptures. The protoplast is forced out through a fracture in the cell wall. This indicates that the *KJK* gene is required to maintain the integrity of the cell wall before the onset of tip growth (Favery et al. 2000). In *Arabis alpina*, *kjk* mutants look virtually hairless (Figure 2.18 B). More detailed analysis revealed that root hairs initiate in the correct position at the apical end of the trichoblast and rupture early upon initiation (Figure 2.18 D). No other defects were observed in *Aakjk* mutants. Plants grow indistinguishable from the wild type and show no obvious defects in fertility. This is reminiscent of former observations in *A. thaliana* (Favery et al. 2000).

Three different mutant lines showing bursting root hairs were isolated from the EMS screens. In the whole genome sequence analysis, they show different mutations in

the *KOJAK* (*KJK*) gene. *Aakjk-1* has a C to T exchange, leading to a threonine (T) to methionine (M) substitution at position 854. *Aakjk-2* has a G to A exchange, translating into a Glycine (G) to Arginine (R) transition at position 248. *Aakjk-3* has a G to A exchange resulting in a stop codon at position 843 (Figure 2.17 A). In Arabidopsis two mutations identified in *Atkjk-1* and *Atkjk-3* locate to similar positions (Favery et al. 2000) (Figure 2.17 B).
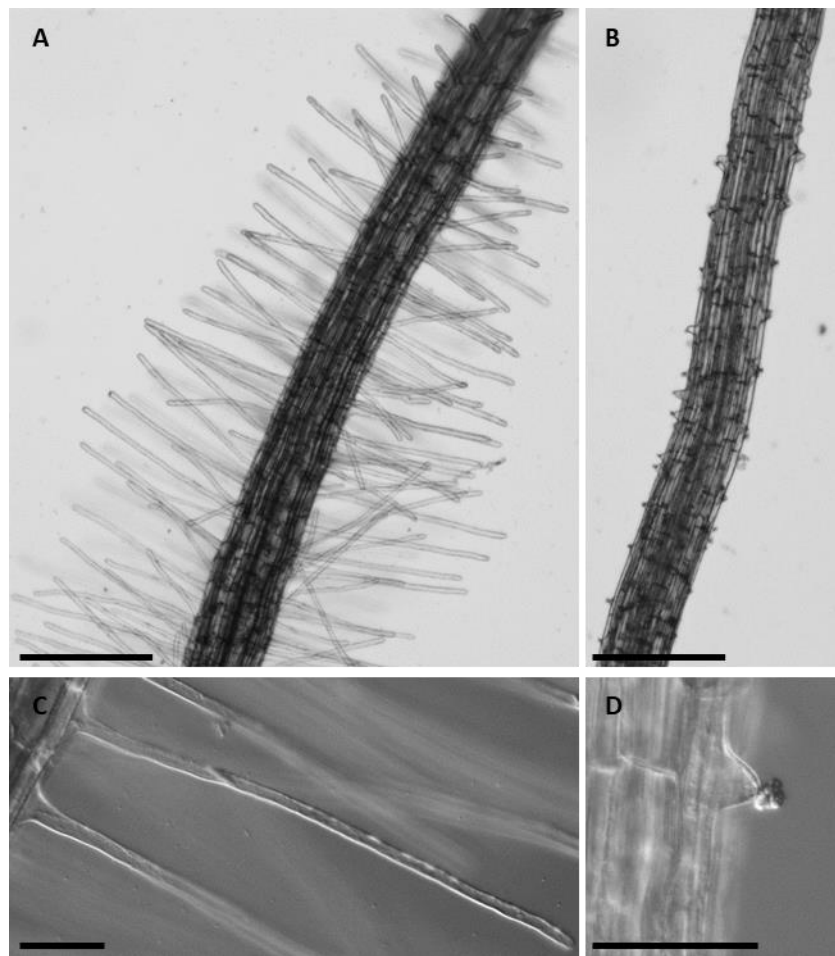
Figure 2.18. *Aakjk* mutant phenotypes. A, Wild type root. **B**, *Aakjk* mutant root (line 1085; see table S10). **C,** DIC picture of wild type root hair. **D,** DIC picture of a bursting hair (line 1185). Scale bar = 250 µm in A and B, 75 µm in C and D.

2.3.2. Analysis of short root hair mutants in *A. alpina*

The whole genome sequence analysis of the sixteen mutants with short root hairs revealed four lines with mutations in the *CAN OF WORMS 1* (*AaCOW1*) and two lines with mutations in the *SPIRRIG* (*AaSPI*) gene.

2.3.2.1. Analysis of *Aacow1* mutants

The *COW1* gene encodes a phosphatidylinositol transfer protein (PITP-like) and is involved in signal transduction and membrane trafficking during tip growth (Grierson et al. 1997 and Böhme et al. 2004). The protein produced by the *COW1* gene in *A. alpina* has 87% similarity to *A. thaliana*. Synteny is also maintained between these two species (Table S11).

Root hairs in *Atcow1* mutants are short, wide and occasionally form as twins. *COW1* acts at the later stages of tip growth; therefore root hair initiation is normal in this mutant (Grierson et al. 1997; Böhme et al. 2004). Detailed analysis of *Aacow1* alleles revealed the same range of phenotypes. Root hairs are clearly shorter and wider as compared to the wild type (Figure 2.20 B, C, and E). Twin hairs are found on the whole mount of primary roots in *Aacow1* alleles (Figure 2.20 D and F). As reported for *A. thaliana*, root hair position and initiation are not affected in *Aacow1* mutants.

Mutations in *AaCOW1* included acceptor splice site mutations, premature stop codons or amino acid exchanges. *Aacow1-1* has a G to A exchange leading to a stop codon after 505 amino acids. The *Aacow1-3* allele has a G to A exchange causing a proline (P) to leucine (L) substitution at position 293. *Aacow1_2* and *Aacow1-4* alleles both have a C to T exchange leading to splice site changes resulting in stop codons at positions 239 and 213 respectively (Figure 2.19 A). One allele in *A. thaliana* (*Atcow1-3*) has been reported to possess a stop codon at position 383 (Böhme et al. 2004) (Figure 2.19 B).
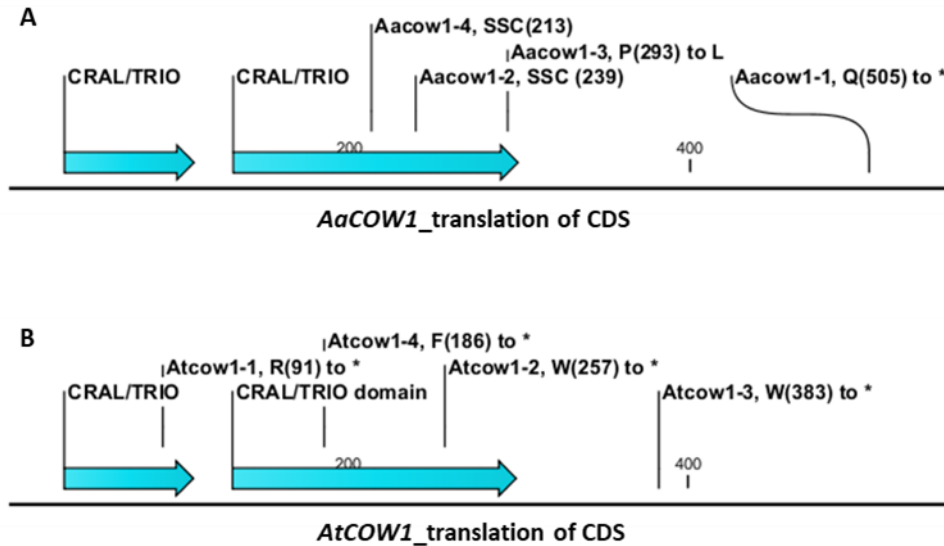
Figure 2.19. Schematic representation of *COW1* amino acid sequence in *A. alpina* and *A. thaliana*. A, *AaCOW1*. **B,** *AtCOW1*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* and *A. thaliana* are marked.

2.3.2.2.   Analysis of *Aaspi* mutants

Two lines with short root hair phenotypes appeared to be *Aaspi* mutants. The Arabidopsis *SPI* gene encodes a WD40/BEACH protein and is involved in membrane trafficking (Saedler et al. 2009). The *SPI* coding sequence in *A. alpina* is 93% identical to the *SPI* coding sequence in *A. thaliana*, and their proteins show 89% sequence similarities. The synteny is also highly maintained between these two species (Table S11).

The SPI protein plays a role in membrane trafficking and RNA stability (Saedler et al. 2009; Steffens et al., 2015). *spi* mutants have fragmented vacuoles in the root hairs. Additionally, they show weak distorted trichome phenotype (Saedler et al. 2009). *Aaspi* mutants show the same range of divergent phenotypes as expected from observations in *A. thaliana*. In addition to their short root hair phenotype, both *Aaspi* alleles have distorted trichomes (Figure 2.22 B, D). A DIC picture of *spi* mutants in *A. alpina* shows that

the root hair cell is filled largely with cytoplasm instead of one big vacuole seen in the wild type (Figure 2.22 E).

Four *spi* alleles in *A. alpina* have been previously identified in a trichome screen (Chopra 2015, PhD thesis). The two alleles found in my root hair screen were named *Aaspi-5* and *Aaspi-6.* They have stop codons at positions 2107 and 1976 of their amino acid sequence, respectively (Figure 2.21 A). Alleles with mutations in similar positions were found in *Atspi* mutants (Saedler et al. 2009) (Figure 2.21 B).
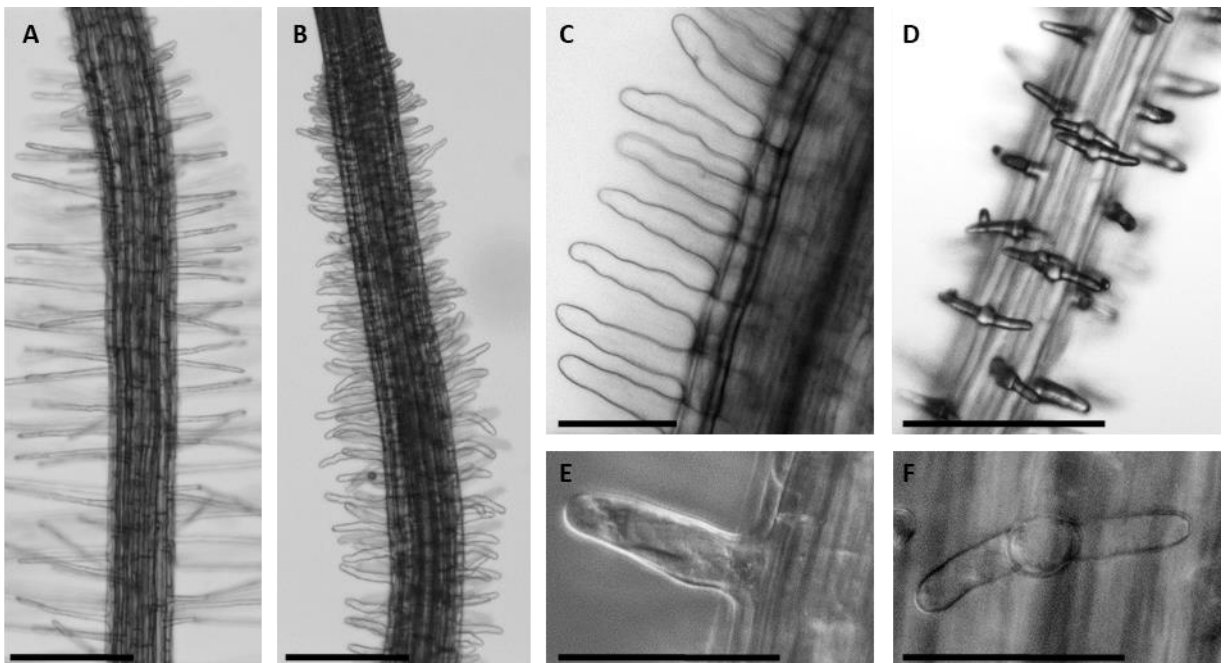
Figure 2.20. *Aacow1* **mutant phenotypes. A,** Wild type root. **B,** *Aacow1* mutant root (line 1183; see table S10). **C,** hairs in *Aacow1* are wide (line 1397). **D,** *Aacow1* root with twin hairs (line 1397). **E,** DIC picture of *Aacow1* hair (line 1051). **F,** twin hairs (line 1051). Scale bar = 250 µm in A, B and D, 75 µm in C, E and F.
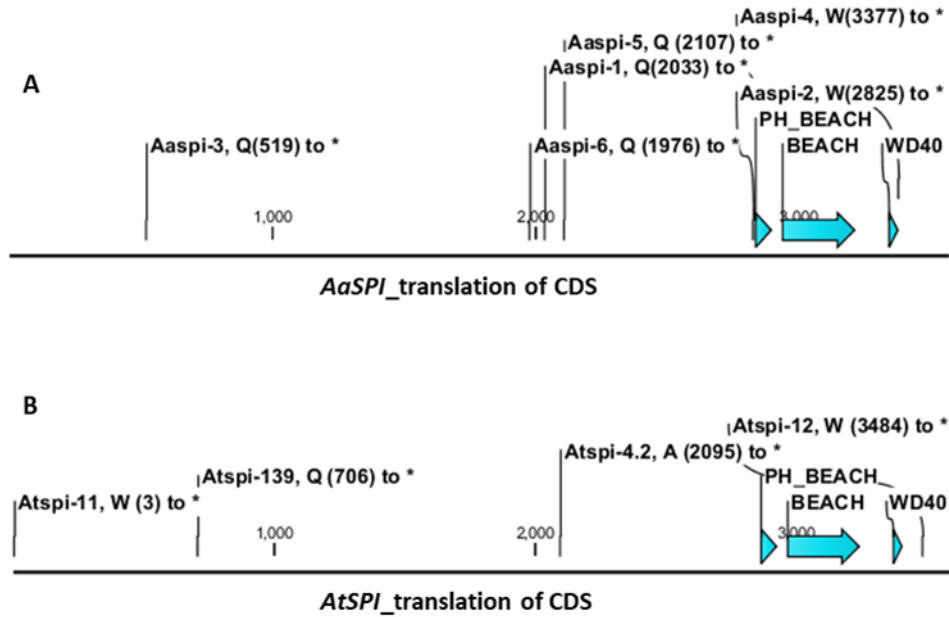
Figure 2.21. Schematic representation of *SPI* amino acid sequence in *A. alpina* and *A. thaliana*. A, *AaSPI*. **B**, *AtSPI*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* and *A. thaliana* are marked.
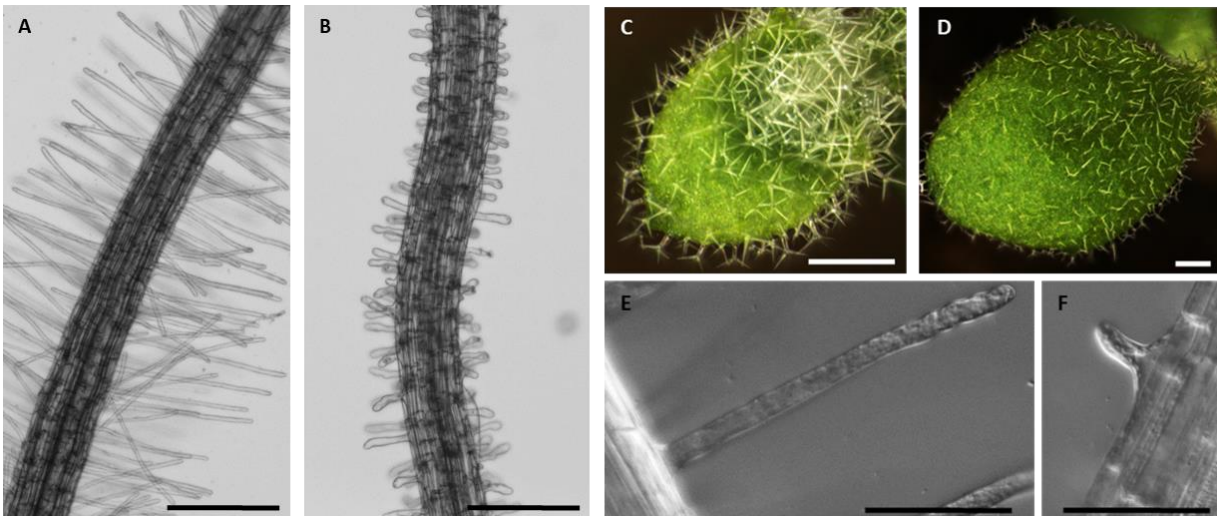
Figure 2.22. *Aaspi* mutant phenotypes. A, Wild type root. **B,** *Aaspi* mutant root (line 1137; see table S10). **C,** Wild type trichome. **D,** *Aaspi* distorted trichome (line 960). **E,** DIC picture of wild type root hair. **F,** DIC picture of *spi* root hair. Scale bar = 250 μm in A and B, 0.5 mm in C and D, 70 μm in E and F.

51

2.3.3. Analysis of branched/wavy root hair mutants in *A. alpina*

2.3.3.1. Analysis of *Aaark1* mutants

Two mutant lines with branched/wavy root hair were identified as *armadillo-repeat kinesin1* (*ark1*) in the whole genome sequence analysis. *ARK1* encodes a root hair growth related, ARM domain-containing kinesin-like protein involved in microtubule organization (Yang et al. 2007). The ARK1 protein shows 83% similarity between *A. thaliana* and *A. alpina*, and synteny is maintained between two species (Table S11).

ARK1 controls cytoskeletal organization during root hair tip growth. In *Atark1* mutants microtubules displayed fragmentation and random orientation which results in wavy/spiral and branched root hairs (Yang et al. 2007; Yoo and Blancaflor 2013). In *Arabis alpina* I observed wavy (Figure 2.24 A, B) and branched (Figure 2.24 C) phenotype for *ark1* mutants.

Both *ark1* alleles found in *A. alpina* produce truncated proteins. *Aaark1-1* has a G to A exchange at position 5751, resulting in a stop codon after 978 amino acids. *Aaark1-2* has a C to T exchange at position 5288, leading to a stop codon after 898 amino acids (Figure 2.23 A). In *Arabidopsis* similar allele has its T-DNA insertion before ARM domain (Yang et al. 2007) (Figure 2.23 B).
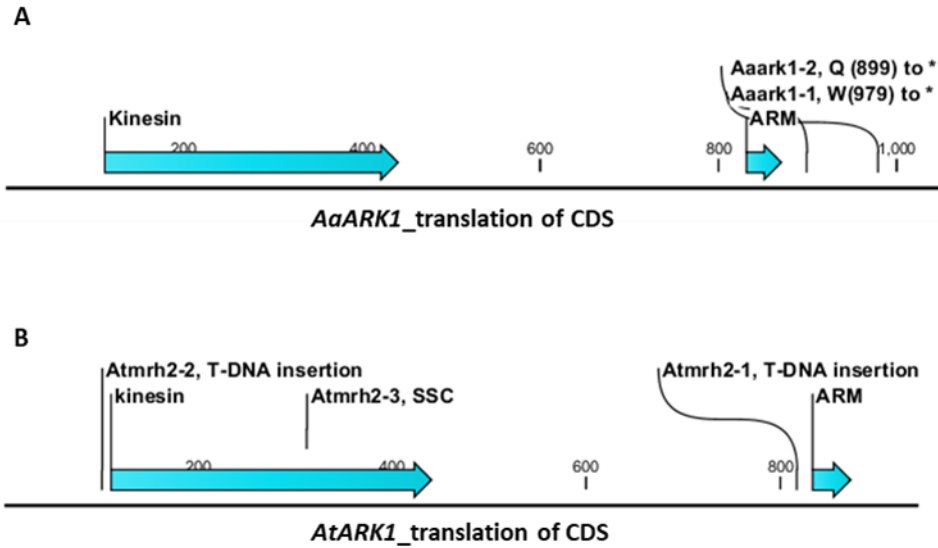
Figure 2.23. Schematic representation of *ARK1* amino acid sequence in *A. alpina* and *A. thaliana*. A, *AaARK1*. **B**, *AtARK1*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* and *A. thaliana* are marked.
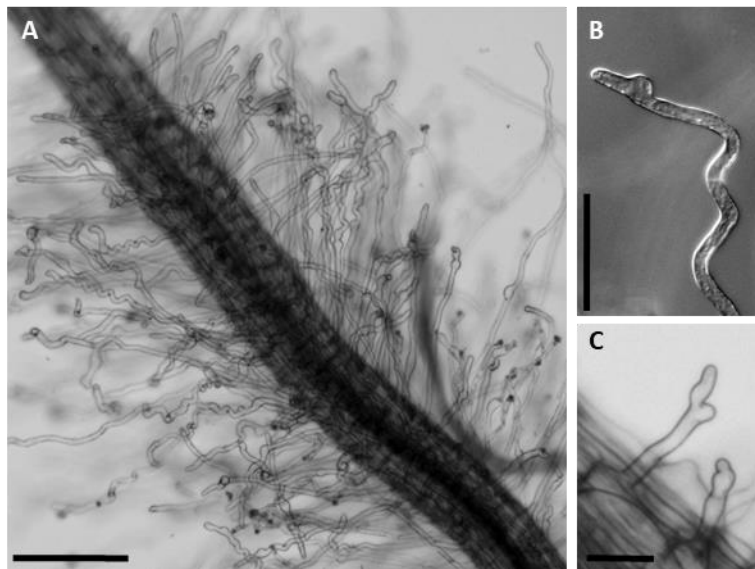
Figure 2.24. *Aaark1* mutant phenotypes. A, *Aaark1* mutant root (line 216; see table S10). **B**, DIC picture of wavy hair of *Aaark1* (line 216). **C,** Branched hair of *Aaark1* (line 1725). Scale bar = 250 µm in A, 70 µm in B and 50 µm in C.

2.3.4. Analysis of root hair mutant alleles carrying amino acid exchanges

2.3.4.1. Analysis of *Aaact8* mutants

Two mutant lines with short root hairs were identified as *actin8* (*act 8*) in the whole genome sequence analysis. One has an Aspartic acid (D) to Asparagine (N) exchange at position 159 (*Aaact8-1*) and the other has a Threonine (T) to Isoleucine (I) exchange at position 204 (*Aaact8-2*) (Figure 2.25 E). Mutations in actin in *A. thaliana* alter cell and organ morphology because of abnormal F-actin cytoskeleton formation (Kandasamy et al. 2009). *ACT2* and *ACT8* are essential for root hair tip growth and *act2act8* double mutants are completely root-hairless (Kandasamy et al. 2009). *ACT2* is missing in the Arabis genome (Willing et al., 2015), therefore it might be that *ACT8* takes over the role in *A. alpina*. Root hairs in *Aaact8* are stunted (Figure 2.25 A) and branched (Figure 2.25 B); additionally they are not properly positioned at the apical end of the epidermal cells (Figure 2.25 D). Similar phenotypes have been described for Arabidopsis actin mutants (Ringli et al. 2002).

2.3.4.2. Analysis of *Aapfn1* mutants

One line with a short root hair phenotype (Figure 2.26 B) was identified as *Aaprofilin1* (*Aapfn1*). It has a Glycine (G) to Glutamic acid (E) exchange at position 64 (Figure 27). Profilin (PFN) is an actin-binding protein involved in the cytoskeleton organization. Arabidopsis PFNs play a role in cell elongation, cell shape maintenance and polarized growth of root hairs (Ramachandran et al., 2000). Overexpression of the *PFN1* produces root hairs that are twice as long as in the wild type (Ramachandran et al., 2000).
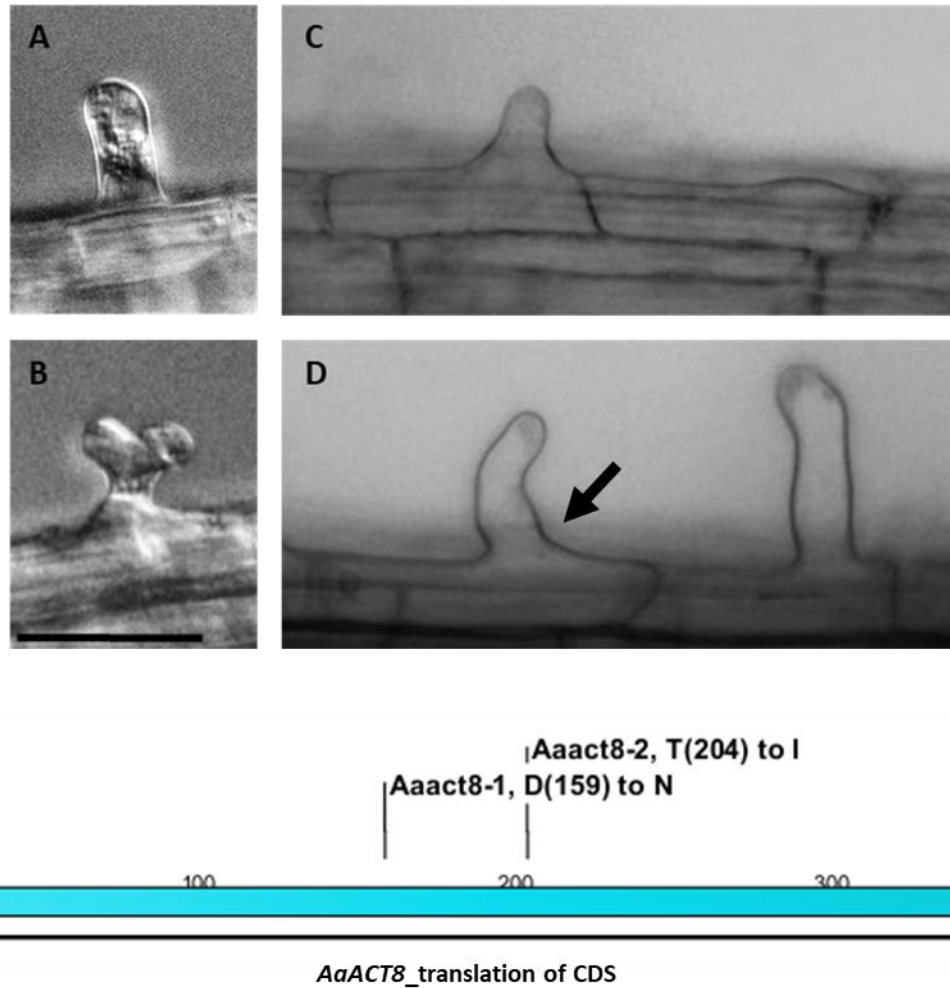
Figure 2.25. *Aaact8* mutant phenotypes. (Line 381; see table S10) **A,** Stunted hair of *Aaact8*. **B,** branched hair of *Aaact8*. **C,** Wild type root hairs emerge at the apical end of the trichoblasts. **D,** In *Aaact8* hair position is affected (The arrow shows the shifted position of the hair). **E,** Schematic representation of *ACT8* amino acid sequence in *A. alpina*. The domain (indicated by blue arrows) was predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* are marked. Scale bar = 50 μm.
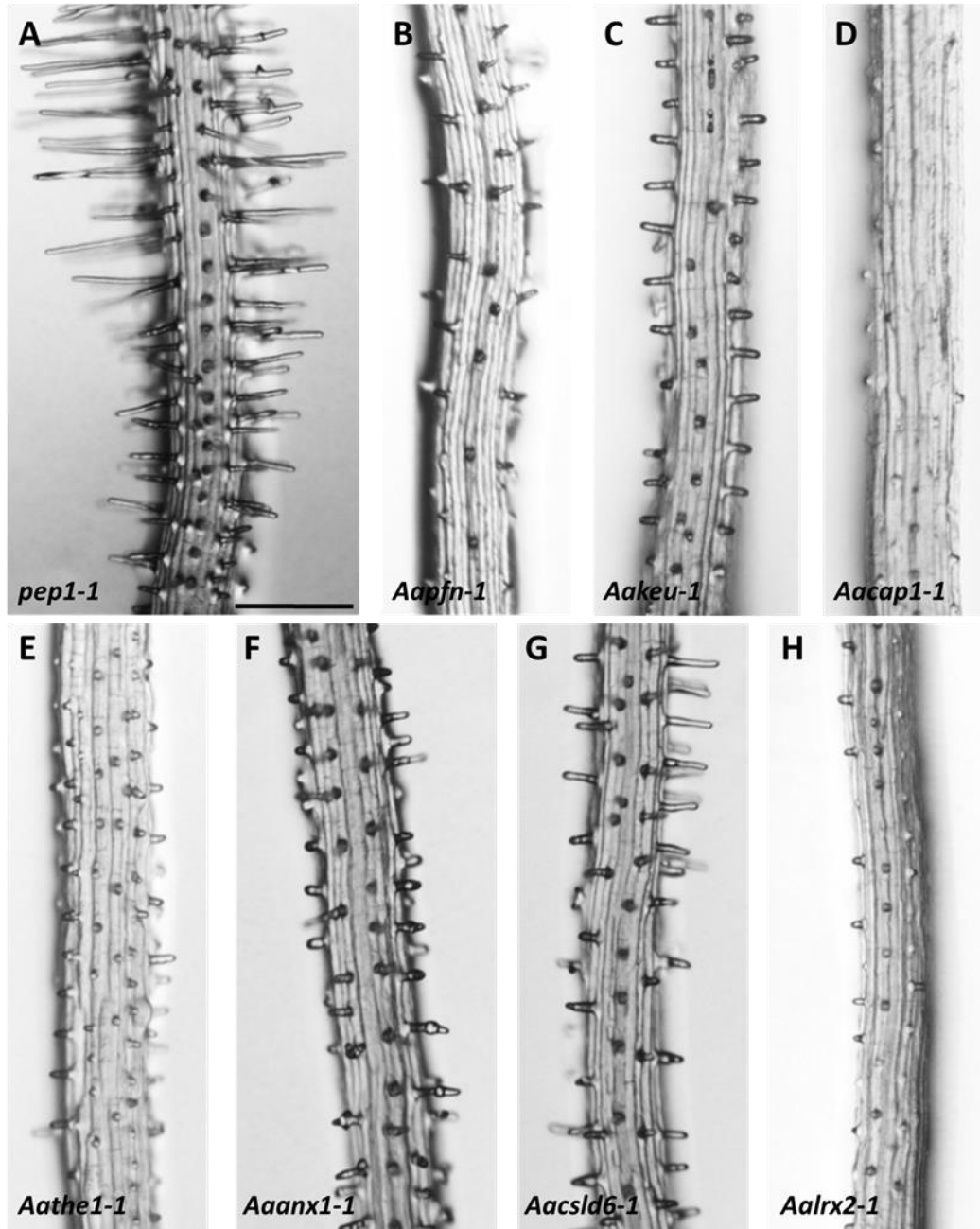
Figure 2.26. Root hair phenotype of mutants with amino acid exchanges. A, wild type root (*pep1-1*). **B**, *Aapfn1-1* (Line 101; see table S10). **C,** *Aakeu-1* (Line 397; see table S10). **D,** *Aacap1-1* (Line 366; see table S10). **E,** *Aathe1-1* (Line 1245; see table S10). **F,** *Aaanx1-1* (Line 1250; see table S10). **G,** *Aacsld6-1* (Line 552; see table S10). **H,** *Aalrx2-1* (Line 1276; see table S10). Scale bar = 250 μm.
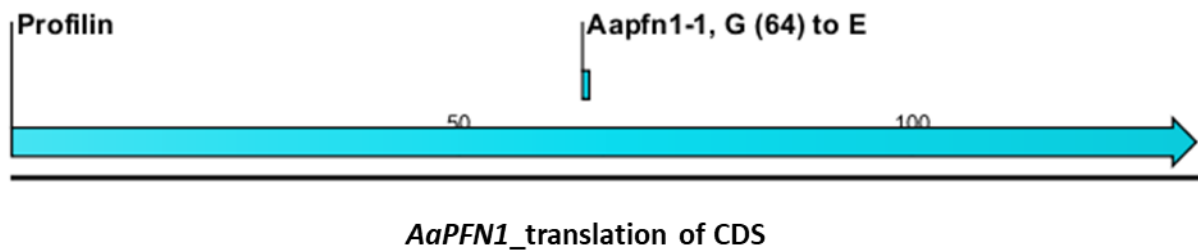
Figure 2.27. Schematic representation of _PFN1_ amino acid sequence in _A. alpina_. The domain (shown in blue arrow) was predicted using Pfam (Finn et al., 2016). The amino acid position changed in the mutant allele in _A. alpina_ is marked.

2.3.4.3.   Analysis of _Aakeu_ mutants

One line with short root hair phenotype presented a Glycine (G) to Arginine (R) substitution at position 487 of a gene homolog to _KEULE_ (_Aakeu-1_) (Figure 2.28). In Arabidopsis, _KEULE_ encodes a Sec1 protein which is a key regulator of vesicle trafficking, important in root hair morphogenesis (Assaad et al. 1996, Assaad et al. 2001). _keule_ mutants in _A. thaliana_ have stunted root hairs which are radially swollen (Assaad et al. 2001). _Aakeu-1_ has short hairs along the whole mount of the root (Figure 2.26 C).
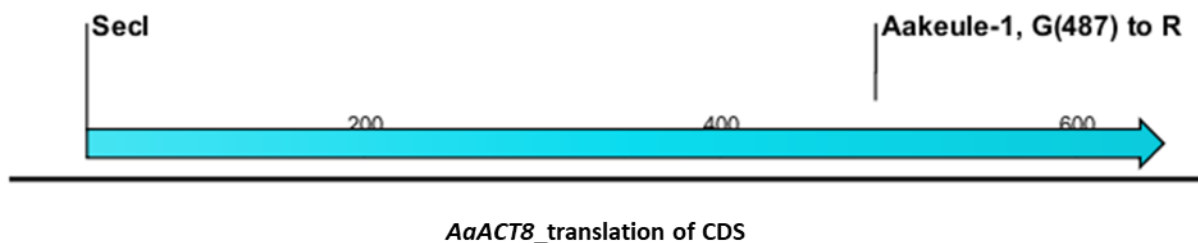
Figure 2.28. Schematic representation of amino acid sequence of _KEULE_ homolog in _A. alpina_. The domain (shown in blue arrow) was predicted using Pfam (Finn et al., 2016). The amino acid position changed in the mutant allele in _A. alpina_ is marked.

2.3.4.4.　　　Analysis of *Aacap1/Aaeru* mutants

One line that only forms bulges instead of root hairs (Figure 2.26 D) was identified as *Aacap1*. It has a Glutamic acid (E) to Lysine (K) substitution at position 465 (*Aacap1-1*) (Figure 2.29). *CAP1* (*Ca²⁺ ASSOCIATED PROTEIN KINASE 1*), also called *ERULUS*, belongs to the *Catharanthus roseus* RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) subfamily of putative cell wall sensor proteins. It regulates root hair tip growth by maintaining the cytoplasmic, tip-focused Ca^{2+} gradient (Bai et al. 2014; Schoenaers et al. 2018). Arabidopsis *cap1* mutants produce bulges that are swollen and have abnormal shapes if they grow further (Bai et al. 2014). Swollen bulges are found in *Aacap1-1* (Figure 2.11 B).
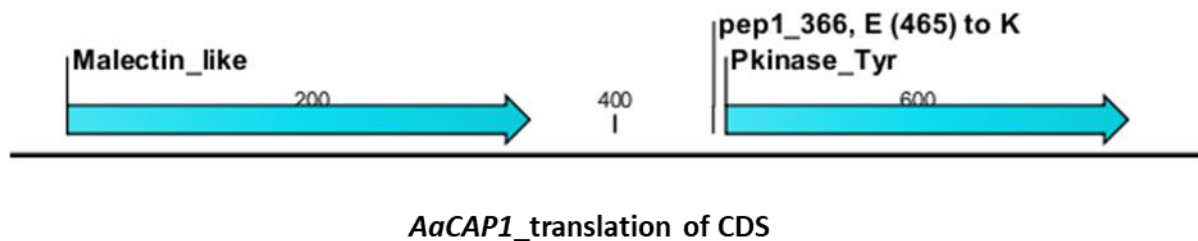
Figure 2.29. Schematic representation of *CAP1/ERU* amino acid sequence in *A. alpina*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid position changed in the mutant allele in *A. alpina* is marked.

2.3.4.5.　　　Analysis of *Aathe1* and *Aaanx1* mutants

Two lines with short root hair phenotypes (Figure 2.26 E and F) presented point mutations in genes homologous to the receptor like kinase (RLK) family. Line 1245 (see table S10) has a Glycine (G) to Arginine (R) exchange at position 614 in *Aatheseus1* (*Aathe1-1*) (Figure 2.30 A). Alleles with amino acid exchanges have been reported for *the1* in *A. thaliana* (Heˊmaty et al. 2007). Line 1250 (see table S10) has an Aspartic acid (D) to

Asparagine (N) substitution at position 813 in *Aaanxur1* (*Aaanx1-1*) (Figure 2.30 B). THESEUS1/FERONIA family receptor like kinases (RLKs) are cell surface regulators that function in cell growth (Cheung and Wu 2011; Duan et al. 2011) and play a role in mediating polarized root hair growth. Severe root hair defects, ranging from collapsed, burst, to short root hair phenotypes have been reported for the mutants (Duan et al. 2011).
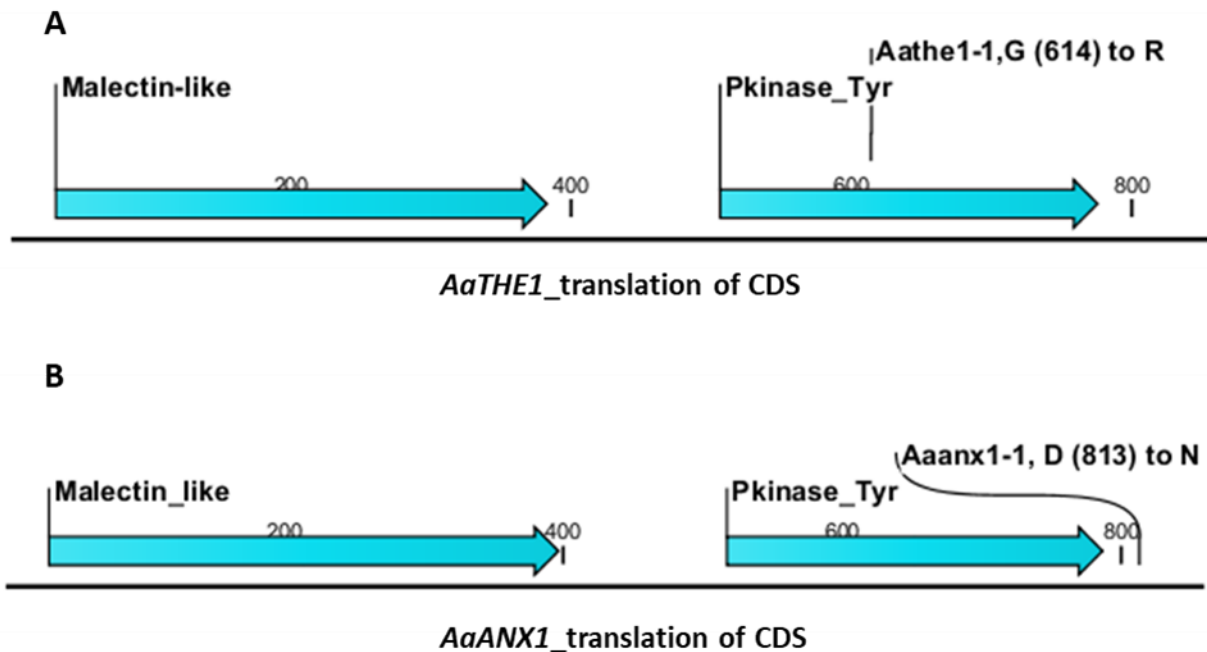
Figure 2.30. Schematic representation of *THE1* and *ANX1* amino acid sequence in *A. alpina*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* are marked.

2.3.4.6.   Analysis of *Aacsld6* mutants

One line showing short root hairs (Figure 2.26 G) was identified as *Aacellulose synthase-like d 6* (*Aacsld6*). It has a Glutamic acid (E) to Lysine (K) substitution at position 289 (Figure 2.31). It is known that CSLD family of putative glycosyltransferases synthesize a polysaccharide that plays a structural role in the cell walls of tip-growing cells (Bernal et

al. 2008). I previously showed that the root hairs in *Aakjk* (*Aacsld3*), another member of the CSLD family, burst. Therefore, it is conceivable that *AaCSLD6* also plays a role in root hair development in *A. alpina*.
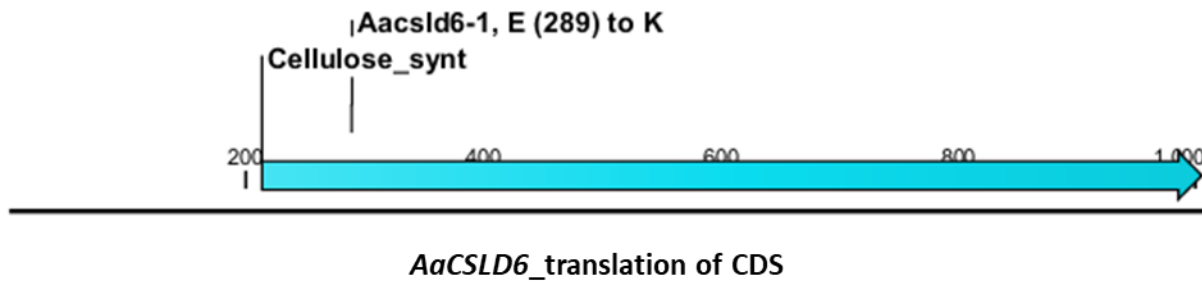
Figure 2.31. Schematic representation of *CSLD6* amino acid sequence in *A. alpina*. The domain (shown in blue arrow) was predicted using Pfam (Finn et al., 2016). The amino acid position changed in the mutant allele in *A. alpina* is marked.

2.3.4.7. Analysis of *Aalrx2* mutants

One line with only bulge formation (Figure 2.26 H) was identified as *Aalrr-extensin* 2 (*Aalrx2*). It has a Proline (P) to Threonine (T) substitution at position 588 (*Aalrx2-1*) (Figure 2.32). *LRX1* and LRX2 of *Arabidopsis thaliana* encode a structural cell wall protein that regulates cell wall development. Both genes are expressed in root hairs and mutants develop distorted hairs (Baumberger et al. 2001; Diet et al. 2006).
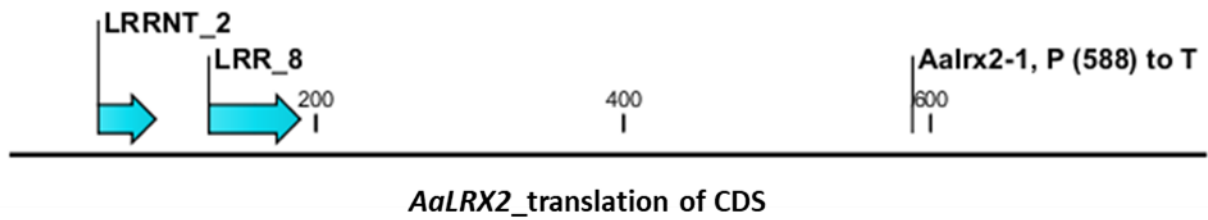
Figure 2.32. Schematic representation of *LRX2* amino acid sequence in *A. alpina*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid position changed in the mutant allele in *A. alpina* is marked.

2.4. Analysis of *AaSCN1* in *A. alpina*

A mutant with a strikingly unique root hair phenotype was isolated from the *Pajares* population. Root hairs in this mutant do not grow as long as in wild type. A detailed analysis revealed that the presence of multiple bulges initiated from each epidermal cell and multiple growing axes are initiated from each bulge (Figure 2.33).
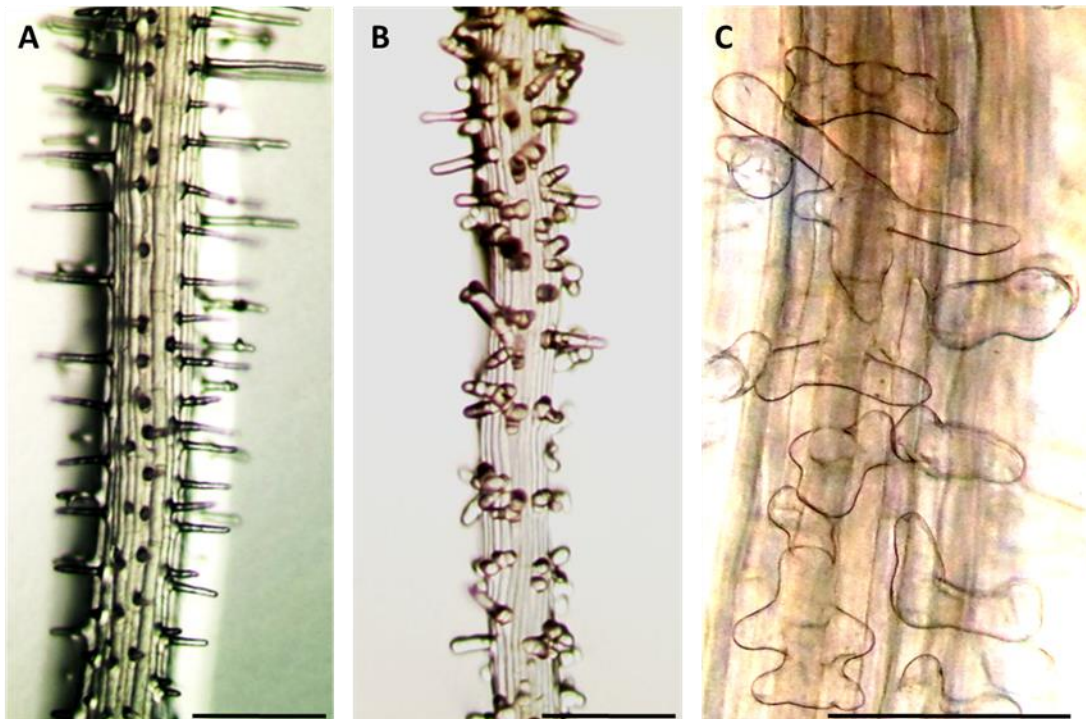
Figure 2.33. *Aascn1* mutant phenotype. A, Wild type root. **B**, *Aascn1* root (line 1384; see table S10). **C,** Higher magnification of root hair phenotype of *Aascn1*. Root hairs grow in different directions. Scale bar = 250 μm in A and B, 100 μm in C.

In Arabidopsis this phenotype is shared by mutants of *TINY ROOT HAIR 1* (*TRH1*) (Rigas et al. 2001) and *SUPERSENTIPEDE1* (*SCN1*) (Carol et al., 2005). Direct sequencing of

the genomic region of *AaTRH1* showed no deviation from wild type, while the sequence of the *AaSCN1* genomic region showed a C to T exchange resulting in a substitution of glutamine to the stop codon at position 46 (Figure 2.34 A). An allele with a similar mutation is also known for *A. thaliana* (*Atscn1-3*) (Carlo et al. 2005) (Figure 2.34 B).

A

Rho GDI | Aascn1-1, Q(46) to *

50   100   150   200

*AaSCN1*_translation of CDS

B

Atscn1-3, R(42) to *

Rho GDI   Atscn1-1, W(86) to *   Atscn1-2, G(182) to E

50   100   150   200

*AtSCN1*_translation of CDS
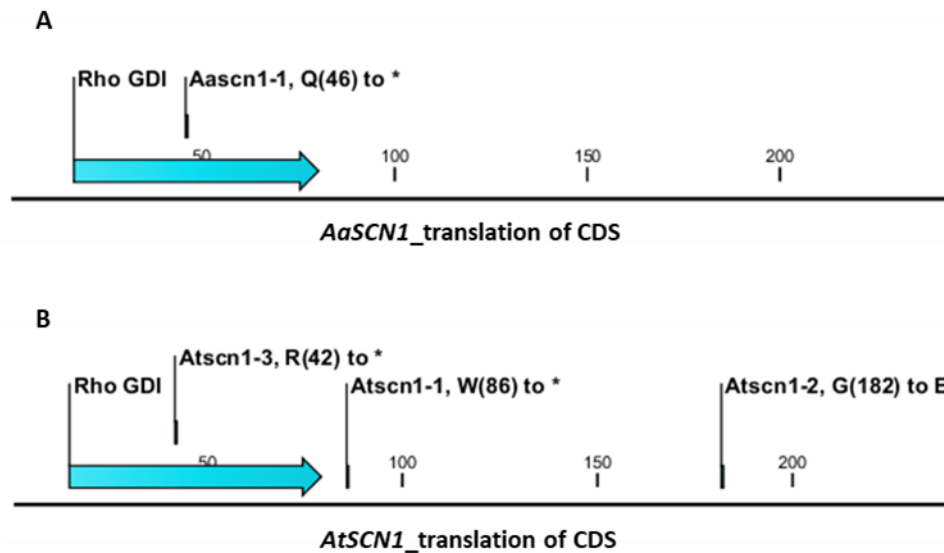
Figure 2. 34. Schematic representation of *SCN1* amino acid sequence in *A. alpina* and *A. thaliana*. A, *AaSCN1*. **B**, *AtSCN1*. The domains (indicated by blue arrows) were predicted using Pfam (Finn et al., 2016). The amino acid positions changed in the mutant alleles in *A. alpina* and *A. thaliana* are marked.

AtSCN1 encodes a RhoGTPase GDP dissociation inhibitor (RhoGDI). It is important to locate RHD2/AtrbohC NADPH oxidase to the tip of root hairs (Carol et al. 2005). The RHD2/AtrbohC NADPH oxidase is required for ROS production, which is essential for root hair elongation (Foreman et al. 2003). *AaSCN1* coding and amino acid sequences show 92 and 84 percent similarity to *AtSCN1* coding and amino acid sequences, respectively. Synteny is also maintained between two species (Table S11). The high similarity of SCN1 protein between Arabis and Arabidopsis together with the similar spectrum of the mutant

phenotype suggest that this mutant is a *scn1* allele that is termed *Aascn1-1* in the following.

2.4.1. Nitrobluetetrazolium (NBT) staining

NBT is reduced to a blue formazan precipitate in the presence of ROS (Fryer et al. 2002). Carol et al. 2002 performed an NBT staining of the wild type and *Atscn1* mutant to determine if the RhoGDI protein is responsible for the ROS localization to a single point during root hair growth. In the wild type, blue color was observed at one single point in the trichoblast and subsequently at the tip of the growing hair. In *Atscn1*, multiple sites of growing tips were stained; confirming the role of *AtSCN1* in spatially regulating apoplastic production of ROS in *A. thaliana*.

I performed the NBT staining in the root hairs of *Pajares* and *Aascn1-1*. In the wild type, I observed the blue staining at one single point of the hair tip (Figure 2.35 A). In the *Aascn1-1* mutant, the blue color was observed in the multiple outgrowths emerging from each hair (Figure 2.35 B).

2.4.2. SCN1 protein localization

SCN1 in *A. thaliana* is highly expressed in the root and stem and, to a lower extent, in the siliques and inflorescence (Carol et al. 2005). Protein localization has not been reported for AtSCN1. I analyzed the localization of the SCN1 protein from Arabis and Arabidopsis. The SCN1-CDS fused to YFP was expressed in *Col-0* under control of the 35S promoter. T2 roots, grown vertically on MS plates, were analyzed for the fluorescent

emission in the root hair. A yellow fluorescence signal is clearly detectable at the tip of growing hairs for both Arabis and Arabidopsis SCN1-YFP (Figure 2.36).
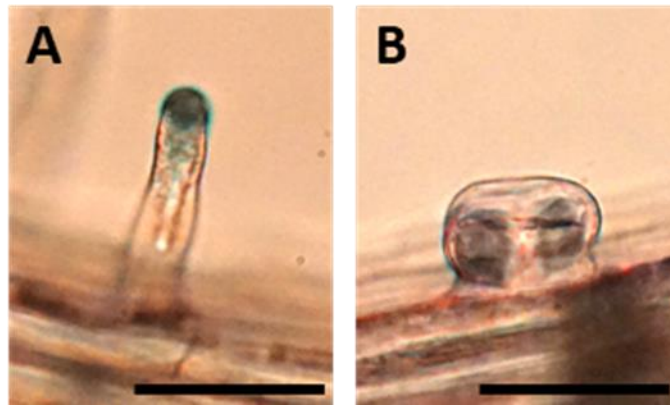
Figure 2.35. ROS localization in *A. alpina* wild type and *scn1* mutant. (Corresponding wild type background is *Pajares*) **A,** NBT staining in the wild type hair. **B**, NBT staining in *Aascn1* hair. Scale bar = 50 μm.
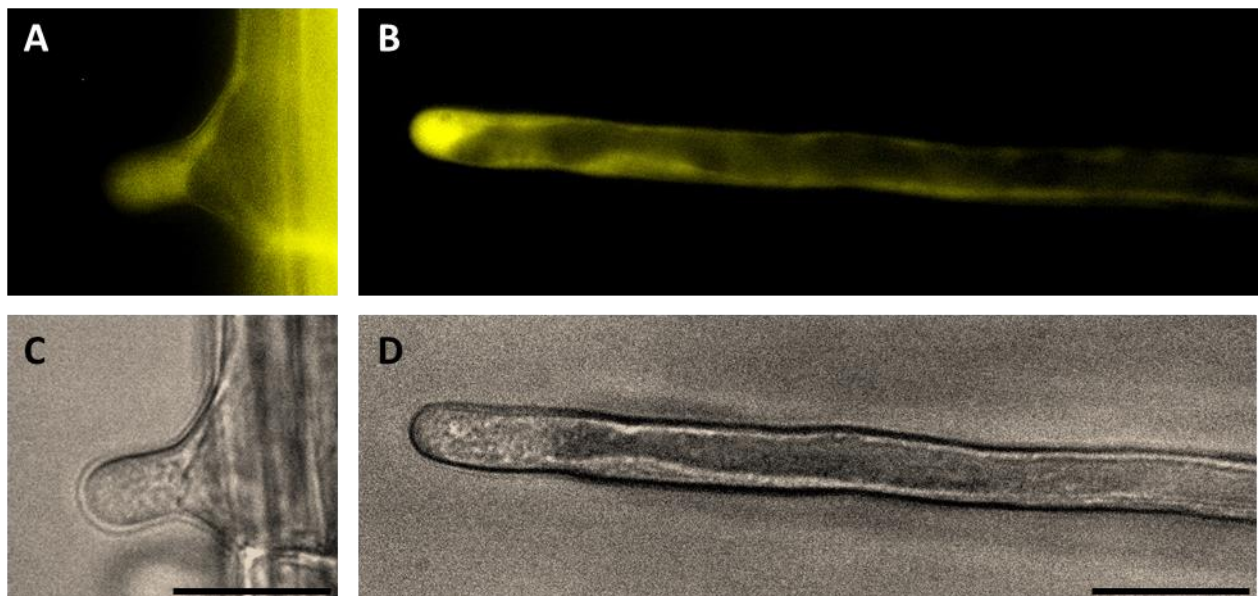
Figure 2.36. SCN1 proteins from *A. alpina* and *A. thaliana* localize at the tip of the hair. A, 35S::*AtSCN1*-CDS fused to YFP at N terminal expressing in *Col-0*. **B**, 35S::*AaSCN1*-CDS fused to YFP at N terminal expressing in *Col-0*. **C,** Bright field image of A. **D,** Bright field image of B. Scale bar = 25 μm.

2.4.3. Rescue experiment

In order to confirm that the phenotype observed in *Aascn1-1* mutant is caused by a mutation in the *AaSCN1* gene, it is necessary to either analyze a second allele or to show rescue by a wild type copy. I attempted to rescue the mutant phenotype by overexpressing *AaSCN1* in *Aascn1-1* using the 35S promoter, however did not recover any transformants in the T1 generation (see 2.1.6.1). Therefore, I performed rescue experiments in *Arabidopsis thaliana*. I reasoned, that a rescue of the *Atscn1-3* (Carol et al. 2005) mutant by wild type *SCN1*-CDS from *A. alpina* but not by the *Aascn1-1* mutant protein will support the idea that this mutation causes the Arabis *scn1* phenotype. Coding sequences were cloned downstream of the 35S promoter in pFAST-R02 (Shimada et al. 2010). The FAST technology is based on the expression of a fluorescent co-dominant screenable marker in dry seeds during dormancy. This facilitated the selection of transformed T1 seeds (Figure 2.37 A) and to analyze their root hair phenotype directly on MS plate. I transformed wild type and the *Aascn1*-mutant into *Atscn1-3* plants. Homozygous, heterozygous and wild type seeds were clearly distinguishable in the T2 population (Figure 2.37 C).

Transgenic plants, expressing Arabidopsis and Arabis *SCN1*-CDS wild type, suppressed the root hair defect of *Atscn1-3* in T1 and T2 generations (Figure 2.38 C, D). This indicates that the AaSCN1 protein is fully functional in Arabidopsis. Transgenic lines expressing the mutant *Aascn1-1* version showed root hair defective phenotype similar to *Atscn1-3* (Figure 2.38 E), suggesting that the mutation found in *Aascn1-1* is causative for the phenotype.

I compared the expression level of the transgenic lines, expressing the *Aascn1*-CDS mutated version, and the lines, expressing the *AaSCN1*-CDS wild type copy by qRT-PCR. One primer was placed a few base pairs upstream of the start codon in the vector sequence to analyze only transgene expression. The expression level in two lines,

66

expressing the mutant *Aascn1* version were not significantly different from the line, expressing *AaSCN1*-CDS wild type copy (Table S12). This indicates that the mutation found in *Aascn1-1* allele renders the protein malfunctioning and causative for the phenotype.
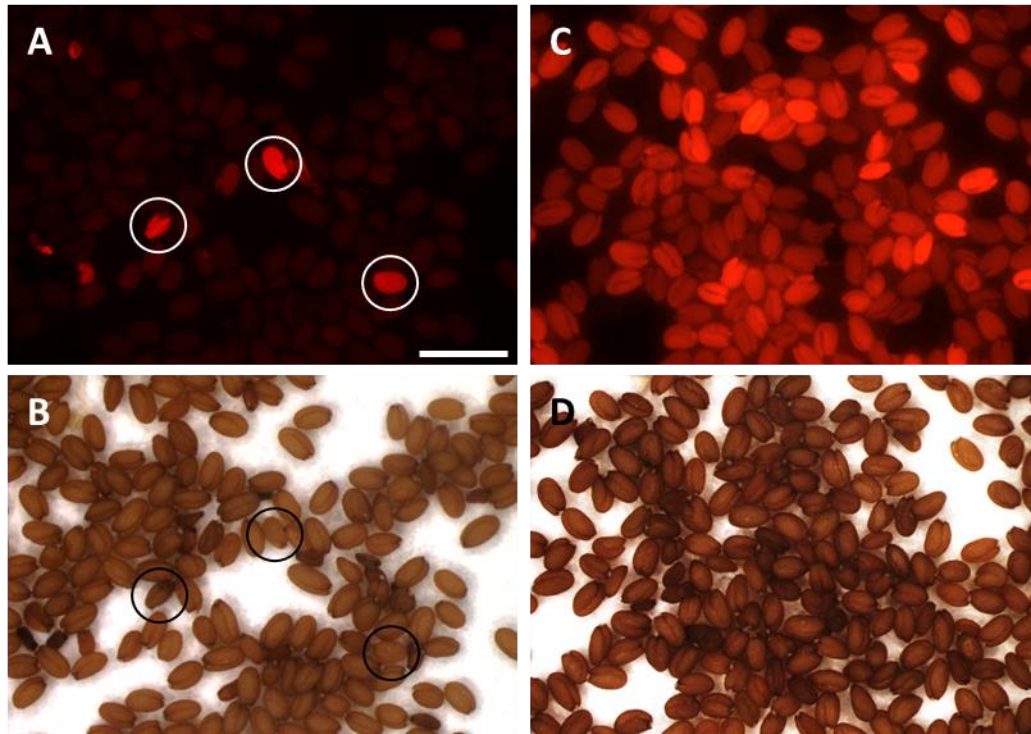
Figure 2.37. Seeds of Arabidopsis plants transformed with pFAST-R02 give red fluorescence. A, The fluorescent T1 seeds are indicated by circles. **B**, The same field as in A. **C,** T2 seed population contained non-fluorescent, moderately fluorescent and strongly fluorescent seeds representing wild type, heterozygous and homozygous seeds. **D,** The same field as in C. Scale bar = 1 mm.
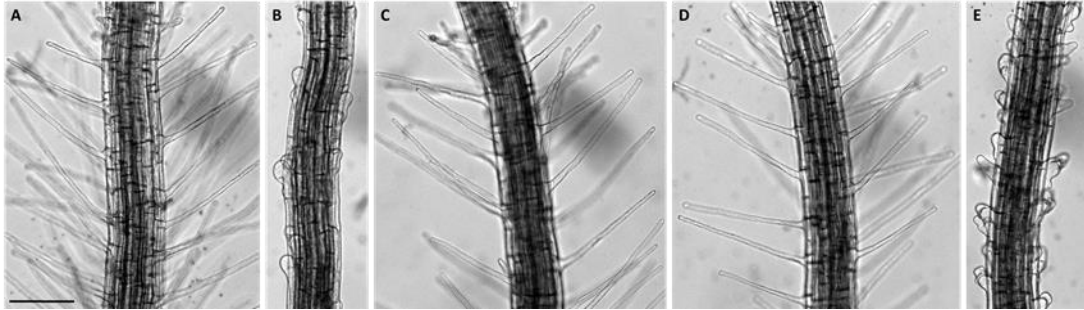
Figure 2.38. Rescue experiment result. A, *Atscn1-3* is in the *Col-0* background (Carol et al., 2005). **B**, *Atscn1-3* root hair phenotype. **C,** 35S::*AtSCN1*-CDS fully rescued the root hair phenotype of *Atscn1-3*. **D,** 35S::*AaSCN1*-CDS fully rescued the root hair phenotype of *Atscn1-3*. **E,** 35S::*Aascn1*-CDS mutated version did not rescue the root hair defect of *Atscn1*-3. Scale bar = 125 μm.

3. Discussion

In this study, I systematically identified genes that are involved in root hair development in *Arabis alpina* by a forward genetic mutagenesis screen and compared them to the genetic inventory in *Arabidopsis thaliana*. In addition, I tried to further investigate trichome patterning in *A. alpina* by creating different transgenic lines. It is generally reasoned that the evolutionary distance between *A. alpina* and *A. thaliana* is close enough to compare orthologous processes and genes, yet distant enough to expect differences. The phenotypic comparison of trichome and root hair development in Arabis and Arbidopsis supports this hypothesis. Trichome and root hair patterning in *A. alpina* are almost indistinguishable from that in *A. thaliana* except for the presence of two differently sized trichome classes and frequently found continuous stretches of root hair cells in the N files (Chopra et al. 2014). Trichome (Chopra 2015, PhD thesis) and root hair morphogenesis (Figure 2.11) in *A. alpina* is almost identical to *A. thaliana*. It is therefore expected to find genetic and molecular deviations explaining the subtle differences in the trichome and root hair patterning, while for all other patterning and morphogenetic aspects, I assumed not to find major differences in the function of the involved genes.

EMS mutagenesis induces random mutations in the genome, therefore the frequency of deleterious mutations should be similar in all genes. In principle, the allele frequency can be used to judge the saturation of the screen. In this study, on average I found 2 mutations for each considered gene, though the allele distribution is asymmetric. The allele frequency of 2.6 have been reported in systematic genetic screen for finding trichome mutants in *A. alpina* (Chopra 2015, PhD thesis).

3.1. Trichome and root hair patterning in *A. alpina*

Trichome and root hair patterning are governed by the same set of genes in *A. thaliana*. Three main activators are required for trichome fate: the WD40 gene *AtTTG1*, the MYB gene *AtGL1* and the redundantly acting bHLH genes *AtGL3* and *AtEGL3* (Hülskamp, 2004; Ishida et al., 2008). Four genes required for non-hair fate are: the WD40 gene *AtTTG1*, the MYB gene *AtWER* and the redundantly acting bHLH genes *AtGL3* and *AtEGL3* (Schiefelbein et al. 2009). As judged by sequence similarity and synteny, orthologous genes of these regulators are present in the *A. alpina* genome (Willing et al. 2015).

***TTG1* plays the same role in trichome and root hair patterning in *A. alpina* and *A. thaliana*.** Two *AaTTG1* alleles were identified in a trichome screen (Chopra et al. 2014) and one was identified in the root hair screen (see 2.1.1). The complete phenotypic spectrum of these alleles, showing all five defects known for *ttg1* mutants in *A. thaliana*, confirms the stable role of *TTG1* in *A. alpina*. WD40 proteins are involved in signal transduction and facilitation of protein-protein interactions. No enzymatic activity has been reported for WD40 proteins. It's been discussed that TTG1 protein may form a surface on which other proteins can interact (Ramsay and Glover 2005). The fact that *TTG1* is expressed globally throughout the whole plant (Walker et al. 1999 and Baudry et al. 2004) would support the idea that the basal expression of *TTG1* is needed for the epidermal pattern formation. My attempts to overexpress *TTG1* in *A. alpina* failed (see 2.1.6.4), but overexpression of *TTG1* in *A. thaliana* wild type does not change the trichome patterning (Payne et al. 2000). Therefore it would be less probable that *TTG1* plays a role in differences of trichome and root hair patterning between *A. thaliana* and *A. alpina*.

***GL3* takes over the role of bHLHs in *A. alpina*.** Seven glabrous mutants isolated from trichome screen (Chopra 2015, PhD thesis) and one hairy mutant isolated from the

root hair screen were identified as different *Aagl3* alleles (see 2.1.2). This is striking, as indicates that *Aagl3* mutants are completely devoid of trichomes and produce ectopic hairs in N-files, to a significantly higher extent than the wild type (Table S17). This is in sharp contrast to *A. thaliana*, where only the simultaneous knockout of *Atgl3egl3* results in glabrous leaves and ectopic root hairs (Zhang et al., 2003; Bernhardt *et al.* 2003). This indicates that *AaGL3* takes over the role of bHLHs and *AaEGL3* has no function in trichome and root hair patterning in *A. alpina*. My attempts to create transgenic lines suppressing *AaEGL3* failed, as I could not recover any transgenic plants (see 2.1.6.2). Chopra in 2015 in her PhD thesis showed that *AaEGL3* can efficiently rescue the *Atgl3egl3* double mutants when expressed under the 35S promoter. This shows that AaEGL3 protein is fully functional and the functional change is likely due to differences in the regulation of expression.

Overexpression of *GL3* acts conversely in *A. alpina* and *A. thaliana* regarding trichome pattern. Overexpression of *AtGL3* in *A. thaliana* wild type causes a higher trichome density and reduced number of root hairs (Payne et al. 2000; Bernhardt et al. 2003). Two transgenic lines with 10 to 30 fold overexpression of *AaGL3* in *A. alpina* wild type show a reduction of trichome density which are fairly similar in size (Figure 2.5). The first possibility to explain their trichome phenotype is that the overexpression of *AaGL3* counteracts intercalating trichome formation. However, both trichome size classes depend on functional *AaGL3* and *AaTTG1*, therefore it would be less probable that only one class of trichomes is sensitive to *AaGL3* overexpression. Based on the theoretical considerations, even a simple patterning model can explain the different response to *GL3* overexpression by changes in the parameter sets. Parameters such as the basal *GL1* expression or the binding strength of *TRY* to *GL3* can simulate the phenotype observed in 35S::*GL3* in *A. alpina* (Personal communication with Anna Deneer, data not shown). The line with a 30 fold overexpression showed reduced number of root hairs (Figure 2.4). This confirms the role of *AaGL3* in specifying non-hair cells in *A. alpina*. The other transgenic

line with a 10 fold increase in *AaGL3* expression does not affect root hair patterning compared to wild type. This indicates that the function of *AaGL3* is dosage dependent in *A. alpina*. Bernhardt et al. 2003 reported that differences in the *35S::GL3* and *35S::EGL3* effects on the root hair numbers might be due to different expression levels of the transgenes.

Overexpression of *AaGL3* cannot suppress *Aattg1* defects in *A. alpina*. *GL3* overexpression restores phenotypic defects of *ttg1* in *A. thaliana*. However, in contrast to R gene from maize, *GL3* is not a strong suppressor of the trichome defects. It was suggested that *AtGL3* needs a functional *TTG1* to fully execute its function in trichome development (Payne et al. 2000). This is supported by the effect of *AtGL3* overexpression on root hair numbers in the wild type and the *Atttg1* mutant. In the wild type, which expresses functional *TTG1*, the reduction of root hairs is much more severe than in *Atttg1* (Bernhardt et al. 2003). No rescue was observed in the transgenic line (35S::*AaGL3* in *Aattg1-2*) with an 8-fold increased expression of *AaGL3* as compared to the background (Figure 2.6). Since overexpression of *AaGL3* in the *A. alpina* wild type resulted in reduced number of trichomes, it is not surprising that its overexpression does not rescue *Aattg1* trichome defects. The same expression level of *AaGL3* in *Aattg1* and the wild type background (Figure 2.6) indicates that *AaGL3* transcription is independent to the presence or absence of *AaTTG1* protein. *GL3* expression in *A. thaliana* was shown to be indifferent to *ttg1*, *gl3* and *gl1* mutations (Payne et al. 2000).

The role of R2R3MYB proteins is still unclear in *A. alpina*. In *A. thaliana*, *gl1* mutants have glabrous leaves (Oppenheimer et al. 1991). It is surprising that no *Aagl1* mutant was found in trichome screen (Chopra 2015, PhD thesis), though this may be due to statistical reasons. My attempts to create transgenic lines suppressing *AaGL1* expression failed, as I could not recover any transgenic plants (see 2.1.6.3). Therefore the role of *GL1* remains unclear in trichome patterning in *A. alpina*. In *A. thaliana*, *wer*

mutants produce ectopic root hairs (Lee and Schiefelbein 1999). None of the mutants with an ectopic hair phenotype were identified as *Aawer*. Overexpression of *WER* in *A. thaliana* induces *GL2* expression in H-position (Lee and Schiefelbein 1999). My attempts to create transgenic lines overexpressing *AaWER* failed (see 2.1.6.6). Therefore the role of *AaWER* remains unclear in *A. alpina* root hair patterning.

Determining the role of R3MYBs in *A. alpina* needs further investigations. Trichome and root hair patterning in *A. thaliana* involves seven redundantly acting R3MYB genes including *AtTRY*, *AtCPC*, *AtETC1*, *AtETC2*, *AtETC3*, *AtTCL1* and *AtTCL2* (Yang and Ye 2013; Grierson et al. 2014). *AtTRY* and *AtCPC* are most relevant as their single mutant shows a phenotype. The other mutants only show an effect when mutated in combination with others (Schellmann et al. 2002; Kirik et al. 2004a and b; Wada et al., 1997). In *A. alpina* genome, *AaETC2* and *AaTCL1* are missing, while an additional *AaCPC* is annotated (Willing et al. 2015). Although my attempts to overexpress *AaTRY* in wild type *A. alpina* failed (see 2.1.6.5), finding two *Aatry* alleles with trichome clusters (Chopra 2015, PhD thesis) confirms the role of *AaTRY* in *A. alpina*. The *CPC* single mutant in *A. thaliana* shows a higher trichome density and reduced number of root hairs (Schellmann et al. 2002). It is not surprising that the *Aacpc* mutant was not found in the trichome screen (Chopra 2015, PhD thesis), as wild-type *A. alpina* plants display a very dense trichome pattern and higher densities can easily be missed under screening conditions. None of the mutants with reduced root hair formation were identified as *Aacpc* or any other R3MYBs tested in the whole genome sequencing. My attempts to create transgenic lines suppressing *AaCPC1* and *AaCPC2* expression failed, as I could not recover any transgenic plants (see 2.1.6.7). Therefore it is difficult to interpret the role of *AaCPC* in *A. alpina*. *ETC2* is known to be responsible for the variation in trichome patterning (Symonds *et al.* at 2011). The accession *ICE50* with 2-fold increase in trichome density compared to *Col-0* was shown to be an *Atetc2* mutant (Jaegle 2015, PhD thesis). The absence of *ETC2* in *A. alpina* might be considered as a hint for its trichome density phenotype. Dr. Chopra transformed wild-

type *A. alpina* with the ~9kb region carrying *ETC2*, *TCL2* and *TCL1* from *A. thaliana*. Analyzing the trichome phenotype of T2 generation showed no deviation from the wild type. However, I could not confirm the presence of the insert by molecular analysis, therefore I did not follow further.

Trichome differentiation in *A. alpina* magnifies the presence of two distinct classes. Trichome and non-hair cell differentiation in *A. thaliana* are regulated by the *AtGL2* (Rerie et al. 1994; Masucci et al., 1996). *Atgl2* mutant trichomes are smaller, less branched, and mostly only form a little bump. This suggests that trichomes are initiated but lose their fate (Rerie et al. 1994). Root hairs in *Atgl2* are found ectopically in N-positions (Masucci et al., 1996). The *Aagl2* mutant phenotype in *A. alpina* differs. While both classes of trichomes are aborted and underdeveloped, the class of small trichomes shows a similar range of phenotypes as seen in *A. thaliana* and the large trichomes appear to be fairly normal. A possible explanation might be that increased size can compensate the requirement for *AaGL2*. This is supported by the previous finding that the *gl2* mutant phenotype in *A. thaliana* can be suppressed in a genetic background in which the trichome size is increased (*try gl2*) (Hülskamp et al. 1994; Bramsiepe et al., 2010). Alternatively, the possibility that other genes like *TTG2* may act in redundancy to *GL2* (Rerie et al. 1994) in trichome differentiation in *A. alpina* cannot be excluded. The increased number of hair formation in the *Aagl2* mutant (Table S17) confirms the role of *AaGL2* in differentiation of non-hair cells in *A. alpina*. In *A. thaliana*, ectopic expression of *GL2* cannot complement the defects of the *gl2* mutant (Ohash et al. 2002). One transgenic line overexpressing *AaGL2* in the *Aagl2-1* background displayed a rescue of trichome defects in T1 generation (Figure 2.9). However the rescue vanished in the next generation despite 38-fold higher expression of *AaGL2* compared to the mutant background (Figure 2.10). This makes it difficult to conclude that 35S::*GL2* acts differently in *A. alpina*. The qRTPCR results revealed that *AaGL2* expression is at the same level in *Aagl2* mutant and the wild type background (Figure 2.10). This is in agreement with the *GL2* expression

analysis in *A. thaliana* that shows *gl2* mutations do not influence the *GL2* expression (Hung et al. 1998).

3.2. Isolation of root hair patterning mutants in *A. alpina*

Two root hair patterning mutants with ectopic hair production were identified as *Aattg1* and *Aagl3* alleles. Identification of mutants with ectopic hairs in *A. alpina* is generally difficult, because of the nature of the wild type root hair phenotype. Therefore some mutants might have been missed under screening conditions. Six mutants with a reduced number of hairs were identified in the root hair screen; none of them showed any mutation in the corresponding candidate genes. This suggests that there might be other mechanisms controlling root hair patterning that need to be identified yet. However, detailed analysis of these mutants might shed a light into identifying the affected genes. For example, measuring the cell length of the N and H files in these mutants can help to recognize the temporal role of affected gene.

3.3. Root hair morphogenesis in *A. alpina*

Root epidermal cell differentiation in *A. alpina* is similar to that of *A. thaliana*. The phenotypic analysis of root hair morphogenesis revealed that root hair development in Arabis is very similar to that in Arabidopsis (Figure 2.11). In *A. thaliana*, hair cells are distinguishable from non-hair cells at an early developmental stages by different characteristics like shorter length (Dolan et al., 1994; Masucci et al., 1996). The cell length measurements in N- and H-positions of wild type roots, revealed that pattern information is provided at an early stage of epidermis development in *A. alpina* (Figure 2.12). In *A. thaliana*, *TTG1* acts at an early developmental stage, therefore all epidermal cells in *ttg1*

mutants have root hair cell characteristics (Galway et al., 1994). *AtGL2* acts at later stages, therefore *gl2* mutants do not completely convert hairless cell type characteristics into another (Masucci et al., 1996). Root epidermal cell length analysis of *Aattg1* and *Aagl2* indicates that all hair cells in *Aattg1* show the hair cell characteristics, while two types of hair bearing cells are distinguishable in *Aagl2* (with hair cell or non-hair cell characteristics).

Root hair morphology mutants in *A. alpina* show the same range of phenotypes as *A. thaliana*. The range of mutants identified in Arabis root hair morphogenesis is similar to that of Arabidopsis (Mathur & Hulskamp 2001; Grierson et al. 2014). Root hair morphogenesis normally proceeds as a well-defined temporal sequence of events: site selection, initiation, bulge formation, tip growth, hair elongation and finally secession of growth (Ryan et al. 2001) (see figure 2.11). I identified mutants forming only bulges, mutants with short, branched and/or wavy hairs, mutants with hairs that are swollen at the base, burst mutants and mutants with several hairs emerging from each epidermal cell (see Figure 2.16). The molecular analysis revealed that morphogenesis mutants in *A. alpina* exhibit specific mutations in genes known to be required in Arabidopsis for the respective phenotype.

Site selection. Two *Aaact8* alleles occasionally exhibit hairs which are not properly positioned at the apical end of the trichoblasts (Figure 2.25). The location of the bulge formation is mainly controlled by auxin and ethylene in *A. thaliana* (Masucci and Schiefelbein 1994). However, the actin cytoskeleton was also shown to influence the prospective site of bulge formation (Ringli et al. 2002).

Only bulge formation. The formation of the bulge is accompanied by the formation of a local high Ca^{2+} concentration at the root tip (Wymer et al. 1997). One mutant with only bulge formation was identified as *Aacap1=Aaeru*. *CAP1* regulates root hair growth by maintaining the cytoplasmic Ca^{2+} gradient (Bai et al. 2014; Schoenaers et al. 2018). The

76

formation of the bulge is also required the tightly regulated expansion of the wall. One mutant with only bulge formation was identified as *Aalrx2*. *LRX1* and *LRX2* encode a structural cell wall protein that regulate cell wall expansion (Baumberger et al. 2001). The third mutant with only bulge formation did not show any mutations in the corresponding sequenced genes.

Swollen at the base. The group of mutants with swollen phenotype could not be identified for any corresponding mutant alleles. The degree of cell wall loosening in Arabidopsis is controlled by *TIP1* and *RHD1* (Ryan et al. 1998; Schiefelbein and Somerville 1990). The phenotype of *rhd1* mutants is suppressed by ethylene (Seifert et al. 2004), implicating that plant hormones continue to contribute to root hair development after the initiation site has been selected. It would be interesting to treat Arabis swollen mutants with ethylene to see if it suppresses the phenotype and subsequently leads us to find the affected genes.

Bursting hairs. The increase of the protoplast volume in the hair cells, requires the expansion of the cell wall. A cell wall structural defect impairs hair elongation and results in a bursting phenotype (Dolan 2001). Three mutants with burst root hair phenotype were identified as different alleles of *Aakjk*. In *A. thaliana*, *KJK* encodes AtCSLD3 which is a member of the cellulose synthase superfamily of proteins required for the formation of the wall during tip growth (Favery et al. 2001).

Multiple swellings. *SCN1* gene in *A. thaliana* affects the number of swellings. It is involved in establishment, sustenance and direction of tip growth; and prevents branching (Parker et al. 2000; Carol et al. 2005). One allele of *Aasnc1* with the full spectrum of expected phenotypes was identified in *A. alpina*.

Short root hairs. Sixteen mutants presented short root hair phenotype. Identification of affected genes via whole genome sequencing appeared to be a great

beneficial for this group. It helped us to overcome the analysis of the large number of candidate genes. Actin and actin-binding proteins control hair elongation via transportation of vesicles and organelles (Miller et al. 1999). Two mutants with short root hairs were identified as *Aaact8*. In *A. thaliana*, mutations in the *ACT2* and *ACT8* exhibit defects in root hair initiation and tip growth (Gilliland et al., 2002; Kandasamy et al. 2009). The corresponding ortholog of *ACT2* is not found in Arabis genome (Willing et al. 2015). Therefore, *ACT8* might take over the role of vegetative actins involved in the root hair development in *A. alpina*. One mutant with short root hair was identified as *Aapfn1*. PFN is an actin-binding protein which plays a role in the cytoskeleton organization (Ramachandran et al., 2000). Polarized expansion of the root hair is achieved by the continuous delivery of newly-synthesized cell wall components. Four different alleles of *cow1* were identified in *A. alpina*. The Arabidopsis *COW1* encodes a phosphatidylinositol transfer protein which is essential for root hair tip growth (Böhme et al. 2004). Two mutants with short hair phenotype were identified as *Aaspi*. The Arabidopsis *SPI* gene encodes a WD40/BEACH protein which is involved in membrane trafficking during tip growth (Saedler et al. 2009). Another mutant with short root hair phenotype presented a mutation in a gene homologous to *AtKEULE*. In *A. thaliana*, *KEULE* encodes a Sec1 protein which is a key regulator of vesicle trafficking (Assaad et al. 1996, Assaad et al. 2001). CSLD family of putative glycosyltransferases are known to be involved in the in the formation of the root hair cell wall (Bernal et al. 2008). One mutant with short root hair, presented a mutation in *AaCSLD6*. RLKs in Arabidopsis have been identified as regulators for various growth-related processes (Hématy et al. 2008). Two mutants with short root hair phenotype were identified as *Aaanx1* and *Aathe1*. Both *ANX1* and *THE1* belongs to the THESEUS1/FERONIA family receptor like kinases (RLKs) and function in polarized cell growth (Cheung and Wu 2011).

Branched/wavy hairs. Microtubules in the root hairs are arranged in a longitudinal orientation (Ryan et al. 2001). ARK1 protein is involved in microtubule organization in root

hairs. Two alleles of *Aaark1* were identified with wavy root hairs that are occasionally branched. A third mutant with branched root hairs still remained to be identified for the affected gene.

The *WER-GL3/EGL3-TTG* transcriptional regulatory complex influences the expression of later-acting genes like *GL2* that controls root-hair or non-hair cell differentiation (Hung et al., 1998). Large-scale identification of downstream genes, preferentially expressed in root hair cells, annotated crucial biological processes in root hair morphogenesis (Jones et al., 2006; Brady et al. 2007). In my root hair screen I could identify alleles affected in almost all highlighted processes. *Aaark1*, *Aacap1*, *Aathe* and *Aaanx1* are involved in kinase activity. *Aakjk*, *Aacsld6* and *lrx2* are involved in cell wall synthesis, *Aascn1* is indirectly involved in calcium ion transport and nicotinamide adenine dinucleotide and/or nicotinamide adenine dinucleotide phosphate (NAD$^+$/NADP$^+$) activity. And finally, *Aacow1*, *Aaspi*, *Aaact8*, *Aapfn1* and *Aakeu* are involved in vesicle docking during exocytosis. However, there are still mutants that show no relevant mutations in the tested genes. It is therefore possible that mutations in additional, non-identified genes lead to the root hair defects in *A. alpina*. Genes regulating the polarity of auxin transport have shown enrichment in the root hair cells (Brady et al. 2007), though I did not identify any mutant related to auxin.

3.4. The *SCN1* gene function is conserved between *A. alpina* and *A. thaliana*

Phenotypic comparison of *scn1* mutants in *A. alpina* and *A. thaliana* revealed the same range of phenotypes. The *AaSCN1* mutant initiates several growth sites from each trichoblasts and subsequently develop multiple axis from each hair (Figure 2.33). This indicates that *SCN1* in *A. alpina* controls the sites at which growth occurs during root hair

morphogenesis. The same function has been described for *SCN1* in *A. thaliana* (Carol et al. 2005). NBT staining showed that ROS is produced in the ectopic points where growth happens in the *Aascn1* mutants, while in the wild type it is focused at one point at the tip of the hair (Figure 2.35). This indicates that the localization of ROS in one single point is controlled by *SCN1* in *A. alpina*, just as in *A. thaliana* (Carol et al. 2005). *SCN1* is strongly expressed in the root epidermis of *A. thaliana* (Carol et al. 2005). I could show that the SCN1 protein of *A. thaliana* and *A. alpina* localizes to the tip of the growing hair (Figure 2.36), where is defined as SCN1 site of action for ROS localization. The rescue of root hair defects in the *Atscn1-3* mutant by 35S::*SCN1* from Arabis and Arabidopsis (Figure 2.38), together with the high similarity of their proteins (See 2.4), indicate that *AaSCN1* is highly conserved during evolution in crucifer and is fully functional in Arabidopsis. No rescue phenotype of lines expressing mutant *Aascn1-1* version in *Atscn1-3* indicates that the mutation found in *AaSCN1* gene is responsible for the phenotype.

3.5. Perspective

The forward genetic approach for investigating trichome and root hair development in *Arabis alpina* has led us to recognize the underlying processes and genes that are similar to that in *A. thaliana*. It also highlighted those that are different. It will be interesting to study in particular trichome and root hair patterning in Arabis in more detail. What is the function of *GL1* for trichome and *WER* for root hair patterning in *A. alpina*? It would worth to repeat transformations to create artificial microRNA and/or CRISPR-Cas lines to further analyze the role of R2R3MYBs in *A. alpina*. Another question remain to be answered is whether *EGL3* play a new role or lost its function completely in *A. alpina*? In addition to further attempts for creating amiRNA and CRISPR-Cas lines targeting *AaEGL3*, a comparison of *EGL3* expression in the wild type and *gl3* mutants in *A. alpina*, using qRT-PCR, might shed a light into its probable function. Overexpression of

AaGL3 in the wild type Arabis indicated that the intercalating trichomes disappear by higher amount of *GL3*. Root hair phenotype of 35S::*AaGL3* showed a dosage dependent phenotype. Therefore it might suggest that the relative level of activators and inhibitors play a temporal role in defining epidermal fates after the first pattern is established. Towards this end, producing transgenic plants overexpressing R3MYBs might help to unravel the mechanisms underlying the large and small trichome classes together with the presence of hairs in N files. The expression pattern of *AaGL2* still remains unknown in *A. alpina*. Analyzing the result of my last transformation of pAaGL2::GFP into *pep1-1* would help us to answer how similar or different the *GL2* expression pattern is between *A. thaliana* and *A. alpina*? The result of root hair morphogenesis so far indicated that similar mechanisms control root hair development in *A. alpina* and *A. thaliana*. However, the group of mutants with the reduced number of hairs and swollen phenotype could not be identified for the responsible genes based on our candidate approaches. It would be difficult to judge, but based on the available information, it might be that the hormones are playing a more drastic role in these two processes in *A. alpina*. One solution would be to analyze them for more candidate genes selected biased for hormonal effects. Generally for the all identified mutants with more than one allele, it would be ideal to perform the allelism test to confirm that the phenotype is caused by the identified genes.

4. Materials and methods

4.1. Plant material and growth condition

All *A. alpina* mutants were isolated from EMS-mutagenized *A. alpina Pajares* and *pep1-1* populations (Wang et al., 2009). For trichome analysis, seeds on soil were stratified in darkness at 4°C for five days and then placed in growth chambers under long day (LD; 16 h light, 8 h darkness) conditions at 21°C. *Pajares* required twelve weeks of vernalization to flower whereas *pep1-1* floweres in ~80 days without vernalization. For root hair analysis, seeds were surface sterilized with chlorine gas (NaOCl (12%) + 2HCl (37%) ≥≥≥ Cl2 + NaCl + H2O) for 3 hours. Sterilized seeds were sown on 1x Murashige-Skoog plates lacking sucrose and stratified at 4°C for five days. Plants were grown on vertically positioned plates for seven days under long day (LD) conditions (16h light, 8h darkness) at 21°C. *Atscn1-3* mutant (Carol et al. 2005) and *A. thaliana* Col-0 were used for inter-species rescue experiments.

4.2. Sequence and synteny analysis

A. alpina gene sequences, obtained from http://www.arabis-alpina.org/, were analysed with the CLC DNA Workbench 5.6.1 (CLC bio, Aarhus, Denmark). gbrows from TAIR 10 (www.arabidopsis.org) along with the assembled *A. alpina* genome were used to confirm the synteny of the selected genes using conserved order and appearance of the neighbouring genes. For sequence analysis by Sanger sequencing, primers were designed outside the CDS of a given *A. alpina* gene to sequence it in mutants (Table S18). LightRUN™sequencing service based on Sanger sequencing from GATC Biotech AG

(European Genome and Diagnostics Centre, Constance, Germany) was used to sequence PCR products and plasmids. For the whole genome sequencing DNA from one to three plants was extracted using Qiagen DNeasy kit. DNA samples were then sent to Cologne Center for Genomics (CCG Köln). The results of whole genome sequencing were preliminary analyzed by Hequan Sun. Annotated SNPs, stringently selected for each mutant with a minimum mutant allele frequency of 0.85 and minimum mutant allele coverage of 3, were followed. EMBL-EBI data base (Pfam 32.0) (http://pfam.xfam.org/) (Finn et al., 2016) was used to search for conserved domains within the protein sequence.

4.3. Constructs and stable plant transformation

The binary vector pFAST-R02 (Shimada et al. 2010) was used to create 35S::*AaGL1*, 35S::*AaWER*, 35S::*AaTRY*, 35S::*AaGL2*, 35S::*AaSCN1* and 35S::*AtSCN1* using the Gateway® system. The coding sequences were cloned in pDONOR$_{201}$ and/or pDONOR$_{207}$ by PCR amplified (Coding sequences of *AaGL1*, *AaWER*, *AaTRY* and *AaGL2* were cloned previously by Dr. Chopra). For construction of 35S::*Aascn1-3*_CDS, the *AaSCN1*-CDS in pDONOR$_{207}$ was re-amplified using site directed mutagenesis (SDM) primers (Table S18), sequenced and cloned in pFAST-R02 (Shimada et al. 2010). For construction of 35S::*AtSCN1*-YFP and 35S::*AaSCN1*-YFP, the Gateway destination vectors pENSG/pEXSG-YFP (Feys et al., 2005) were used for N-terminal/C-terminal fusions with YFP. The proAaGL2::GFP-ER construct was created using classical and gateway cloning techniqes. 2116 bp fragment up-stream of *AaGL2* start have been previously cloned in pJET 2.1 (Chopra 2015, phD thesis). The pAaGL2 fragment was replaced with 35S promoter in pAMPAT-CaMV35S-GW using XhoI and AscI. The GFP-ER was then placed in front of pAaGL2 from pENTR-GFP-GW (available from the lab) using gateway system.

4.3.1. Construction of 35S::amir*AaEGL3*, *AaGL1*, *AaGL2* and *AaCPC1CPC2*

Artificial microRNAs targeting *AaEGL3*, *AaGL1*, *AaCPC1*, *AaCPC2* and *AaGL2* (for sequences see Table S19) were designed using Web MicroRNA Designer (wmd3.weigelworld.org tool). *amiRAaEGL3*, *amiRAaGL1* and *amiRAaGL2* were constructed by targeted mutagenesis of *mir319a* (pRS300, wmd3.weigelworld.org) using four primers (I-IV) each listed in table S18. This PCR amplicons were cloned into pDONOR$_{201}$ and/or pDONOR$_{207}$ using primers to add attB1/attB2 adaptors (table S18), sequenced and transferred into the pFAST binary vector (Shimada et al. 2010). 35S::amir*AaCPC1CPC2* targets *AaCPC1* and *AaCPC2* genes simultaneously. Two microRNA sequences were separated by a linker of 88 bp (Yan et al. 2012) (Figure S1). The complete fragment flanking by attL1/attL2 adaptors was subjected to gene synthesis, delivered in pUC57-Kan, and cloned into pFAST (Shimada et al. 2010).

4.3.2. Construction of *U6p::sgRNA* targeting *AaEGL3*, *AaGL1* and *AaCPC1CPC2*

To find target sites for CRISPR/Cas, I searched for NGG or CCN motif in cDNAs and take 20nt unique target sequences (Table S18). The target sequences were analyzed to avoid off-targets in Arabis genome using Local Blast in CLC. *U6p::sgRNA* cassettes carrying two different target specific guide sequences (Hyun et al. 2015) were designed in CLC (Figure S2) and subjected to sequence synthesis. Fragments were cloned in pJET and then cloned in pYB196 binary vector (Hyun et al. 2015) using BamHI and SpeI.

The constructs were introduced in the respective plant backgrounds (see table S6) by *Agrobacterium*-mediated transformation (strain GV3101 and GV3101-pMP90RK for pAMPAT vectors) using floral dip (Clough and Bent, 1998).

4.4. qRT-PCR experiments

qRT-PCR analysis was done in T1 and T2 generations of transgenic plants to analyse the level of expression compared to the respective background. For T1 analysis, I collected three leaves from each plant 30 days after germination and studied each leaf with two technical replicas. For T2 analysis, total RNA from leaves of 3 biological replicates was isolated and followed with three technical replicates. *RAN3* (Wang et al., 2009) in *A. alpina* and *PP2A* (Frerigmann et al., 2016) in *A. thaliana* were used as a reference gene (primers used in qPCR are provided in table S19). The expression level was calculated using $2-\Delta\Delta CT$ equation (Livak & Schmittgen 2001).

4.5. Trichome and root hair analysis in 35S::*AaGL3* plants

Root hair and trichome analysis was done with individual T2 plants. In a first step seeds were grown on agar plates and the number of root hairs determined in the root hair zone equivalent to that described in Arabidopsis (Grierson and Schiefelbein, 2002) in a one millimetre long section. Thereafter plants were transferred on soil and the third leaf was analysed for trichome density when it reached a size of 0.6 to 1 cm. Because of the high trichome density trichomes were counted in a region of 2.4 μm^2 in the middle of the leaf on both sites of the leaf.

4.6. Root hair counting and root epidermal cell length measurements

The number of root hairs determined in the root hair zone equivalent to that described in Arabidopsis (Grierson and Schiefelbein, 2002) in a one millimetre long section. The position of H-files was determined by their position with respect to the underlying cortex cells on 7 day old plate-grown seedlings.

4.7. Photography and microscopy

Photographs of the whole leaves and seeds were taken using a Leica stereomicroscope (MZ 16F) with the Multi-Focus and Montage option. Photographs of the roots and the root hairs were taken using a Leica microscope DMRA2 and Leica DM5000B fluorescence microscope (Leica Microsystems, Wetzlar). For SEM pictures leaves were mounted on a SEM stub; and further analysis was carried out by Hans-Peter Bollhagen.

Nitroblue tetrazolium chloride (Sigma) was used to stain for the site of superoxide production (Fryer et al. 2002). Seedlings (7 days old) grown on solid medium were covered with freshly made NBT solution (0.5 mg/ml NBT in 0.1 M potassium phosphate pH 7) for ten minutes. Roots were imaged under bright-field illumination on a Leica microscope DMRB. For epidermal cell length analysis, roots were stained with 100 mg/ml of propidium iodide for 1 min followed by washing the samples with water. Optical sections of PI-stained roots were obtained by confocal laser scanning microscopy (CLSM; Leica TCS SPE). Seed coat mucilage production was studied by Ruthenium red (0.1 mg/ml) in water. For staining seeds I wetted them with ruthenium red solution and made a direct observation. All Images were processed using Fiji (Schindelin et al., 2012).

5. Bibliography

Albani MC, Castaings L, Wotzel S, Mateos JL, Wunder J, Wang R, Reymond M, Coupland G (2012) PEP1 of Arabis alpina is encoded by two overlapping genes that contribute to natural genetic variation in perennial flowering. PLoS Genet **8(12)**:e1003130.

Alfano F, Russell A, Gambardella R, Duckett JG (1993) The actin cytoskeleton of the liverwort Riccin Juitans-effects of cytochalasin-B and aluminum ions on rhizoid tip growth. J Plant Physiol **142:** 569-574.

Ansell SW, Grundmann M, Russell SJ, Schneider H, Vogel JC (2008) Genetic discontinuity, breeding-system change and population history of Arabis alpina in the Italian Peninsula and adjacent Alps. Mol Ecol **17(9):**2245-2257.

Ansell SW, Grundmann M, Russell SJ, Schneider H, Vogel JC: Genetic discontinuity, breeding-system change and population history of Arabis alpina in the Italian Peninsula and adjacent Alps. (2008) *Mol Ecol*, **17**(9):2245-2257.

Assaad F. F. (2001) A key regulator of vesicle trafficking required for root hair morphogenesis. 12TH INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH. TAIR accession, Publication: 501709723. https://www.arabidopsis.org/servlets/TairObject?type=publication&id=501709723

Assaad F. F. Mayer U. Wanner G. Jurgens G. (1996) The KEULE gene is involved in cytokinesis in Arabidopsis. Mol Gen Genet **253:** 267-277.

Assaad, F.F., Y. Huet, U. Mayer, and G. Jürgens. (2001) The cytokinesis gene KEULE encodes a Sec1 protein which binds the syntaxin KNOLLE. J. Cell Biol.**152**: 531-543.

Bai, L., Zhou, Y., Ma, X., Gao, L., and Song, C. P. (2014) Arabidopsis CAP1-mediated ammonium sensing required reactive oxygen species in plant cell growth. Plant Signal. Behav. **9:** e29582.

Balkunde R, Pesch M, Hulskamp M. (2010) Trichome patterning in Arabidopsis thaliana from genetic to molecular models. Curr Top Dev Biol 91:299–321.

Baluska, F., Salaj, J., Mathur, J., Braun, M., Jasper, F., Samaj, J., Chua, N. H., Barlow, P. W., and Volkmann, D. (2000) Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilinsupported F-actin meshworks accumulated within expansin-enriched bulges. Dev. Biol. **227:** 618-632.

Bancroft, I., J. D. G. Jones and C. Dean. (1993) Heterologous transposon tagging of the DRL1 locus in Arabidopsis. Plant Cell **5:** 631–638.

Bates, T. R., and Lynch, J. P. (1996). Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant Cell Environ.* **19**, 529-538.

Baudry A, Heim MA, Dubreucq B, Caboche M, Weisshaar B, et al. (2004) TT2, TT8, and TTG1 synergistically specify the expression of BANYULS and proanthocyanidin biosynthesis in Arabidopsis thaliana. Plant J **39:** 366–380.

Baumberger, N., Ringli, C., and Keller, B. (2001). The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in Arabidopsis thaliana. *Genes Dev.* **15**, 1128-1139.

Bedhomme M., Mathieu C., Pulido A., Henry Y., Bergounioux C. (2009). Arabidopsis monomeric G proteins, markers of early and late events in cell differentiation. Int. J. Dev. Biol. **53** 177–185.

Beilstein, M.A., Nagalingum, N.S., Clements, M.D., Manchester, S.R., and Mathews, S. (2010) Dated molecular phylogenies indicate a Miocene origin for Arabidopsis thaliana. Proc Natl Acad Sci U S A **107,** 18724-18728.

Berger, F., Hung, C.-Y., Dolan, L., and Schiefelbein, J. (1998) Control of cell division in the root epidermis of Arabidopsis thaliana. Dev. Biol. **194,** 235-245.

Bergonzi S, Albani MC, Themmat PEVL, Nordström KJV, Wang R, Schneeberger K, Moerland PD, Coupland G (2013) Mechanisms of agedependent response to winter temperature in perennial flowering of Arabis alpina. Science.

Bernal AJ, Yoo CM, Mutwil M, Jensen JK, Hou G, Blaukopf C, Sorensen I, Blancaflor EB, Scheller HV, Willats WG. (2008) Functional analysis of the cellulose synthase-like genes CSLD1, CSLD2, and CSLD4 in tip-growing Arabidopsis cells Plant Physiol, **148,** 1238-1253.

Bernhardt C, Zhao M, Gonzalez A, Lloyd A, Schiefelbein J (2003) The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis. Development **132:** 291–298.

Bibikova TN, Blancaflor EB, Gilroy S (1999) Microtubules regulate tip growth and orientation in root hairs of Arabidopsis thaliana. Plant J **17:** 657-665.

Bibikova, T.N., Jacob, T., Dahse, I., and Gilroy, S. (1998) Localized changes in apoplastic and cytoplasmic pH are associated with root hair development in Arabidopsis thaliana. Development **125:** 2925–2934.

Böhme, K., Li, Y., Charlot, F., Grierson, C., Marrocco, K., Okada, K., Laloue, M. and Nogue, F. (2004) The Arabidopsis COW1 gene encodes a phosphatidylinositol transfer protein essential for root hair tip growth. The Plant Journal **40,** 686–698.

Bouyer, D., Geier, F., Kragler, F., Schnittger, A., Pesch, M., Wester, K., Balkunde, R., Timmer, J., Fleck, C., and Hulskamp, M. (2008) Two-dimensional patterning by a trapping/depletion mechanism: the role of TTG1 and GL3 in Arabidopsis trichome formation. PLoS biology **6,** e141.

Brady, S. M., Orlando, D. A., Lee, J. Y., Wang, J. Y., Koch, J., Dinneny, J. R., Mace, D., Ohler, U., and Benfey, P. N. (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. Science **318:** 801-806.

Bruex A, Kainkaryam RM, Wieckowski Y, Kang YH, Bernhardt C, Xia Y, Zheng X, Wang JY, Lee MM, Benfey P et al. (2012) A gene regulatory network for root epidermis cell differentiation in Arabidopsis. PLoS Genetics **8:** e1002446.

Buer, C & Djordjevic, M (2009) Architectural phenotypes in the transparent testa mutants of Arabidopsis thaliana', Journal of Experimental Botany, **60 (3)** 751-763.

Camilleri C, Azimzadeh J, Pastuglia M, Bellini C, Grandjean O, Bouchez D. (2002) The Arabidopsis TONNEAU2 Gene Encodes a Putative Novel Protein Phosphatase 2A Regulatory Subunit Essential for the Control of the Cortical Cytoskeleton. The Plant Cell. **14(4)** 833-845.

Carol, RJ., Takeda, S., Linstead, P., Durrant, MC., Kakesova, H., Derbyshire, P., Drea, S., Zarsky, V., and Dolan, L. (2005) A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *nature* **438,** 1013-1016.

Cernac A., Lincoln C., Lammer D. and Estelle M. (1997) The SAR1 gene of Arabidopsis acts downstream of the AXR1 gene in auxin response. Development **124,** 1583-1591.

Chant, J. (1999) Cell polarity in yeast. Ann. Rev. Cell Dev. Biol. **15:** 365-391.

Chaudhry, F., Guérin, C., von Witsch, M., Blanchoin, L., and Staiger, C.J. (2007) Identification of Arabidopsis cyclase-associated protein 1 as the first nucleotide exchange factor for plant actin. Mol Biol Cell **18:** 3002-3014.

Chen, J., Stefano, G., Brandizzi, F., and Zheng, H. (2011) Arabidopsis RHD3 mediates the generation of the tubular ER network and is required for Golgi distribution and motility in plant cells. J. Cell Sci. **124:** 2241-2252.

Cheung A Y. and Wu H. M. (2011) THESEUS 1, FERONIA and relatives: a family of cell wall-sensing receptor kinases? Current Opinion in Plant Biology **14,** 632–641

Cho, H. T. and Cosgrove, D. J. (2002) Regulation of Root Hair Initiation and Expansin Gene Expression in Arabidopsis. The Plant Cell, **14,** 3237–3253.

Chopra, D (2015) Genetic analysis of trichome and root hair development in *Arabis alpina*. PhD thesis.

Chopra, D., Wolff, H., Span, J., Schellmann, S., Coupland, G., Albani, M C., Schrader, A., and Hülskamp, M. (2014) Analysis of *TTG1* function in *Arabis alpina*. BMC Plant Biology **14(16),** 1471-2229.

Clough, S.J., and Bent, A.F. (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J **16,** 735-743.

Cnops, G., Wang, X., Linstead, P., Van Montagu, M., Van Lijsebettens, M., and Dolan, L. (2000) *TORNADO1* and *TORNADO2* are required for the specification of radial and circumferential pattern in the *Arabidopsis* root. *Development* **127**, 3385-3394.

Cui H, Kong D, Wei P, et al. (2014) SPINDLY, ERECTA, and Its Ligand STOMAGEN Have a Role in Redox-Mediated Cortex Proliferation in the Arabidopsis Root. Molecular Plant. **7(12):**1727-1739.

Cutter EG: The Epidermis. In: *Plant Anatomy.* (1978) London: Clowes & Sons; **pp:** 94-106.

De Simone, S., Oka, Y., and Inoue, Y. (2000) Effect of light on root hair formation in Arabidopsis thaliana phytochrome-deficient mutants. J. Plant Res. **113**, 63-69.

Deeks, M.J., Rodrigues, C., Dimmock, S., Ketelaar, T., Maciver, S.K., Malhó, R., and Hussey, P.J. (2007) Arabidopsis CAP1 - a key regulator of actin organisation and development. J. Cell Sci. **120:** 2609-2618.

Dewitte W, Scofield S, Alcasabas AA, et al. (2007) Arabidopsis CYCD3 D-type cyclins link cell proliferation and endocycles and are rate-limiting for cytokinin responses. Proceedings of the National Academy of Sciences, USA **104,** 14537–14542.

DiCristina, M.D., Sessa, G., Dolan, L., Linstead, P., Baima, S., Ruberti, I., and Morelli, G. (1996) The Arabidopsis Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development. Plant J. **10:** 393-402.

Diet A, Link B, Seifert GJ, et al. (2006) The Arabidopsis root hair cell wall formation mutant lrx1 is suppressed by mutations in the RHM1 gene encoding a UDP-L-rhamnose synthase. *Plant Cell*. **18(7):**1630-41.

Digiuni S, Schellmann S, Geier F, et al. (2008) A competitive complex formation mechanism underlies trichome patterning on *Arabidopsis* leaves. *Molecular Systems Biology*. **4:**217.

Djakovic, S., Dyachok, J., Burke, M., Frank, M.J., and Smith, L.G. (2006) BRICK1/HSPC300 functions with SCAR and the ARP2/3 complex to regulate epidermal cell shape in Arabidopsis. Development **133** 1091–1100.

Dolan L and Costa S (2001) Evolution and genetics of root hair stripes in the root epidermis, Journal of Experimental Botany, **52:** 413–417.

Dolan, L. (2001) How and where to build a root hair. Curr. Opin. Plant Biol. **4,** 550–554.

Dolan, L., Duckett, C., Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, S. and Roberts, K. (1994) Clonal relations and patterning in the root epidermis of Arabidopsis. Development **120,** 2465-2474.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. and Scheres, B. (1993) Cellular organisation of the Arabidopsis thaliana root. Development **119,** 71-84.

Duan QH, Kita D, Li C, Cheung AY, Wu HM (2011) FERONIA receptor like kinase regulates RHO GTPase signaling of root hair development. Proc Natl Acad Sci U S A 107:17821-17826.

Esch JJ, Chen M, Sanders M, Hillestad M, Ndkium S, et al. (2003) A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in Arabidopsis. Development **130:** 5885–5894.

Esch, J.J., Oppenheimer, D.G., and Marks, M.D. (1994) Characterization of a weak allele of the GL1 gene of Arabidopsis thaliana. Plant MOI. Biol. **24,** 203-207.

Eshraghi L, Anderson JP, Aryamanesh N, McComb JA, Shearer B, Hardy GSJE. (2014) Suppression of the auxin response pathway enhances susceptibility to Phytophthora cinnamomi while phosphite-mediated resistance stimulates the auxin signalling pathway. BMC Plant Biology. **14:**68.

Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Steer, M., Shaw, P., and Dolan, L. (2001) *KOJAK* encodes a cellulose synthase-like protein required for root hair cell morphogenesis in Arabidopsis. *Genes Dev.* **15**, 79-89.

Feys BJ, Wiermer M, Bhat RA, Moisan LJ, Medina-Escobar N, Neu C, Cabral A, Parker JE (2005) Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. Plant Cell **17:** 2601–2613.

Finn, R.D. Coggill, P. Eberhardt, R.Y. Eddy, S.R. Mistry, J. Mitchell, A.L. Potter, S.C. Punta, M. Qureshi, M. Sangrador-Vegas, A. Salazar, G.A. Tate, J. Bateman A. (2016) Nucleic Acids Research: The Pfam protein families database: towards a more sustainable future. Database Issue 44:D279-D285.

Fischer, U., Ikeda, Y., Ljung, K., Serralbo, O., Singh, M., Heidstra, R., Palme, K., Scheres, B., and Grebe, M. (2006) Vectorial information for Arabidopsis planar polarity is mediated by combined AUX1, EIN2, and GNOM activity. Curr. Biol, **16:** 2143–2149.

Folkers, U., Berger, J. & Hu¨lskamp, M. (1997) Cell morphogenesis of trichomes in Arabidopsis: differential regulation of primary and secondary branching by branch initiation regulators and cell size. Development **124,** 3779–3786.

Foreman, J., Demidchik, V., Bothwell, J. H. F., Mylona, P., Miedema, H., Torres, M. A., Linstead, P., Costa. S., Brownlee, C., Jones, J. D. G., Davies, J. M., and Dolan, L. (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422,** 442–-446.

Frerigmann, H., Pislewska-Bednarek, M., Sanchez-Vallet, A., Molina, A., Glawischnig, E., Gigolashvili, T. and Bednarek, P. (2016) Regulation of pathogen-triggered tryptophan metabolism in *Arabidopsis thaliana* by MYB transcription factors and indole glucosinolate conversion products. *Mol. Plant*, **9**, 682–695.

Fryer, M. J., Oxborough, K., Mullineaux, P. M. & Baker, N. R. (2002) Imaging of photooxidative stress responses in leaves. J. Exp. Bot. 53, 1249–-1254.

Galway ME, Heckman JW Jr, Schiefelbein JW (1997) Growth and ultrastructure of Arabidopsis root hairs: the rhd3 mutation alters vacuole enlargement and tip growth. Planta **201:**209-218

Galway ME, Lane DC, Schiefelbein JW (1999) Defective control of growth rate and cell diameter in tip-growing root hairs of the rhd4 mutant of Arabidopsis thaliana. Can J Bot **77:**494-507.

Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, et al. (1994) The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. Dev Biol **166:** 740–754.

Gilliland, L.U., Kandasamy, M.K., Pawloski, L.C., and Meagher, R.B. (2002) Both vegetative and reproductive actin isovariants complement the stunted root hair phenotype of the Arabidopsis act2-1 mutation. Plant Physiol. **130:** 2199-2209.

Grierson C, Nielsen E, Ketelaarc T, Schiefelbein J. (2014) Root Hairs. *The Arabidopsis Book / American Society of Plant Biologists*. **12:**e0172.

Grierson, C. S. Roberts, K. Feldmann, K. A. Dolan L. (1997) The COW1 Locus of Arabidopsis Acts after RHD2, and in Parallel with RHD3 and TIP1, to Determine the Shape, Rate of Elongation, and Number of Root Hairs Produced from Each Site of Hair Formation. Plant Physiol. **115:** 981-990.

Grierson, C., and Schiefelbein, J. (2002) Root hairs. In: Somerville C, Meyerowitz EM, eds. The Arabidopsis book. *Rockville, MD: American Society of Plant Biologists*.

Guimil S and Dunand C (2007) Cell growth and differentiation in Arabidopsis epidermal cells, Journal of Experimental Botany, **58(14)** 3829–3840.

Guo W, Chen S, Hussain N, Cong Y, Liang Z, Chen K. (2015) Magnesium stress signaling in plant: Just a beginning. Plant Signaling & Behavior. **10(3):**e992287.

Han S, Green L, Schnell DJ (2009) The signal peptide peptidase isrequired for pollen function in Arabidopsis. Plant Physiol **149:** 1289–1301.

Hassan, H., Scheres, B., and Blilou, I. (2010) JACKDAW controls epidermal patterning in the Arabidopsis root meristem through a non-cellautonomous mechanism. Development **137:** 1523-1529.

Hayashi, S., Ishii, T., Matsunaga, T., Tominaga, R., Kuromori, T., Wada, T., Shinozaki, K., and Hirayama, T. (2008) The glycerophosphoryl diester phosphodiesterase-like proteins SHV3 and its homologs play important roles in cell wall organization. Plant Cell Physiol. **49:** 1522-1535.

He´maty K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou J-P, Hofte H. (2007) A receptor-like kinase mediates the response of Arabidopsis cells to inhibition of cellulose synthesis. Curr Biol 17:922-931.

Hématy K, Höfte H (2008) Novel receptor kinases involved in growth regulation. Curr Opin Plant Biol **11:**321–328.

Hemsley, P.A., Kemp, A.C., and Grierson, C.S. (2005) The TIP GROWTH DEFECTIVE S-acyl transferase regulates plant cell growth in Arabidopsis. Plant Cell **17:** 2554–2563.

Herman, P.L. and Marks, M.D. (1989) Trichome development in *Arabidopsis thaliana*. II. Isolation and complementation of the *GLABROUS1* gene, Plant Cell. **1,** 1051–1055.

Herrmann A, Felle HH (1995) Tip growth in root hair-cells of Sinapis alba 1-significance of interna1 and externa1 Ca2+ and pH. New Phytol **129:** 523-533.

Heslop-Harrison J, Heslop-Harrison Y, Cresti M, Ciampolini F (1991) Ultra structural features of pollen tubes of Endymion nonscriptus modified by cytochalasin D. Sex Plant Reprod **4:** 73-80.

Heydlauff J and Groß-Hardt (2014). Love is a battlefield: programmed cell death during fertilization. J Exp Bot **65,** 1323-30.

Hirano T, Matsuzawa T, Takegawa K, Sato MH. (2011) Loss-of-Function and Gain-of-Function Mutations in FAB1A/B Impair Endomembrane Homeostasis, Conferring Pleiotropic Developmental Abnormalities in Arabidopsis. Plant Physiology. **155(2):**797-807.

Hong RL, Sommer RJ (2006) Pristionchus pacificus: a well-rounded nematode. *Bioessays,* **28**(6):651-659.

Huang L., Shi X., Wang W., Ryu K H., and Schiefelbein J (2017) Diversification of Root Hair Development Genes in Vascular Plants. Plant Physiology **174,** 1697–1712.

Hülskamp, M. & Schnittger, A. (1998) Spatial regulation of trichome formation in Arabidopsis thaliana. Semin. Cell Dev. Biol. 9, 213–220.

Hülskamp, M. (2004) Plant trichomes: a model for cell differentiation. Nat Rev Mol Cell Biol **5**(6):471-480.

Hülskamp, M., Misera, S., Jürgens, G. (1994) Genetic dissection of trichome cell development in Arabidopsis. Cell **76,** 555–566.

Hung, C.-Y., Lin, Y., Zhang, M., Pollock, S., Marks, M.D., and Schiefelbein, J. (1998) A common position-dependent mechanism controls cell-type patterning and GLABRA2 regulation in the root and hypocotyl epidermis of Arabidopsis. Plant Physiol. **117,** 73–84.

Hyun, Y., Kim, J., Cho, S.W., Choi, Y., Kim, J.-S. and Coupland, G. (2015) Site-directed mutagenesis in *Arabidopsis* thaliana using dividing tissue-targeted RGEN of the CRISPR/Cas system to generate heritable null alleles. *Planta*, **241**, 271–284.

ILGENFRITZ, H., BOUYER, D., SCHNITTGER, A., MATHUR, J., KIRIK, V., SCHWAB, B., CHUA, N.-H., JÜRGENS, G. and HULSKAMP, M. (2003). The Arabidopsis STICHEL Gene is a Regulator of Trichome Branch Number and Encodes a Novel Protein. Plant Physiology. Plant Physiol. **131,** 643-655.

Ishida T, Kurata T, Okada K, Wada T (2008) A genetic regulatory network in the development of trichomes and root hairs. Annu Rev Plant Biol **59:** 365–386.

Jaegle, B. (2015) Natural variation of a gene network regulating trichome patterning, seed coat mucilage and proanthocyanidin production in *Arabidopsis thaliana*. PhD thesis.

Jakoby MJ, Falkenhan D, Mader MT, Brininstool G, Wischnitzki E, Platz N, Hudson A, Hulskamp M, Larkin J, Schnittger A. (2008) Transcriptional profiling of mature Arabidopsis trichomes reveals that NOECK encodes the MIXTA-like transcriptional regulator MYB106. Plant Physiol. **148(3):**1583–602.

Jakoby MJ, Weinl C, Pusch S, et al. (2006) Analysis of the Subcellular Localization, Function, and Proteolytic Control of the Arabidopsis Cyclin-Dependent Kinase Inhibitor ICK1/KRP1. Plant Physiology. **141(4):**1293-1305.

Johnson, H. B. (1975) Plant pubescence: an ecological perspective. *Bot. Rev.* **41**, 233–258.

Jones A.R., Kramer E.M., Knox K., Swarup R., Bennett M.J., Lazarus C.M., Leyser H.M., and Grierson C.S. (2009). Auxin transport through non-hair cells sustains root-hair development. Nat. Cell Biol. **11:**78-84.

Jones DL, Shaft JE, Kochian LV (1995) Role of calcium and other ions in directing root hair tip growth in Limnobium stoloniferum. Planta **197:**672-680.

Jones MA, Raymond MJ, Smirnoff N (2006) Analysis of the root-hair morphogenesis transcriptome reveals the molecular identity of six genes with roles in root-hair development in Arabidopsis. Plant J **45:** 83–100.

Jones, A.R., Raymond, M.J., Yang, Z., and Smirnoff, N. (2007) NADPH oxidase-dependent reactive oxygen species formation required for root hair growth depends on ROP GTPase. J. Exp. Bot. **58:** 1261-1270.

Jones, M.A., Shen, J.J., Fu, Y., Li, H., Yang, Z. and Grierson, C.S. (2002) The Arabidopsis Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. Plant Cell, **14,** 763– 776.

Kandasamy M.K., McKinney E.C., Meagher R.B. (2009). A single vegetative actin isovariant overexpressed under the control of multiple regulatory sequences is sufficient for normal Arabidopsis development. Plant Cell **21:** 701–718.

Kang, Y.H., Song, S.K., Schiefelbein, J., and Lee, M.M. (2013) Nuclear trapping controls the position-dependent localization of CAPRICE in the root epidermis of Arabidopsis. Plant Physiol. **163:** 193-204.

Kanter, U.; Hauser, A.; Michalke, B.; Dräxl, S.; Schäffner, A. R. (2010) Caesium and strontium accumulation in shoots of Arabidopsis thaliana – genetic and physiological aspects. Journal of Experimental Botany **61(14),** 3995–4009.

Karnik R., Grefen C., Bayne R., Honsbein A., Köhler T., Kioumourtzoglou D., Williams M., Bryant N.J., and Blatt, M.R. (2013) Arabidopsis Sec1/Munc18 protein SEC11 is a competitive and dynamic modulator of SNARE binding and SYP121-dependent vesicle traffic. Plant Cell **25:** 1368-1382.

Kasili R, Walker JD, Simmons LA, Zhou J, De Veylder L, Larkin JC. (2010) SIAMESE Cooperates With the CDH1-like Protein CCS52A1 to Establish Endoreplication in Arabidopsis thaliana Trichomes. Genetics. **185(1):**257-268.

Ketelaar, T., Galway, M.E., Mulder, B.M., and Emons, A.M. (2008) Rates of exocytosis and endocytosis in Arabidopsis root hairs and pollen tubes. J Microsc. **231:** 265-273.

Kieber, J. J., Rotheberg, M., Roman, G., Feldmann, K. A. and Ecker J. (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell **72,** 427-441.

Kirchner J, Woehlke G, Schliwa M (1999) Universal and unique features of kinesin motors: insights from a comparison of fungal and animal conventional kinesins. Biol Chem **380:**915-921.

Kirik V, Lee MM, Wester K, Herrmann U, Zheng Z, et al. (2005) Functional diversification of MYB23 and GL1 genes in trichome morphogenesis and initiation. Development **132:** 1477–1485.

Kirik V, Schnittger A, Radchuk V, Adler K, Hulskamp M. (2001) Ectopic expression of the Arabidopsis AtMYB23 gene induces differentiation of trichome cells. Dev Biol **235:** 366–377.

Kirik V, Simon M, Hulskamp M, Schiefelbein J (2004a) The ENHANCER OF TRY AND CPC1 (ETC1) gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. Dev Biol **268:** 506–513.

Kirik V, Simon M, Wester K, Schiefelbein J, Hulskamp M (2004b) ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis. Plant Mol Biol **55:** 389–398.

Kirik, V. et al. (2002) Functional analysis of the tubulin-folding cofactor C in Arabidopsis thaliana. Curr. Biol. **12,** 1519–1523.

Koch MA, Kiefer C, Ehrich D, Vogel J, Brochmann C, Mummenhoff K (2006) Three times out of Asia Minor: the phylogeography of Arabis alpina L. (Brassicaceae). Mol Ecol **15(3):**825-839.

Kohno T, Shimmen T (1987) Cazt-induced fragmentation of actin filaments in pollen tubes. Protoplasma **141:** 177-179.

Kohno T, Shimmen T (1988) Mechanism of Ca2+ inhibition of cytoplasmic streaming in lily pollen tubes. J Cell Sci **91:** 501-509.

Kohno T, Shimmen T (1988a) Accelerated sliding of pollen tube organelles along Characeae actin bundles regulated by Ca2+. J Cell Biol **106:** 1539-1543.

Koornneef M (1981) The complex syndrome of ttg mutants. Arabidopsis. Inf. Serv., **18,** 45–51.

Koornneef, M. Dellaert, W.M. and van der Veen, J.H. (1982) EMS- and radiation-induced mutation frequencies at individual loci in Arabidopsis thaliana (L.). Heynh. Mutat. Res. **93,** 109–123.

Kost B, Mathur J, Chua N-H (1999) Cytoskeleton in plant development. Curr Opin Plant Biol **2:**462-470.

Kulich, I., Vojtíková, Z., Glanc, M., Ortmannová, J., Rasmann, S., and Žárský, V. (2015) Cell wall maturation of Arabidopsis trichomes is dependent on exocyst subunit EXO70H4 and involves callose deposition. Plant Physiol. **168,** 120–131.

Kuroha T, Tokunaga H, Kojima M, et al. (2009) Functional Analyses of LONELY GUY Cytokinin-Activating Enzymes Reveal the Importance of the Direct Activation Pathway in Arabidopsis. The Plant Cell. **21(10):**3152-3169.

Kwak, S. H., Shen, R., and Schiefelbein, J. (2005) Positional signaling mediated by a receptor-like kinase in Arabidopsis. Science **307:** 1111-1113.

Kwak, S.H., and Schiefelbein, J. (2007) The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis. Dev. Biol. **302** 118–131.

Larkin JC, Walker JD, Bolognesi-Winfield AC, Gray JC, Walker AR (1999) Allele-specific interactions between ttg and gl1 during trichome development in Arabidopsis thaliana. Genetics **151:** 1591–1604.

Larkin JC, Young N, Prigge M, Marks MD (1996) The control of trichome spacing and number in Arabidopsis. Development **122:** 997–1005.

Larkin, J. C., Brown, M. L. & Schiefelbein, J. (2003) How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis. Annu. Rev. Plant Biol. **54,** 403–430.

Larkin, J. C., D. G. Oppenheimer, A. M. Lloyd, E. T. Paparozzi and M. D. Marks (1994b) Roles of the GLABROUS1 and TRANS PARENT TESTA GLABRA genes in Arabidopsis trichome development. Plant Cell **6,** 1065–1076.

Larkin, J. C., M. D. Marks, J. Nadeau and F. Sack, (1997) Epidermal cell fate and patterning in leaves. Plant Cell **9:** 1109–1120.

Larkin, J.C., Oppenheimer, D.G., Pollock, S., and Marks, H.D. (1993) Arabidopsis GLABROUSl gene requires downstream sequences for function. Plant Cell **5,** 1739-1748.

Larkin, J.C., Oppenhelmer, D.G., and Marks, M.D. (1994a) The GL7 gene and the trichome developmental pathway in Arabidopsis fhaliana. In Plant Transcription Factors, L. Nover, ed (Berlin: Springer- Verlag), **pp**. 259-275.

Lee, M. M. & Schiefelbein, J. (2002) Cell patterning in the Arabidopsis root epidermis determined by lateral inhibition with feedback. Plant Cell **14,** 611–618 (2002).

Lee, M.M. and Schiefelbein, J. (1999) WEREWOLF, a MYB-related protein in Arabidopsis, is a position-dependent regulator of epidermal cell patterning. Cell **99,** 473–483

Lee, M.M. and Schiefelbein, J. (2001) Developmentally distinct MYB genes encode functionally equivalent proteins in Arabidopsis. Development **128,** 1539–1546.

Lee, Y.-R.J., and Liu, B. (2004) Cytoskeletal motors in Arabidopsis. Sixty-one kinesins and seventeen myosins. Plant Physiol **136:** 3877–3883.

Lew, R.R. (1996) Pressure regulation of the electrical properties of growing *Arabidopsis thaliana L* root hairs. Plant Physiol. **112:** 1089-1100.

Leyser HMO, Pickett FB, Dharmasiri S, Estelle M (1996) Mutations in the AXR3 gene of Arabidopsis result in altered auxin response including ectopic expression from the SAUR-AC1 promoter. Plant J **10:** 403–413.

Li C, Yeh FL, Cheung AY, Duan Q, Kita D, Liu MC, Maman J, Luu EJ, Wu BW, Gates L, et al. (2015) Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. eLife **4:** 4.

Li SF, Milliken ON, Pham H, Seyit R, Napoli R, Preston J, Koltunow AM, Parish RW (2009) The Arabidopsis MYB5 transcription factor regulates mucilage synthesis, seed coat development, and trichome morphogenesis. Plant Cell **21:** 72–89.

Liao HZ, Zhu MM, Cui HH, Du XY, Tang Y, Chen LQ, Ye D, Zhang XQ. (2016) MARIS plays important roles in Arabidopsis pollen tube and root hair growth. JIBP **58,** 927-940.

Livak, K. J. and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Method **25**, 402–408.

Lloyd CW, Pearce KJ, Rawlins DJ, Ridge RW, Shaw PJ (1987) Endoplasmic microtubules connect the advancing nucleus to the tip of legume root hairs, but F-actin is involved in basipetal migration. Cell Motil Cytoskeleton **8:** 27-36.

Lloyd, A.M., Walbot, V. and Davis, R.W. (1992) Arabidopsis and Nicotiana anthocyanin production activated by maize regulators R and C1. Science, **258,** 1773–1775.

Lukowitz, W., U. Mayer, and G. Jürgens. (1996) Cytokinesis in the Arabidopsis embryo involves the syntaxin-related KNOLLE gene product. Cell **84:** 61-71.

Lyons, E., Pedersen, B., Kane, J., Alam, M., Ming, R., Tang, H., Wang, X., Bowers, J., Paterson, A., Lisch, D., and Freeling, M. (2008) Finding and comparing syntenic regions among Arabidopsis and the outgroups papaya, poplar, and grape: CoGe with rosids. Plant Physiol **148,** 1772-1781.

Mandaokar, A., and Browse, J. (2009) MYB108 acts together with MYB24 to regulate jasmonate-mediated stamen maturation in Arabidopsis. Plant Physiol. **149,** 851–862.

Marchler-M, Bauer, A., M. K. Derbyshire, N. R. Gonzales, S. Lu, F. Chitsaz, L. Y. Geer, R. C. Geer, J. He, M. Gwadz, D. I. Hurwitz, C. J. Lanczycki, F. Lu, G. H. Marchler, J. S. Song, N. Thanki, Z. Wang, R. A. Yamashita, D. Zhang, C. Zheng and S. H. Bryant (2015) "CDD: NCBI's conserved domain database." Nucleic Acids Research 43(D1): D222MD226.

Marks MD and Esch JJ (1992) Trichome formation in Arabidopsis as a genetic model system for studying cell expansion. Curr Top Plant Biochem Physiol **11:** 131–142.

Marks MD and Esch JJ (2003) Initiating inhibition. Control of epidermal cell patterning in plants. EMBO Rep **4:** 24–25.

Marks, M.D., and Feldmann, K.A. (1989) Trichome development in Arabidopsis thaliana. 1. T-DNA tagging of the GLABROUSI gene. Plant Cell **1,** 1043-1050.

Masucci JD, Schiefelbein JW (1994) The rhd6 mutation of Arabidopsis thaliana alters root hair initiation through an auxinassociated and ethylene-associated process. Plant Physiol **106:** 1335-1346.

Masucci, J. D., Rerie, W. G., Foreman, D. R., Zhang, M., Galway, M. E., Marks, M. D. and Schiefelbein, J. W. (1996). The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of Arabidopsis thaliana. Development **122,** 1253-1260.

Masucci, J.D., and Schiefelbein, J.W. (1996) Hormones act downstream of TTG and GL2 to promote root hair outgrowth during epidermis development in the Arabidopsis root. *Plant Cell* **8,** 1505-1517.

Mathur J, Mathur N, Kernebeck B, Hülskamp M. (2003a) Mutations in Actin-Related Proteins 2 and 3 Affect Cell Shape Development in Arabidopsis. The Plant Cell. **15(7):**1632-1645.

Mathur J. (2005) Conservation of boundary extension mechanisms between plants and animals. The Journal of Cell Biology. **168(5):**679-682.

Mathur, J. and Hülskamp, M. (2002) Microtubules and microfilaments in cell morphogenesis in higher plants. *Curr. Biol.* **12**, R669 -R676.

Mathur, J., Mathur, N., Kirik, V., Kernebeck, B., Srinivas, B.P., and Hulskamp, M. (2003b) Arabidopsis CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. Development **130,** 3137–3146.

Mauricio, R. & Rausher, M. D. (1997) Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* **51**, 1435–1444.

Miller DD, Bisseling T, Emons AMC (1999) The role of actin in root hair morphogenesis: studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. Plant J **17:**141-154.

Miller DD, Norbert CA, de Ruijter NCA, Emons AMC (1997) From signal to form: aspects of the cytoskeleton-plasma membrane-cell wall continuum in root hair tips. J Exp Bot **48:**1881-1896.

Molendijk, A.J., Bischoff, F., Rajendrakumar, C.S., Friml, J., Braun, M., Gilroy, S. and Palme, K. (2001) Arabidopsis thaliana Rop GTPases are localized to tips of root hairs and control polar growth. EMBO J. **20**, 2779–2788.

Monshausen G.B., Bibikova T.N., Messerli M.A., Shi C., and Gilroy S. (2007) Oscillations in extracellular pH and reactive oxygen species modulate tip growth of Arabidopsis root hairs. Proc. Natl. Acad. Sci. U S A. **104:** 20996-21001.

Morohashi, K., Zhao, M., Yang, M., Read, B., Lloyd, A., Lamb, R., and Grotewold, E. (2007) participation of the Arabidopsis bHLH factor GL3 in trichome initiation regulatory events. Plant physiology **145,** 736-746.

Muto, H., Watahiki, M.K., Nakamoto, D., Kinjo, M. and Yamamoto, K.T. (2007) Specificity and similarity of functions of the Aux/IAA genes in auxin signaling of Arabidopsis revealed by promoterexchange experiments between MSG2/IAA19, AXR2/IAA3 and SLR/ IAA14. Plant Physiol. **144:** 187–196.

Nagpal, P., Walker, L. M., Young, J. C., Sonawala, A., Timpte, C., Estelle, M., and Reed, J. W. (2000) AXR2 encodes a member of the Aux/IAA protein family. *Plant Physiol*. **123**, 563-573.

Nayidu, N.K., Kagale, S., Taheri, A., Withana-Gamage, T.S., Parkin, I.A.P., Sharpe, A.G., and Gruber, M.Y. (2014) Comparison of Five Major Trichome Regulatory Genes in Brassica villosa with Orthologues within the Brassicaceae. Plos One 9.

O'Lexy R, Kasai K, Clark N, Fujiwara T, Sozzani R, Gallagher KL. (2018) Exposure to heavy metal stress triggers changes in plasmodesmatal permeability via deposition and breakdown of callose. Journal of Experimental Botany. 69(15):3715-3728.

Ohashi Y, Oka A, Rodrigues-Pousada R, Possenti M, Ruberti I, Morelli G, Aoyama T (2003) Modulation of phospholipid signaling by GLABRA2 in root-hair pattern formation. Science **300:** 1427–1430.

Ohashi, Y., Ruberti, I., Morelli, G. & Aoyama, T. (2002) Entopically additive expression of GLABRA2 alters the frequency and spacing of trichome initiation. Plant J. **21,** 5036–5046.

Oppenheimer DG (1998) Genetics of plant cell shape. Current Opinion in Plant Biology **1:**520–524.

Oppenheimer DG, Pollock MA, Vacik J, et al. (1997) Essential role of a kinesin-like protein in Arabidopsis trichome morphogenesis. Proceedings of the National Academy of Sciences of the United States of America. **94(12):** 6261-6266.

Oppenheimer, D. G., P. L. Herman, S. Sivakumaran, J. Esch and M. D. Marks (1991) A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. Cell **67**, 483–493.

Orman-Ligeza B, Parizot B, de Rycke R, et al. (2016) RBOH-mediated ROS production facilitates lateral root emergence in Arabidopsis. Development (Cambridge, England). **143(18):**3328-3339.

Oyama, T., Shimura, Y. and Okada, K. (2002) The IRE gene encodes a protein kinase homologue and modulates root hair growth in Arabidopsis. Plant J. **30,** 289–299.

Pacheco-Escobedo MA, Ivanov VB, Ransom-Rodríguez I, et al. (2016) Longitudinal zonation pattern in *Arabidopsis* root tip defined by a multiple structural change algorithm. *Annals of Botany*. **118(4):**763-776.

Park, E., and Nebenfur, A. (2013) Myosin XIK of *Arabidopsis thaliana* accumulates at the root hair tip and is required for fast hair growth. PLoS One **8:** e76745.

Park, S., Szumlanski, A.L., Gu, F., Guo, F., and Nielsen, E. (2011) A role for CSLD3 during cell-wall synthesis in apical plasma membranes of tip-growing root-hair cells. Nat. Cell Biol. **13:** 973-980.

Parker, J.S., Cavell, A.C., Dolan, L., Roberts, K., and Grierson, C.S. (2000) Genetic interactions during root hair morphogenesis in Arabidopsis. Plant Cell **12**, 1961-1974.

Payne, C.T., F. Zhang and A. M. Lloyd (2000) GL3 encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with *GL1* and *TTG1*. Genetics, **156,** 1349–1362.

Pei, W., Du, F., Zhang, Y., He, T., and Ren, H. (2012) Control of the actin cytoskeleton in root hair development. Plant Sci **187:** 10-18.

Pesch M, Hulskamp M (2004) Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. Current Opin Genet Dev **14:** 422–427.

Pesch, M., and Hulskamp, M. (2009) One, two, three...models for trichome patterning in Arabidopsis? Current opinion in plant biology **12,** 587-592.

Pesch, M. Dartan, B. Birkenbihl, R. Somssich, I.E. Hulskamp M. (2014) *Arabidopsis* TTG2 regulates *TRY* expression through enhancement of activator complex-triggered activation Plant Cell, 26 pp. 4067-4083.

Pitts RJ, Cernac A, Estelle M (1998) Auxin and ethylene promote root hair elongation in Arabidopsis. Plant J **16:** 553–560.

Ramachandran, S., Christensen, H. E. M., Ishimaru, Y., Dong, C. H., Chao-Ming, W., Cleary, A. L., and Chua, N. H. (2000) Profilin plays a role in cell elongation, cell shape maintenance, and flowering in arabidopsis. Plant Physiol. **124**, 1637-1647.

Ramon M, Rolland F, Sheen J. (2008) Sugar Sensing and Signaling. The Arabidopsis Book / American Society of Plant Biologists. **6:**e0117.

Ramsay, N.A. et al. (2003) Two basic-helix–loop–helix genes (MYC-146 and GL3) from Arabidopsis can activate anthocyanin biosynthesis in a white-flowered Matthiola incana mutant. Plant Mol. Biol. **52,** 679–688.

Ramsay, N.A., and Glover, B.J. (2005) MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends in plant science **10,** 63-70.

Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red far-red light receptor phytochrome-b alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5:** 147–157.

Reiss HD, Herth W (1979) Calcium gradients in tip growing plant cells visualized by chlorotetracycline fluorescence. Planta **146:** 6 15-62 1.

Rerie, W. G., Feldmann, K. A. & Marks, M. D. (1994) The glabra 2 gene encodes a homeo domain protein required for normal trichome development in Arabidopsis. Genes Dev. **8,** 1388–1399.

Ridge, R. W., Uozumi, Y., Plazinski, J., Hurley, U. A., and Williamson, R. E. (1999) Developmental transitions and dynamics of the cortical ER of Arabidopsis cells seen with green fluorescent protein. Plant Cell Physiol. **40:** 1253-1261.

Rigas, S., Debrosses, G., Haralampidis, K., Vicente-Agullo, F., Feldmann, K. A., Grabov, A., Dolan, L., and Hatzopoulos, P. (2001) TRH1 encodes a potassium transporter required for tip growth in Arabidopsis root hairs. *Plant Cell* **13**, 139-151.

Ringli, C., Baumberger, N., Diet, A., Frey, B., and Keller, B. (2002) ACTIN2 is essential for bulge site selection and tip growth during root hair development of Arabidopsis. Plant Physiol. **129:** 1464-1472.

Rishmawi L, Sun H, Schneeberger K, Hülskamp M, Schrader A (2014) Rapid identification of a natural knockout allele of ARMADILLO REPEAT-CONTAINING KINESIN1 that causes root hair branching by mapping-by-sequencing. Plant Physiol **166:** 1280–1287.

Roth S, Hartenstein V. (2008) Development of Tribolium castaneum. *Dev Genes Evol*, **218**(3-4):115-118.

Rudel D, Sommer RJ. (2003) The evolution of developmental mechanisms. Dev Biol **264(1),** 15–37.

Rui Y, Xiao C, Yi H, et al. (2017) POLYGALACTURONASE INVOLVED IN EXPANSION3 Functions in Seedling Development, Rosette Growth, and Stomatal Dynamics in Arabidopsis thaliana. The Plant Cell. **29(10):**2413-2432.

Ryan E, Grierson CS, Cavell A, Steer M, Dolan L (1998) TIP1 is required for both tip growth and non-tip growth in Arabidopsis. New Phytol **138:**49-58.

Ryan, E Steer, M Dolan, L. (2001) Cell biology and genetics of root hair formation in Arabidopsis thaliana. Protoplasma **215**:140-149

Saedler, R., Jakoby, M., Marin, B., Galiana-Jaime, E., and Hulskamp, M. (2009) The cell morphogenesis gene SPIRRIG in Arabidopsis encodes a WD/BEACH domain protein. Plant J. **59,** 612–621.

Sakai, T., van der Honing, H.S., Nishioka, M., Uehara, Y., Takahashi, M., Fujisawa, N., Saji, K., Seki, M., Shinozaki, K., Jones, M.A., Smirnoff, N., Okada, K., and Wasteneys, G.O. (2008) Armadillo repeat-containing kinesins and a NIMA-related kinase are required for epidermal-cell morphogenesis in Arabidopsis. Plant J. **53:** 157-171.

Sakamoto T, Inui YT, Uraguchi S, Yoshizumi T, Matsunaga S, Mastui M, Umeda M, Fukui K, Fujiwara T. (2011) Condensin II alleviates DNA damage and is essential for tolerance of boron overload stress in Arabidopsis. Plant Cell **23:** 3533–3546.

Salazar-Henao JE, Vélez-Bermúdez IC, Schmidt W. (2016) The regulation and plasticity of root hair patterning and morphogenesis. Development **143,** 1848–1858.

Schellmann S, Hulskamp M. (2005) Epidermal differentiation: trichomes in Arabidopsis as a model system, The International Journal of Developmental Biology, **49,** 579-584.

Schellmann S, Schnittger A, Kirik V, Wada T, Okada K, et al. (2002) TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. EMBO J **21:** 5036–5046.

Scheres B (2002) Plant patterning: TRY to inhibit your neighbors. Curr Biol **12:** R804–R806.

Schiefelbein J, Kwak S-H, Wieckowski Y, Barron C, Bruex A. (2009) The gene regulatory network for root epidermal cell-type pattern formation in Arabidopsis. Journal of Experimental Botany **60(5),** 1515–1521.

Schiefelbein JW, Shipley A, Rowse P (1992) Calcium influx at the tip of growing root hair cells of Arabidopsis thaliana. Planta **187:** 455-459.

Schiefelbein, J. (2003) Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot. Curr. Opin. Plant Biol. **6,** 74–78.

Schiefelbein, J. W. and Somerville, C. (1990) Genetic control of root hair development in Arabidopsis thaliana. Plant Cell **2,** 235-243.

Schiefelbein, J., Galway, M., Masucci, J., and Ford, S. (1993) Pollen tube and root-hair tip growth is disrupted in a mutant of Arabidopsis thaliana. Plant Physiol. **103**, 979-985.

Schiefelbein, J.W. (2000) Constructing a plant cell. The genetic control of root hair development. Plant Physiol. **124,** 1525–1531.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012) Fiji: an open-source platform for biological-image analysis. Nat. Methods **9,** 676–682. doi: 10.1038/nmeth.2019

Schneider, K., Wells, B., Dolan, L., and Roberts, K. (1997) Structural and genetic analysis of epidermal cell differentiation in Arabidopsis primary roots. *Development* **124,** 1789-1798.

Schneider, K.,Mathur, J., Boudonck, K.,Wells, B., Dolan, L., and Roberts, K. (1998) The ROOT HAIRLESS 1 gene encodes a nuclear protein required for root hair initiation in Arabidopsis. Genes & Dev. **12:** 2013–2021.

Schnittger A, Hülskamp M. (2002) Trichome morphogenesis: a cell-cycle perspective. Philosophical Transactions of the Royal Society B: Biological Sciences.**357 (1422):**823-826.

Schnittger, A., U. Folkers, B. Schwab, G. Jürgens and M. Hülskamp (1999) Generation of a Spacing Pattern: The Role of TRIPTYCHON in Trichome Patterning in Arabidopsis. The Plant Cell **11**(6): 1105-1116.

Schoenaers, S. et al. (2018) The auxin-regulated CrRLK1L kinase ERULUS controls cell wall composition during root hair tip growth. Curr. Biol. **28**: 722-732.e6.

Schrader A, Welter B, Hulskamp M, Hoecker U and Uhrig JF. (2013) MIDGET connects COP1-dependent development with endoreduplication in Arabidopsis thaliana. The Plant Journal **75,** 67–79.

Schwab B, Folkers U, Ilgenfritz H, Hülskamp M. (2000) Trichome morphogenesis in Arabidopsis. Philosophical Transactions of the Royal Society B: Biological Sciences. **355(1399):**879-883.

Seifert, G.J., Barber, C., Wells, B., and Roberts, K. (2004). Growth regulators and the control of nucleotide sugar flux. Plant Cell **16:** 723–730.

Seifert, G.J., Barber, C., Wells, B., Dolan, L., and Roberts, K. (2002) Galactose biosynthesis in Arabidopsis: genetic evidence for substrate channeling from UDP-D-galactose into cell wall polymers. Curr. Biol. **12:** 1840–1845.

Shi M. Z., Xie D. Y. (2014) Biosynthesis and metabolic engineering of anthocyanins in Arabidopsis thaliana. Recent Pat. Biotechnol. **8,** 47–60.

Shimada, T L. Shimada T and Hara-Nishimura I (2010) A rapid and non-destructive screenable marker, FAST, for identifying transformed seeds of Arabidopsis thaliana. The Plant Journal **61**, 519–528.

Šimková, K., Moreau, F., Pawlak, P., Vriet, C., Baruah, A., Alexandre, C., et al. (2012) Integration of stress-related and reactive oxygen species-mediated signals by Topoisomerase VI in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U.S.A. **109,** 16360–16365.

Simon, M., Lee, M. M., Lin, Y., Gish, L., and Schiefelbein, J. (2007) Distinct and overlapping roles of single-repeat MYB genes in root epidermal patterning. Dev Biol **311:** 566-78.

Smith, T.F. Gaitatzes, C. Saxena, K. and Neer, E. J. (1999) The WD repeat: a common architecture for diverse functions. Trends Biochem. Sci. **24,** 181–185

Sommer R: The future of evo-devo:model systems and evolutionary theory. 2009 *Nature Reviews Genetics*, **10**:416-422.

Song, S.K., Ryu, K.H., Kang, Y.H., Song, J.H., Cho, Y.H., Yoo, S.D. Schiefelbein, J., and Lee, M.M. (2011) Cell fate in the Arabidopsis root epidermis is determined by competition between WEREWOLF and CAPRICE. Plant Physiol. **157:** 1196-208.

Spannagl,M., K. Mayer, J. Durner, G.Haberer and A. Fröhlich (2011) "Exploring the genomes: From Arabidopsis to crops."Journal of Plant Physiology **168(1)**:3M8.

Steffens A, Bräutigam A, Jakoby M, Hülskamp M (2015) The BEACH Domain Protein SPIRRIG Is Essential for Arabidopsis Salt Stress Tolerance and Functions as a Regulator of Transcript Stabilization and Localization. PLoS Biol **13(7):** e1002188.

Sugimoto-Shirasu K, Roberts GR, Stacey NJ, McCann MC, Maxwell A., and Roberts K. (2005) RHL1 is an essential component of the plant DNA topoisomerase VI complex and is required for ploidy-dependent cell growth. Proc. Natl. Acad. Sci. USA **102:** 18736-18741.

Symonds, V.V., Hatlestad, G., and Lloyd, A.M. (2011) Natural allelic variation defines a role for AT*MYC1*: trichome cell fate determination. PLoS genetics *7*, e1002069.

Szymanski, D. B., Lloyd, A. M. & Marks, M. D. (2000) Progress in the molecular genetic analysis of trichome intiation and morphogenesis in Arabidopsis. Trends Plant Sci. **5,** 214–219.

Szymanski, D.B., Jilk, R.A., Pollock, S.M., and Marks, D. (1998). Control of *GL2* expression in *Arabidopsis* leaves and trichomes. Development **125,** 1161–1171.

Taylor, N.G. (2011) A role for Arabidopsis dynamin related proteins DRP2A/B in endocytosis; DRP2 function is essential for plant growth. Plant Mol Biol **76:**117–129.

Teng, S., Keurentjes, J., Bentsink, L., Koornneef, M., and Smeekens, S. (2005) Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the MYB75/PAP1 gene. Plant Physiol. **139,** 1840–1852.

Thole, J.M., Vermeer, J.E., Zhang, Y., Gadella, T.W., and Nielsen, E. (2008) Root hair defective4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. Plant Cell **20:** 381-395.

Thompson, Elinor & Wilkins, Christopher & Demidchik, Vadim & M Davies, Julia & J Glover, Beverley. (2009) An Arabidopsis flavonoid transporter is required for anther dehiscence and pollen development. Journal of experimental botany. **61.** 439-51.

Toller A., Brownfield L., Neu C., Twell D., Schulze-Lefert P. (2008) Dual function of Arabidopsis glucan synthase-like genes GSL8 and GSL10 in male gametophyte development and plant growth. Plant J. **54,** 911-923.

Tominaga R, Iwata M, Sano R, Inoue K, Okada K, et al. (2008) Arabidopsis CAPRICE-LIKE MYB 3 (CPL3) controls endoreduplication and flowering development in addition to trichome and root hair formation. Development **135:** 1335–1345.

Tominaga, R., Iwata, M., Okada, K. and Wada, T. (2007) Functional analysis of the epidermal-specific MYB genes CAPRICE and WEREWOLF in Arabidopsis. Plant Cell **19:** 2264-77.

Tominaga-Wada R and Wada T (2014) Regulation of root hair cell differentiation by R3 MYB transcription factors in tomato and Arabidopsis. Front. Plant Sci. **5:**91.

Tominaga-Wada, R., Ishida, T., and Wada, T. (2011) New Insights into the Mechanism of Development of Arabidopsis Root Hairs and Trichomes. Int Rev Cel Mol Bio **286,** 67-106.

Tsai HH, Schmidt W (2017) Mobilization of iron by plant-borne coumarins. Trends Plant Sci **22:** 538–548.

Umeda M, Umeda-Hara C, Uchimiya H. (2000) A cyclin-dependent kinase-activating kinase regulates differentiation of root initial cells in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America. **97(24):**13396-13400.

Vernoud, V., Horton, A.C., Yang, Z., and Nielsen, E. (2003) Analysis of the small GTPase gene superfamily of Arabidopsis. Plant Physiol. **131:** 1191-1208.

Very, A. A., and Davies, J. M. (2000) Hyperpolarization-activated calcium channels at the tip of Arabidopsis root hairs. Proc. Natl. Acad. Sci. USA **97:** 9801-9806.

Wada T, Kunihiro A, Tominaga-Wada R (2014) Arabidopsis CAPRICE (MYB) and GLABRA3 (bHLH) Control Tomato (Solanum lycopersicum) Anthocyanin Biosynthesis. PLoS ONE **9(9):** e109093.

Wada, T., Kurata, T., Tominaga, R., Koshino-Kimura, Y., Tachibana, T., Goto, K., Marks, M.D., Shimura, Y. and Okada, K. (2002) Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation. Development **129,** 5409–5419.

Wada, T., Tachibana, T., Shimura, Y., and Okada, K. (1997) Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, *CPC*. *Science* **277,** 1113-1116.

Wagner GJ, Wang E, Shepherd RW (2004) New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann Bot (Lond)*, **93**(1):3-11.

Walker, A. R., P. A. Davison, A. C. BolognesiMWinfield, C. M. James, N. Srinivasan, T. L. Blundell, J. J. Esch, M. D. Marks and J. C. Gray (1999) The *TRANSPARENT TESTA GLABRA1* Locus, Which Regulates Trichome Differentiation and Anthocyanin Biosynthesis in Arabidopsis, Encodes a WD40 Repeat Protein. The Plant Cell **11**: 1337-1349.

Wang R, Farrona S, Vincent C, Joecker A, Schoof H, Turck F, Alonso-Blanco C, Coupland G, Albani MC. (2009) PEP1 regulates perennial flowering in Arabis alpina. Nature **459(7245)**:423–427.

Wang S, Kwak SH, Zeng Q, Ellis BE, Chen XY, et al. (2007) TRICHOMELESS1 regulates trichome patterning by suppressing GLABRA1 in Arabidopsis. Development **134:** 3873–3882.

Wang S., Chen J. G. (2014) Regulation of cell fate determination by single-repeat R3 MYB transcription factors in Arabidopsis. Front. Plant Sci. **5:**133.

Wang W, Wang L, Chen C, Xiong G, Tan XY, Yang KZ, Wang ZC, Zhou Y, Ye D, Chen LQ (2011) Arabidopsis CSLD1 and CSLD4 are required for cellulose deposition and normal growth of pollen tubes. J Exp Bot **62:** 5161–5177.

Wang, H.-Y., Lockwood, S.K., Hoeltzel, M.F., and Schiefelbein, J.W. (1997) The ROOT HAIR DEFECTIVE3 gene encodes an evolutionarily conserved protein with GTP-binding motifs and is required for regulated cell enlargement in Arabidopsis. Genes Dev. **11,** 799-811.

Wang, S., Barron, C., Schiefelbein, J., and Chen, J.G. (2010) Distinct relationships between GLABRA2 and single-repeat R3 MYB transcription factors in the regulation of trichome and root hair patterning in Arabidopsis. New Phytol. **185:** 387-400.

Wang, X., Cnops, G., Vanderhaeghen, R., De Block, S., Van Montagu, M., and Van Lijsebettens, M. (2001) AtCSLD3, a cellulose synthase-like gene important for root hair growth in Arabidopsis. *Plant Physiol.* **126**, 575-586.

Wilkins A. S. (2002) The evolution of developmental pathways. Sunderland, Massachusetts: Sinauer Associates.

Willing E M, Rawat V, Mandáková T, Maumus F, James G V, Nordström K J.V., Becker C, Warthmann N, Chica C, Szarzynska B, Zytnicki M, Albani M C, Kiefer C, Bergonzi S, Castaings L, Mateos J L., Berns M. C., Bujdoso N, Piofczyk T, de Lorenzo L, Barrero-Sicilia C, Mateos I, Piednoël M, Hagmann J, Chen-Min-Tao R, Iglesias-Fernández R, Schuster S. C., Alonso-Blanco C, Roudier F, Carbonero P, Paz-Ares J, Davis S. J., Pecinka A, Quesneville H, Colot V, Lysak M A., Weigel D, Coupland G and Schneeberger K. (2015) Genome expansion of Arabis alpina linked with retrotransposition and reduced symmetric DNA methylation. *Nature Plants* **volume1**, Article number: 14023

Wilson A, Pickett FB, Turner JC, Estelle M (1990) A dominant mutation in Arabidopsis confers resistance to auxin, ethylene, and abscisic acid. Mol Gen Genet **222:** 377–383.

Wymer, C.L., Bibikova, T.N., and Gilroy, S. (1997) Cytoplasmic free calcium distributions during the development of root hairs of Arabidopsis thaliana. Plant J. **12:** 427–439.

Xu J, Yang J-Y, Niu Q-W, Chua N-H. (2006) Arabidopsis DCP2, DCP1, and VARICOSE Form a Decapping Complex Required for Postembryonic Development. The Plant Cell. **18(12):**3386-3398.

Xu, C. R., Liu, C., Wang, Y. L., Li, L. C., Chen, W. Q., Xu, Z. H., and Bai, S. N. (2005) Histone acetylation affects expression of cellular patterning genes in the Arabidopsis root epidermis. Proc. Natl. Acad. Sci. U S A **102:** 14469-14474.

Yan J, Gu Y, Jia X, Kang W, Pan S, Tang X, Chen X, Tang G (2012) Effective small RNA destruction by the expression of a short tandem target mimic in *Arabidopsis*. Plant Cell **24:**415–427.

Yang G, Gao P, Zhang H, Huang S, Zheng Z-L (2007) A Mutation in MRH2 Kinesin Enhances the Root Hair Tip Growth Defect Caused by Constitutively Activated ROP2 Small GTPase in Arabidopsis. PLoS ONE **2(10):** e1074.

Yang, C. & Ye, Z. (2013) Trichomes as models for studying plant cell differentiation Cell. Mol. Life Sci. 70: 1937.

Yoo C, and Blancaflor E (2013) Overlapping and divergent signaling pathways for ARK1 and AGD1 in the control of root hair polarity in Arabidopsis thaliana. Frontiers in Plant Science. **4:** 528.

Yuen C.Y.L., Sedbrook J. C., Perrin R. M., Carroll K. L. and Masson P. H. (2005) Loss-of-Function Mutations of ROOT HAIR DEFECTIVE3 Suppress Root Waving, Skewing, and Epidermal Cell File Rotation in Arabidopsis. Plant Physiology, **138,** 701–714.

Zhang F, Gonzalez A, Zhao M, Payne CT, Lloyd A (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. Development **130:** 4859–4869.

Zhang S, Pan Y, Tian W, Dong M, Zhu H, Luan S, Li L. (2017) Arabidopsis CNGC14 Mediates Calcium Influx Required for Tip Growth in Root Hairs. Molecular Plant. **10,** 1004–1006.

Zhang X, Dyachok J, Krishnakumar S, Smith LG, Oppenheimer DG. (2005) IRREGULAR TRICHOME BRANCH1 in Arabidopsis Encodes a Plant Homolog of the Actin-Related Protein2/3 Complex Activator Scar/WAVE That Regulates Actin and Microtubule Organization. The Plant Cell. **17(8):**2314-2326.

Zhang Y., He J., McCormick S. (2009) Two *Arabidopsis* AGC kinases are critical for the polarized growth of pollen tubes. Plant J. **58,** 474–484.

Zhang, M., Wu, F., Shi, J., Zhu, Y., Zhu, Z., Gong, Q., and Hu, J. (2013) ROOT HAIR DEFECTIVE3 family of dynamin-like GTPases mediates homotypic endoplasmic reticulum fusion and is essential for Arabidopsis development. Plant Physiol. **163:** 713-720.

Zhang, Y., Xiao, Y., Du, F., Cao, L., Dong, H., and Ren, H. (2011) Arabidopsis VILLIN4 is involved in root hair growth through regulating actin organization in a Ca2+-dependent manner. New Phytol. **190:** 667–682.

Zhao, M., Morohashi, K., Hatlestad, G., Grotewold, E., and Lloyd, A. (2008) The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. Development (Cambridge, England) **135,** 1991-1999.

6. Supplementary data

									AT5G24557			
									AT5G24550			
	AT5G24480				AT5G24510				AT5G24540			
AT5G24470	/	AT5G24490		AT5G24500	/	AtTTG1 (AT5G24520)		AT5G24530	///	AT5G24560		AT5G24570
Aa_G45380	Aa_G45370	Aa_G45360		Aa_G45350	Aa_G45340	AaTTG1 (Aa_G45330)		Aa_G45320		Aa_G45310		Aa_G45300
									AT5G41280			
									AT5G41290			
									AT5G41300		AT5G41265	
AT5G41340		AT5G41330		AT5G41320		AtGL3 (AT5G41315)		AT5G41310	///	AT5G41270	/	AT5G41260
Aa_G547840	Aa_G547870	Aa_G547880		Aa_G547890		AaGL3 (Aa_G547900)		Aa_G668820		Aa_G603470		Aa_G603480
	Aa_G547860					reverse right and left border						
	Aa_G547850											
AT1G63610		AT1G63630		AT1G63640		AtEGL3 (AT1G63650)		AT1G63660		AT1G63670		AT1G63680
Aa_G234540		Aa_G234530		Aa_G234520		AaEGL3 (Aa_G234510)	Aa_G234500	Aa_G234490		Aa_G234480		Aa_G234470
						reverse right and left border						
				AT3G27895								
			AT3G27883	AT3G27900								
			AT3G27884	AT3G27906								
			AT3G27886	AT3G27910								
AT3G27870		AT3G27880	///	AT3G27890	////	AtGL1 (AT3G27920)		AT3G27925		AT3G27930	AT3G27940	AT3G27950
Aa_G34170		Aa_G34180		Aa_G34190	Aa_G34200	AaGL1 (Aa_G34210)		Aa_G34220	Aa_G34230	Aa_G34240	/	Aa_G34250
AT5G14720		AT5G14730		AT5G14740		AtWER (AT5G14750)		AT5G14760		AT5G14770		AT5G14780
Aa_G7880		Aa_G7890	Aa_G7900	Aa_G7910		AaWER (Aa_G7920)	Aa_G7930	Aa_G7940		Aa_G7950		Aa_G7960
				AT5G53180								
				AT5G53190			AT5G53205		AT5G53220	AT5G53240		
AT5G53150		AT5G53160		AT5G53170	//	AtTRY (AT5G53200)	/	AT5G53210	/	AT5G53230	/	AT5G53250
Aa_G94200		Aa_G94220		Aa_G94230	Aa_G156720	AaTRY (Aa_G156710)	Aa_G156700	Aa_G156670		Aa_G156660		Aa_G156650
							Aa_G156680					AT2G21450
											AT2G46440	
AT2G46380		AT2G46390		AT2G46400		AtCPC (AT2G46410)		AT2G46420		AT2G46430	/	AT2G46450
Aa_G196820		Aa_G196830		Aa_G196840		AaCPC1 (Aa_G196850)		Aa_G196860		Aa_G196870	Aa_G196880	Aa_G196890
							AT1G01410					
							AT1G01400					
							AT1G01390					
AT1G01355		AT1G01360		AT1G01370		AtETC1 (AT1G01380)	///	AT1G01420		AT1G01430		AT1G01440
Aa_G105940		Aa_G105930		Aa_G105920		AaETC1 (Aa_G105910)		Aa_G105900		Aa_G105890		Aa_G105880
AT1G02250		AT1G02260										
AT4G01037		AT4G01040		AT4G01050		AtETC3 (AT4G01060)		AT4G01070		AT4G01080		AT4G01090
Aa_G67880		Aa_G67890		Aa_G67900		AaETC3 (Aa_G67910)		Aa_G67920		Aa_G67930	Aa_G67940	Aa_G67960
AT2G30400		AT2G30410		AT2G30420		AtTCL2 (AT2G30424)		AT2G30430		AT2G30432		AT2G30440
				Aa_G645470		AaTCL2 (Aa_G645450)		Aa_G645460		Aa_G645470		
							AT1G79850					
AT1G79810		AT1G79820		AT1G79830		AtGL2 (AT1G79840)	/	AT1G79860		AT1G79870		AT1G79880
Aa_G239170		Aa_G239160		Aa_G239150		AaGL2 (Aa_G239140)		Aa_G239130		Aa_G239120		Aa_G239110
AT2G37230		AT2G37240		AT2G37250		AtTTG2 (AT2G37260)		AT2G37270		AT2G37280		AT2G37290
Aa_G29970		Aa_G29980		Aa_G29990		AaTTG2 (Aa_G300000)		Aa_G30010	Aa_G30020	Aa_G30030		Aa_G30040
								AT5G41690				

Table S1. Synteny for epidermal patterning genes found in *A. alpina* genome. The left and right borders correspond to the flanking genes and present synteny. Gray boxes = homologous to *A. thaliana*, green boxes = non homologous to *A. thaliana*, black boxes = new gene in *A. alpina* and / = *A. thaliana* gene absent in *A. alpina* (updated from Chopra 2015, PhD thesis).

	Ct target (AaGL3)	AV Ct target	Ct REF (AaRAN3)	AV Ct REF	ΔCt	AV ΔCt control	ΔΔCt	2^-ΔΔCt	Average fold change	fold change rank	Sum of ranks	Count	U statistic	Critical value	Significant
pep1-1 Pep_1.1.	27.97	27.91	23.29	23.02	4.89		0.79	0.58		1	6	3	0	0	*
Pep_1.2.	27.85		22.76												
Pep_2.1.	28.95	28.85	24.14	24.30	4.55	4.10	0.45	0.73	1.22	2					
Pep_2.2.	28.74		24.45												
Pep_3.1.	25.45	26.10	22.94	23.23	2.87		-1.23	2.35		3					
Pep_3.2.	26.74		23.52												
line 1-T1 AaGL3_1.1.1.	23.216	23.12	23.595	23.54	-0.43		-4.53	23.04		5	15	3	9		
AaGL3_1.1.2.	23.015		23.486												
AaGL3_1.2.1.	28.417	28.34	29.894	29.86	-1.52		-5.62	49.27	30.41	6					
AaGL3_1.2.2.	28.261		29.828												
AaGL3_1.3.1.	26.764	26.53	26.741	26.67	-0.14		-4.24	18.92		4					
AaGL3_1.3.2.	26.302		26.607												
pep1-1 Pep_1.1.	27.97	27.91	23.29	23.02	4.89		0.79	0.58		1	6	3	0	0	*
Pep_1.2.	27.85		22.76												
Pep_2.1.	28.95	28.85	24.14	24.30	4.55	4.10	0.45	0.73	1.22	2					
Pep_2.2.	28.74		24.45												
Pep_3.1.	25.45	26.10	22.94	23.23	2.87		-1.23	2.35		3					
Pep_3.2.	26.74		23.52												
line 2-T1 AaGL3_2.1.1.	28.47	28.47	26.99	26.96	1.51		-2.60	6.04		5	15	3	9		
AaGL3_2.1.2.	28.47		26.94												
AaGL3_2.2.1.	26.86	26.88	24.22	24.19	2.69		-1.41	2.66	9.79	4					
AaGL3_2.2.2.	26.91		24.16												
AaGL3_2.3.1.	32.99	32.99	33.11	33.26	-0.27		-4.37	20.68		6					
AaGL3_2.3.2.	Undetermine		33.42												

Table S2. qRTPCR result of 35S::*AaGL3* in *pep1-1* in T1. Only one individual per line was analyzed.

AaRAN3		Ct 1	Ct 2	Ct 3	Average Ct	SD												
pep1-1 pep1-2		23.44	23.62	23.83	23.63	0.20												
pep1-3		24.59	24.40	24.32	24.44	0.14												
pep1-5		24.50	23.99	23.90	24.13	0.33												
pep1-6		23.40	23.18	23.24	23.27	0.12												
Line 1_T2 1-8		28.53	28.52	28.78	28.61	0.15												
1-9		26.93	26.71	26.89	26.84	0.12												
1-22		25.33	25.75	25.63	25.57	0.22												
1-29		23.48	23.76	23.61	23.62	0.14												
AaGL3		Ct 1	Ct 2	Ct 3	Average Ct	SD	ΔCt	SD		ΔΔCt	2^-ΔΔCt	Ave. fold change	SEM	Fold change ranks	Sum of ranks	Count	U statistic	Critical value	Significant
pep1-1 pep1-2		31.72	32.39	33.00	32.37	0.64	8.74	0.67	8.38	0.36	0.78	1.03	0.17	1	10	4	0	1	*
pep1-3		32.77	31.83	34.28	32.96	1.24	8.52	1.25		0.14	0.91			2					
pep1-5		32.24	31.90	33.58	32.57	0.89	8.44	0.95		0.06	0.96			3					
pep1-6		31.07	31.73	30.52	31.11	0.61	7.83	0.62		-0.55	1.46			4					
Line 1_T2 1-8		31.89	31.74	32.08	31.90	0.17	3.29	0.23		-5.09	34.12	24.00	5.43	8	26	4	16		
1-9		30.56	30.90	31.05	30.84	0.25	4.00	0.28		-4.39	20.95			6					
1-22		30.28	29.95	30.71	30.31	0.38	4.74	0.44		-3.64	12.46			5					
1-29		26.64	27.35	27.51	27.17	0.46	3.55	0.48		-4.83	28.49			7					

Table S3. qRTPCR result of 35S::*AaGL3* in *pep1-1* line1-T2. Four individuals per line was analyzed.

AaRAN3		Ct 1	Ct 2	Ct 3	Average Ct	SD												
pep1-1 pep1-2		23.44	23.62	23.83	23.63	0.20												
pep1-3		24.59	24.40	24.32	24.44	0.14												
pep1-5		24.50	23.99	23.90	24.13	0.33												
pep1-6		23.40	23.18	23.24	23.27	0.12												
Line 2_T2 2-3		22.86	23.07	23.24	23.06	0.19												
2-8		25.33	25.58	25.49	25.47	0.13												
2-21		27.22	27.08	27.69	27.33	0.32												
2-28		25.39	25.71	26.09	25.73	0.35												
AaGL3		Ct 1	Ct 2	Ct 3	Average Ct	SD	ΔCt	SD		ΔΔCt	2^-ΔΔCt	Ave. fold change	SEM	Fold change ranks	Sum of ranks	Count	U statistic	Critical value	Significant
pep1-1 pep1-2		31.72	32.39	33.00	32.37	0.64	8.74	0.67	8.38	0.36	0.78	1.03	0.17	1	10	4	0	1	*
pep1-3		32.77	31.83	34.28	32.96	1.24	8.52	1.25		0.14	0.91			2					
pep1-5		32.24	31.90	33.58	32.57	0.89	8.44	0.95		0.06	0.96			3					
pep1-6		31.07	31.73	30.52	31.11	0.61	7.83	0.62		-0.55	1.46			4					
Line 2_T2 2-3		29.28	30.98	29.41	29.89	0.95	6.83	0.97		-1.55	2.94	7.41	3.11	5	26	4	16		
2-8		29.95	30.06	29.86	29.96	0.10	4.49	0.16		-3.89	14.87			8					
2-21		34.01	30.68	33.60	32.76	1.81	5.43	1.84		-2.95	7.74			7					
2-28		31.53	31.55	33.18	32.09	0.95	6.36	1.01		-2.03	4.08			6					

Table S4. qRTPCR result of 35S::*AaGL3* in *pep1-1* line2-T2. Four individuals per line were analyzed.

	pep1-1		Line1		Line2	
	Number of root hairs	Number of trichomes	Number of root hairs	Number of trichomes	Number of root hairs	Number of trichomes
1	37	ND	30	56.5	34	10
2	50	61.5	20	29.5	43	ND
3	49	69	22	25.5	36	9.5
4	36	92.5	3	ND	26	47
5	44	61	23	13	33	14
6	34	59.5	6	32.5	41	27.5
7	36	60	25	59.5	32	12.5
8	48	41.5	9	6.5	32	6
9	45	56.5	10	8	33	19
10	30	ND	27	78	29	49.5
11	43	80	30	66	33	ND
12	31	ND	10	12.5	40	13.5
13	34	ND	17	46.5	53	17
14	37	54	6	ND	41	ND
15	29	72	10	42.5	38	12
16	38	57.5	7	ND	41	ND
17	31	58	8	26	39	34.5
18	24	62	42	ND	46	15.5
19	31	83.5	29	ND	57	44.5
20	30	66.5	5	ND	44	49
21	38	ND	4	33.5	43	3
22	37	68.5	5	6.5	29	24
23	29	85.5	8	64.5	26	72
24	37	50.5	15	ND	29	13.5
25	32	51	26	ND	30	ND
26	34	68	6	ND	27	16
27	33	55	31	69	25	25.5
28	30	ND	10	63	31	7
29	34	ND	2	7	34	61
30	28	ND	5	ND	30	10
Mean	35.63	64.25				
SD	6.54	12.48				

Table S5. Root hair and trichome counting for two transgenic lines of 35S::*AaGL3* and the respective wild type (*pep1-1*) in T2. 30 individuals per line were analyzed. Mean and SD were not determined for transgenic lines because of the mixed genetic background we expect in T2.

108

	Transformed constructs	Background used for transformation	Number of T1 survivals	PCR for insert confirmation (T1)
1	35S::AaTTG1-CDS	*pep1-1*	11	negative
2	35S::AaGL3-CDS	*pep1-1*	17	positive (3 lines)
3	35S::AaEGL3-CDS	*pep1-1*	1	negative
4	35S::AaGL1-CDS	*pep1-1*	11	negative
5	35S::AaWER-CDS	*pep1-1*	10	negative
6	35S::AaTRY-CDS	*pep1-1*	11	negative
7	amiR1-AaEGL3	*pep1-1*	4	negative
8	amiR2-AaEGL3	*pep1-1*	2	negative
9	amiR3-AaGL1	*pep1-1*	9	negative
10	amiR8-AaGL1	*pep1-1*	17	negative
11	amiR-AaCPC1CPC2	*pep1-1*	3	negative
12	amiR6-AaGL2	*pep1-1*	0	ND
13	amiR14-AaGL2	*pep1-1*	0	ND
14	35S::AaGL3-CDS	*Aattg1-2*	3	positive (1 line)
15	35S::AaGL1-CDS	*Aatry-1*	1	negative
16	35S::AaGL2-CDS	*Aagl2-1*	10	positive (1 line)
17	35S::AaSCN1-CDS	*Aascn1-3*	0	ND
18	p6::AaGL1-CRISPR	*pep1-1*	0	ND
19	p6::AaEGL3-CRISPR	*pep1-1*	0	ND
20	p6::AaCPC1CPC2-CRISPR	*pep1-1*	0	ND
21	pAaGL2::GFP-ER	*pep1-1*	under basta selection	
22	35S::AtSCN1-CDS	*Atscn1-3*	10-30	positive
23	35S::AaSCN1-CDS	*Atscn1-3*	10-30	positive
24	35S::Aascn1-3_CDS	*Atscn1-3*	10-30	positive
25	35S::YFP-AtSCN1-CDS	*Col-0*	30-40	ND
26	35S::AtSCN1-CDS-YFP	*Col-0*	30-40	ND
27	35S::YFP-AaSCN1-CDS	*Col-0*	30-40	ND
28	35S::AaSCN1-CDS-YFP	*Col-0*	30-40	ND

Table S6. List of transformations in *A. alpina* and *A. thaliana*.

	Reduced number of root hairs
35	no root hair
53	very few almost no hair, considerably smaller leaves than wt, but grows normally
235/244	reduced, wt normal, very little amount of seeds
292	reduced and swollen at base, even hyp reduced, normal seedling
357	root hair less, only very few much further of the tip, small plant, leaves with less or no sarration, not big after 6 months
587	reduced and short root hair, normal seedling and root lenght
616	reduced, very thin root, dwarf, tiny leaves
646	no root hair, very tiny seedling
704	reduced, not grown, swollen, normal seedling and root
699	reduced
985	reduced, unusual, normal seedling
1017	reduced
1089	reduced
1101	No hair
1105	reduced, pattern deffect
1171	root hair less
1185	root hair less, in closer look there is initiation, but it is not out growing
1198	reduce
1201	reduce
1266	reduce, firt half empty, second uper half wt
1288	reduced, unusual pattern deffect, small normal seedling
1358	no hair, root like dead, but green leaves, small tiny seedling
1372	no hair
1388	reduced, short, all over, even hyp
1954	reduced, root like dead but normal seedling
1972	reduced, not strong, but even at hyp
1996	reduced
	Excessive number of root hairs
139	more partial root hairs, small seedling, growing very high without flowering
156	more partial
177	excessive, not strong
240	ectopic
252	ectopic, normal seedling, wt normal
311	excessive, unusual, at the tip tidy but excessive, small seedling and short root, round thick leaves, whitout sarration
337	excessive, siliqes are formed but empty, instead of seeds, brown dusty dots
345	ectopic
358	ectopic, not strong, but root looks excessive by eyes, 1: small plant wiht tiny leaves
420	excessive, normal seedling
483	ectopic root hairs, small seedling
555	excessive
574	excessive and branched, small seedling
589	excessive and swollen at the base, small seedling and short root
607	excessive, no meristematic zone, strong
611	excessive
615	excessive, 2 weeks old and very short root, round leaves, maybe allel for 311
744	excessive
745	excessive
749	excessive
756	excessive, small meristematic region
788	excessive
963	excessive, small meristematic region, short root
1082	excessive, swollen at the base
1165	excessive, friquently 3 files of root hairs
1190	excessive, pattern deffect
1198	excessive
1211	excessive, small meristematic zone
1301	excessive
1329	excessive, late flowering, dwarf, tiny leaves
1333	excessive
1353	excessive, cell file deffect, small normal seedling
1358	excessive, unusual, normal seedling

	Short root hairs
101	not grown hairs
223	short, thick root hair,even at hyp, small seedling and short root
318	not grown hairs, all over the root, wt normal
366	reduced, not grown hairs, all over the root, very small leaves with different shape and sarration, some leaves with less chlorophyl
381	short, thick, branched all over
389	short, thick, all over
392	reduced number of root hairs and not elongated, after 3 months still vegetative, with big leaves like Pj, but healthy, very little amount of seeds
394	short root hairs all over, small seedling
395	short and reduced number of root hairs all over, siliqes and leaves purple
396	short root hairs all over, sometimes branched
397	short root hairs all over, sometimes branched
402	short root hairs all over
413	short and thick root hairs all over, small, light green seedling
493	short root hairs all over the root, small, normal seedling, rather small plant after 2 months, still small after 5 months
498	short root hair all over, small seedling
502	short root hair all over the root, late flowering, after 5 months only one give seeds
512	short, not grown root hair
552	short all over, even hyp, normal seedling
588	short all over, even hyp, normal seedling
696	short root hair
881	short all over, no hair at the hyp
721	short all over, not strong but even hyp
928	short and reduced
960	short
966	short all over even at the hyp
988	short, swollen all over, pattern deffect, normal, small seedling
1001	short all over
1051	short and branched
1070	short and thick, hyp reduced
1089	short
1104	short, even hyp, dwarf, leaves different with flat surface, tiny siliqs, late flowering
1108	short, small seedling and short root, rhs are kind of burst
1112	short all over
1137	short all over, even hyp
1139	short all over
1151	short all over
1179	short, not grown, swollen
1183	short all over
1186	short, only swollen
1187	short, small yellow seedling, all over, even hyp
1216	short all over, small siliqs
1233	short all over
1250-1	short and branched (check for scn1)
1250-2	short all over, even hyp
1267	short and reduced
1269	short all over
1276	short all over
1305	short and reduced
1337	short all over
1354	short, medium root hairs all over
1357	short and branched
1365	short and thick, sometimes branched (you will fine three alles for this phenotype)
1367	short and branched, all over (another morphology mutant)
1397	short and branched (another morphology mutant)
1401	short and excessive a bit
1427	short, small, tiny seedling
1960	short
1966	short
2002	short all over, even hyp, not strong

	Branched root hairs
16	branched
55	branched
58	branched, not strong
69	branched, not strong, 4 is strong, 4: yellow leaves with veins green, 2: after 8 months still not dry, late flowering, 3: small and no seed
74	branched, strong, dried leaves are a bit purple
92	branched, strong
95	branched
104	branched
171	branched, 1: dead like annual, the whole plant died after giving seed
195	branched, not strong
197	branched
199	branched, strong
210	branched
211	branched, wt also was branched but not strongly
216	branched and short hairs (unusual)
217	branched
220	branched, wt show branching but other mutants don't show branching
225	branched
226	branched, not strong but 3 show, 1: not dry yet 2: died after seeding
246	branched
247	branched, strong
249	branched, short plant, round leaves, no seeds
250	branched
263	branched
287	branched all over, normal seedling and root lenght, plenty show branching, 1: die after giving seeds
292	branched
299	branched
308	branched
341	branched
398	branched all over
551	branched
565	branched
576	branched
589	branched, strong all over
601	branched, strong even in elongated parts with elongated branches
610	branched and short hairs, all over, 2 weeks old
614	branched and short hairs, 2 weeks old and medium root lenght
699	branched
714	branched and short
736	branched, short and thick
1036	branched, not like wt branching, long root hair and cool branches
1183	branched
1288	branched
1315	branched
1344	branched
1373	branched
1382	branched and short
1390	branched and short all over (another morphology mutant)
1419	branched, all over, quite a few show the same phenotype
1432	branched

	Swollen root hairs
84	swollen at the base of the root hairs, not strong
117	swollen at the base of the root hairs, small seedling
133	swollen
326	swollen at the base of the root hairs
327	swollen at the base of the root hairs and branched at the base, round leaves with no sarration and light green
427	swollen at the base of the root hair strongly, yellow leaf, samll seedling, short root
433	swollen, reuced, normal sedling, swolne at hyp part and generally short root hair, small plant with slow growth rate, big long leaves
469	swollen at the base, small seedling and short root, small plant and slow growth rate
503	swollen at the base, small, normal seedling
584	swollen at the base, short root
617	swollen at the base
683	swollen at the base, very very tiny and yellow seedling
719	swollen
725	swollen at the base, small meristematic zone, normal seedling and root
742	swollen, short, sometimes branched
793	swollen
799	swollen, thick and short
816	swollen
1015	swollen and short
1081	swollen, not grown, some grow hairs but mostly only swollen
1127	swollen at the base, all over
1264	swollen at the base, very tiny seedling
1297	swollen at the base
1301	swollen at the base
1321	swollen at the base
1354	swollen at the base
1370	swollen at the base, branched
1426	swollen, not strong, all over
1959	swollen at the base
1984	swollen at the base
	Mixed different phenotypes
609	long hairs, two weeks old
914	patterning deffect, small seedling, growth problem in root
963	patterning deffect, normal root and seedling, growth problem
997	pattern deffect, branched and thick and long rh, unusual
1034	No pattern, unusual, small seedling
1085	swollen at the end or middle of the root hairs, normal seedling
1172	pattern deffect, reduced
1217	pattern deffect, reduced
1253	pattern deffect, very tiny seedling
1287	swollen at the end of the root hair, not all but some show
1288	first root hair, then only big bulbs, small normal seedling
1310	swollen at the end or middle of the root hairs, unusual rh morphology
1355	swollen at the end and long root hair
1382	long hairs, small meristematic zone, small normal seedling
1387	pattern and file deffect, branched
1419	unusual all over, reduced at hyp
1419	unusual 2, not grown, reduced, root like dead but normal seedling

Table S7. Root Hair Screen result of M2 Generation in *pep1-1* population.

	Gene ID		Reference	Alpina_v5.1 ortholog	Athal gene isoform
1	ERH1	unknown	Schneider et al. 1997	unknown	unknown
2	ERH2/POM1	AT1G05850	Schneider et al. 1997	Aa_G220660	AT1G05850.1
3	ERH3	AT1G80350	Schneider et al. 1997	Aa_G312130	AT1G80350.1
4	CTR1	AT5G03730	Kieber et al. 1993	Aa_G226400	AT5G03730.2
5	TRN1	AT5G55540	Cnops et al. 2000	Aa_G48430	AT5G55540.1
6	TRN2	AT5G46700	Cnops et al. 2000	Aa_G26310	AT5G46700.1
7	RHL1	AT1G48380	Schneider et al. 1997	Aa_G110800	AT1G48380.2
8	RHL2	AT5G02820	Schneider et al. 1997	Aa_G260770	AT5G02820.1
9	RHL3/HYP6	AT3G20780	Schneider et al. 1997	Aa_G133270	AT3G20780.1
10	AXR2/IAA7	AT3G23050	Bates and Lynch 1996	Aa_G423890	AT3G23050.1
11	AXR3/IAA17	AT1G04250	Leyser et al. 1996	NA	AT1G04250.1
12	RHD6	AT1G66470	Masucci and Schiefelbein 1994	Aa_G288090	AT1G66470.1
13	TRH1	AT4G23640	Rigas et al. 2001	Aa_G135350,Aa_G147500,Aa_G147520,Aa_G189640,Aa_G204130, Aa_G240860,Aa_G263930,Aa_G328750,Aa_G365580,Aa_G468700, Aa_G52320,Aa_G66630,Aa_G677910,Aa_G8080,Aa_G8090	AT4G23640.1
14	TIP1	AT5G20350	Parker et al. 2000, Ryan et al. 1998, Schiefelbein et al. 1993	Aa_G105100,Aa_G15720,Aa_G490430	AT5G20350.1
15	RHD1	AT1G64440	Schiefelbein and Somerville 1990	Aa_G270090,Aa_G275300,Aa_G500540	AT1G64440.1
16	RHD2	AT5G51060	Schiefelbein and Somerville 1990, Foreman et al., 2003	Aa_G103490,Aa_G153070,Aa_G19200,Aa_G275560,Aa_G324280,Aa_G33950,Aa_G353720,Aa_G407850,Aa_G477180,Aa_G699380	AT5G51060.1
17	SHV1	unknown	Parker et al. 2000	unknown	unknown
18	SHV2	AT5G49270	Parker et al. 2000	Aa_G16800,Aa_G20530,Aa_G250690,Aa_G468260,Aa_G512260,Aa_G589230	AT5G49270.1
19	SHV3	AT4G26690	Parker et al. 2000	Aa_G250600,Aa_G31480,Aa_G31600,Aa_G48350,Aa_G618320,Aa_G250610	AT4G26690.1
20	KJK	AT3G03050	Favery et al 2001, Wang et al 2001	Aa_G111780,Aa_G136880,Aa_G150190,Aa_G152760,Aa_G162220, Aa_G183690,Aa_G20930,Aa_G264900,Aa_G353330,Aa_G487080,Aa_G489300,Aa_G553710,Aa_G6600,Aa_G9880,Aa_G89560	AT2G33100.1,AT3G03050.1, AT4G38190.1
21	KEULE	AT1G12360	Assaad et al., 1996, Assaad et al., 2001	Aa_G106090,Aa_G246090,Aa_G35070	AT1G12360.1
22	CEN1	unknown	Parker et al. 2000	unknown	unknown
23	CEN2	unknown	Parker et al. 2000	unknown	unknown
24	CEN3	unknown	Parker et al. 2000	unknown	unknown
25	SCN1	AT3G07880	Parker et al. 2000	Aa_G258560	AT3G07880.1
26	BST1	AT5G65090	Parker et al. 2000	NA	AT5G65090.1
27	COW1	AT4G34580	Grierson et al., 1997	Aa_G110270,Aa_G287860,Aa_G351950,Aa_G547700,Aa_G179550, Aa_G287840,Aa_G351940,Aa_G348690,Aa_G93390	AT4G34580.1
28	RHD3	AT3G13870	Schiefelbein and Somerville 1990, Galway et al. 1997, Wang et al., 1997	Aa_G243330,Aa_G254510,Aa_G579230,Aa_G10200,Aa_G579240	AT3G13870.1
29	RHD4	AT3G51460	Schiefelbein and Somerville 1990, Galway et al. 1999, Galway and Schiefelbein 1999	Aa_G113750,Aa_G122720	AT3G51460.1
30	LRX1	AT1G12040	Baumberger et al 2001	Aa_G298900,Aa_G488340	AT1G12040.1
31	AUX1	AT2G38120	Masucci and Schiefelbein 1996	Aa_G121500,Aa_G172230,Aa_G36610,Aa_G612100,Aa_G666030	AT2G38120.1
32	AXR1	AT1G05180	Masucci and Schiefelbein 1996	Aa_G235380,Aa_G243640	AT1G05180.1
33	EIN2	AT5G03280	Pitts et al., 1998, Schiefelbein and Somerville 1990	Aa_G259850	AT5G03280.1
34	ETR1/EIN1	AT1G66340	Pitts et al., 1998, Schiefelbein and Somerville 1990	Aa_G149690,Aa_G241240	AT1G66340.1
35	PFN1	AT2G19760	Ramachandran et al 2000	Aa_G331450	AT2G19760.1
36	ETO1	AT3G51770	Yuen et al., 2005, Pitts et al. 1998	Aa_G188720,Aa_G236050,Aa_G93870	AT3G51770.2
37	PHYA	AT1G09570	De Simone et al. 2000	Aa_G176300,Aa_G380660,Aa_G44320,Aa_G516460,Aa_G56930	AT1G09570.1,AT2G18790.1
38	PHYB	AT2G18790	Reed et al. 1993, De Simone et al. 2000	Aa_G176300,Aa_G380660,Aa_G44320,Aa_G516460,Aa_G56930	AT1G09570.1,AT2G18790.1
39	SAR1	AT1G33410	Cernac et al. 1997	Aa_G353110	AT1G33410.1
40	FER	AT3G51550	Cheung A Y. and Wu H. M. 2011	Aa_G120780,Aa_G120790,Aa_G443210,Aa_G508110,Aa_G122900, Aa_G138820,Aa_G351890,Aa_G377420,Aa_G414150,Aa_G4360,Aa_G448330,Aa_G50380,Aa_G54560,Aa_G550770,Aa_G632240,Aa_G204820,Aa_G448810	AT3G51550.1,AT5G61350.1
41	MRI	AT2G41970	Liao et al., 2016	Aa_G67270	AT2G41970.1
42	LLG1	AT5G56170	Li et al., 2015	Aa_G216680,Aa_G640210	AT5G56170.1
43	CNGC9	AT4G30560	Kanter et al. 2010	Aa_G166810,Aa_G179720,Aa_G196870,Aa_G196890,Aa_G306080, Aa_G31700,Aa_G325220,Aa_G325430,Aa_G381520,Aa_G41500,Aa_G420580,Aa_G432050,Aa_G462570,Aa_G502960,Aa_G67850,Aa_G196910,Aa_G196880,Aa_G196900	AT2G24610.1,AT4G30560.1
44	CNGC14	AT2G24610	Zhang et al., 2017	Aa_G166810,Aa_G179720,Aa_G196870,Aa_G196890,Aa_G306080, Aa_G31700,Aa_G325220,Aa_G325430,Aa_G381520,Aa_G41500,Aa_G420580,Aa_G432050,Aa_G462570,Aa_G502960,Aa_G67850,Aa_G196910,Aa_G196880,Aa_G196900	AT2G24610.1,AT4G30560.1
45	TTG1	AT5G24520	Galway et al. 1994	Aa_G45330	AT5G24520.1

46	GL3	AT5G41315	Bernhardt et al. 2003	Aa_G234510,Aa_G547900	AT1G63650.1,AT5G41315.1
47	EGL3	AT1G63650	Bernhardt et al. 2003	Aa_G234510,Aa_G547900	AT1G63650.1,AT5G41315.1
48	TT8	AT4G09820	Baudry et al. 2004	Aa_G390510	AT4G09820.1
49	MYC1	AT4G00480	Huang et al. 2017	Aa_G511730,Aa_G586660	AT4G00480.2
50	GL1	AT3G27920	Larkin et al. 1994b	Aa_G34210	AT3G27920.1
51	WER	AT5G14750	Lee and Schiefelbein 1999	Aa_G7920	AT5G14750.1
52	MYB23	AT5G40330	Esch et al. 2003	Aa_G247540	AT5G40330.1
53	PAP1	AT1G56650	Teng et al. 2005	Aa_G691680	AT1G56650.1,AT1G66390.1
54	PAP2	AT1G66390	Wang and Chen 2014	Aa_G691680	AT1G56650.1,AT1G66390.1
55	TT2	AT5G35550	Baudry et al. 2004	Aa_G363010	AT5G35550.1
56	MYB5	AT3G13540	Li et al. 2009	Aa_G463630	AT3G13540.1
57	TRY	AT5G53200	Schellmann et al., 2002	Aa_G156710	AT5G53200.1
58	CPC	AT2G46410	Wada et al., 1997, Wada et al. 2002	Aa_G196850,Aa_G33400	AT2G46410.1
59	ETC1	AT1G01380	Kirik et al., 2004 a	Aa_G105910	AT1G01380.1
60	ETC2	AT2G30420	Kirik et al., 2004 b	NA	AT2G30420.1
61	ETC3	AT4G01060	Salazar-Henao et al. 2016	Aa_G67910	AT4G01060.1
62	TCL1	AT2G30432	Wang et al., 2007	NA	AT2G30432.1
63	TCL2	AT2G30424	Tominaga-Wada and Wada 2014	Aa_G645450	AT2G30424.1
64	GL2	AT1G79840	Masucci et al. 1996	Aa_G115050,Aa_G235430,Aa_G257080,Aa_G298510,Aa_G604430,Aa_G15850,Aa_G239140,Aa_G247160,Aa_G303440,Aa_G557370,Aa_G57930,Aa_G243670,Aa_G724610,Aa_G78490	AT1G79840.2
65	TTG2	AT2G37260	Bruex et al. 2012	Aa_G30000	AT2G37260.1
66	ARK1	AT3G54870	Yoo and Blancaflor 2013	Aa_G10870,Aa_G94580,Aa_G381380	AT3G54870.1
67	MID	AT5G24630	Schrader et al. 2013	Aa_G45230	AT5G24630.6
68	CAP1	AT5G61350	Bai et al. 2014	Aa_G120780,Aa_G120790,Aa_G443210,Aa_G508110,Aa_G122900,Aa_G138820,Aa_G351890,Aa_G377420,Aa_G414150,Aa_G4360,Aa_G448330,Aa_G50380,Aa_G54560,Aa_G550770,Aa_G632240,Aa_G204820,Aa_G448810	AT3G51550.1,AT5G61350.1
69	AtGSL10	AT3G07160	(L. Huang et al., 2009)	Aa_G105250,Aa_G132950,Aa_G154650,Aa_G155200,Aa_G159720,Aa_G22770,Aa_G340260,Aa_G373290,Aa_G647680,Aa_G78770,Aa_G79390,Aa_G350910	AT2G13680.1,AT3G07160.1
70	CSLD4	AT4G38190	Toller et al. 2008	Aa_G111780,Aa_G136880,Aa_G150190,Aa_G152760,Aa_G162220,Aa_G183690,Aa_G20930,Aa_G264900,Aa_G353330,Aa_G487080,Aa_G489300,Aa_G553710,Aa_G6600,Aa_G9880,Aa_G89560	AT2G33100.1,AT3G03050.1,AT4G38190.1
71	CSLD1	AT2G33100	(Bernal et al., 2008)	Aa_G111780,Aa_G136880,Aa_G150190,Aa_G152760,Aa_G162220,Aa_G183690,Aa_G20930,Aa_G264900,Aa_G353330,Aa_G487080,Aa_G489300,Aa_G553710,Aa_G6600,Aa_G9880,Aa_G89560	AT2G33100.1,AT3G03050.1,AT4G38190.1
72	ATREV3	AT1G67500	Sakamoto et al. 2011	Aa_G449670	AT1G67500.2
73	MYB108	AT3G06490	Mandaokar and Browse et al., 2009	Aa_G49930	AT3G06490.1
74	MYB21	AT3G27810	Mandaokar and Browse et al., 2009	Aa_G570800	AT3G27810.1
75	MYB24	AT5G40350	Mandaokar and Browse et al., 2009	NA	AT5G40350.1
76	ATSGP2	AT3G21700	Bedhomme et al., 2009	Aa_G171660	AT3G21700.3
77	TRANSPARENT TESTA 4	AT5G13930	Buer et al. 2009	Aa_G289990,Aa_G52330,Aa_G52340	AT5G13930.1
78	AGC KINASE 1.5	AT3G12690	Yan Zhang et al., 2009	Aa_G111340,Aa_G12270,Aa_G520000,Aa_G63480,Aa_G151470,Aa_G27390,Aa_G40140,Aa_G467090,Aa_G641230,Aa_G69790,Aa_G336910,Aa_G377620	AT3G12690.1
79	HVA22D	AT4G24960	Yan Zhang et al., 2009	Aa_G130040	AT4G24960.1
80	ATSPP	AT2G03120	Han et al., 2009	Aa_G171920	AT2G03120.1
81	scrambeled	AT1G11130	Kwak and Schiefelbein 2007	Aa_G290210,Aa_G293170,Aa_G371040,Aa_G504530,Aa_G56290,Aa_G290220,Aa_G384420,Aa_G572760	AT1G11130.1
82	MYB113	AT1G66370	Shi and Xie 2014	NA	AT1G66370.1
83	MYB114	AT1G66380	Wada et al., 2014	NA_in_wb_table	unknown
84	CPL3	AT4G01060	Tominaga et al. 2008	Aa_G67910	AT4G01060.1
85	AN	AT1G01510	Schwab et al. 2000	Aa_G105790,Aa_G105800	AT1G01510.1
86	wurm	AT3G27000	Schwab et al. 2000	Aa_G120920	AT3G27000.1
87	kak	AT4G38600	Hulskamp et al. 1994	Aa_G138090	AT4G38600.1
88	cycb1	AT4G37490	Schnittger and Hülskamp 2002	Aa_G153290	AT4G37490.1
89	cyclin-dependent kinases	AT2G27960	Umeda et al. 2000	NA	AT2G27960.1
90	AtPLDζ1	AT3G16785	Ohashi et al. 2003	Aa_G51080,Aa_G97020	AT3G16785.1
91	SIAMESE (SIM)	AT5G04470	Kasili et al. 2010	Aa_G73130	AT5G04470.1
92	CYCB1;2	AT5G06150	Pacheco-Escobedo et al. 2016	Aa_G216070,Aa_G570060,Aa_G570070	AT5G06150.1
93	CYCD3;1	AT4G34160	Dewitte et al. 2007	Aa_G97330	AT4G34160.1
94	HYPOCOTYL6 (HYP6)	AT3G20780	Ishida et al. 2008	Aa_G133270	AT3G20780.1
95	CONSTITUTIVE PATHOGEN RESPONSE5 (CPR5)	AT5G64930	Schellmann and Hulskamp 2005	Aa_G20740	AT5G64930.1

96	INHIBITOR/INTERACTOR OF CYCLIN-DEPENDENT KINASES/KIP-RELATED PROTEINS (ICK/KRP)	AT1G49620	Jakoby et al. 2006	Aa_G362410	AT1G49620.1
97	poc	AT2G42260	Wada et al. 2002	Aa_G230820	AT2G42260.1
98	rfi	unknown	Guimil and Dunand 2007	unknown	unknown
99	spindly (spy)	AT3G11540	Cui et al. 2014	Aa_G216050	AT3G11540.1
100	TUBULIN FOLDING COFACTOR (TFC)A	AT2G30410	Kirik et al. 2002	Aa_G257690	AT2G30410.1
101	TFCC	AT4G39920	Schellmann and Hulskamp 2005	Aa_G486330	AT4G39920.1
102	FASS/TONNEAU2	AT5G18580	Camilleri et al. 2002	Aa_G280280	AT5G18580.1
103	SPIKE	AT1G13870	Bancroft et al. 1993	Aa_G569530	AT1G13870.1
104	ZWICHEL (ZWI)	AT5G65930	Oppenheimer et al. 1997	Aa_G113650	AT5G65930.3
105	STICHEL (STI)	AT2G02480	Ilgenfritz et al., 2003	Aa_G435350	AT2G02480.1
106	GLASSY HAIR (GLH)	AT1G25540	Kulich et al. 2015	Aa_G596310	AT1G25540.1
107	chardonnay (CDO)	AT4G31390	Oppenheimer 1998	Aa_G399020	AT4G31390.1
108	STACHEL (STA)	unknown	Folkers et al. 1997	unknown	unknown
109	ITB	AT2G38440	Zhang et al. 2005	Aa_G402790	AT2G38440.1
110	NOECK	AT3G01140	Jakoby et al. 2008	Aa_G14650	AT3G01140.1
111	DIS1	AT1G13180	Mathur et al. 2003a	Aa_G584930	AT1G13180.1
112	DIS2	AT1G30825	Zhang et al. 2005	Aa_G216410	AT1G30825.1
113	DIS3	AT2G38440	Guimil and Dunand 2007	Aa_G402790	AT2G38440.1
114	ALIEN	unknown	Mathur et al. 2003a	unknown	unknown
115	CROOKED	AT4G01710	Mathur et al. 2003b	Aa_G37300	AT4G01710.1
116	GNARLED	AT2G35110	Guimil and Dunand 2007	Aa_G344510	AT2G35110.1
117	KLUNKER	AT5G18410	Mathur 2005	Aa_G655880	AT5G18410.1
118	BRICK1	AT2G22640	Djakovic et al. 2006	NA	AT2G22640.1
119	SPIRRIG	AT1G03060	Saedler et al. 2009	Aa_G180060,Aa_G228370	AT1G03060.1
120	QRT2	AT3G07970	Rui et al. 2017	Aa_G258660	AT3G07970.1
121	XRI1	AT5G48720	Šimková et al. 2012	Aa_G139060	AT5G48720.2
122	RPT5B	AT1G09100	Ramon et al. 2008	Aa_G50940	AT1G09100.1,AT3G05530.1
123	RPT5A	AT3G05530	Eshraghi et al. 2014	Aa_G50940	AT1G09100.1,AT3G05530.1
124	URH1	AT2G36310	Kuroha et al. 2009	Aa_G282280	AT2G36310.1
125	ATMGT9	AT5G64560	Guo et al. 2015	Aa_G21130,Aa_G309270	AT5G64560.1
126	DUO3	AT1G64570	Heydlauff and Groß-Hardt 2014	Aa_G683720	AT1G64570.1
127	REF3	AT2G30490	Tsai and Schmidt 2017	Aa_G552340	AT2G30490.1
128	MSG2	AT3G15540	Muto et al. 2007	Aa_G424060	AT3G15540.1
129	FAB1A	AT4G33240	Hirano et al. 2011	Aa_G214060,Aa_G288250,Aa_G367170,Aa_G56340	AT3G14270.1,AT4G33240.1
130	FAB1B	AT3G14270	Hirano et al. 2011	Aa_G214060,Aa_G288250,Aa_G367170,Aa_G56340	AT3G14270.1,AT4G33240.1
131	FFT	AT4G25640	Thompson et al. 2009	Aa_G29660,Aa_G299940	AT4G25640.2
132	CALS5	AT2G13680	Wang et al. 2011	Aa_G105250,Aa_G132950,Aa_G154650,Aa_G155200,Aa_G159720,Aa_G22770,Aa_G340260,Aa_G373290,Aa_G647680,Aa_G78770,Aa_G79390,Aa_G350910	AT2G13680.1,AT3G07160.1
133	CALS9	AT3G07160	O'Lexy et al. 2018	Aa_G105250,Aa_G132950,Aa_G154650,Aa_G155200,Aa_G159720,Aa_G22770,Aa_G340260,Aa_G373290,Aa_G647680,Aa_G78770,Aa_G79390,Aa_G350910	AT2G13680.1,AT3G07160.1
134	PDAT1	AT5G13640	(Zhang M et al., 2009)	Aa_G209420,Aa_G611350	AT5G13640.1
135	DGAT1	AT2G19450	(Zhang M et al., 2009)	Aa_G143440	AT2G19450.1
136	LTP5	AT3G51600	(Chae K et al ., 2009)	Aa_G122970	AT3G51600.1
137	GAPCP-2	AT1G16300	(Muñoz-Bertomeu J et al ., 2009)	Aa_G217200,Aa_G563200	AT1G16300.1
138	ATUGP1	AT3G03250	(Park JI et al., 2010)	Aa_G267910,Aa_G56660	AT3G03250.1,AT5G17310.2
139	ATUGP2	AT5G17310	(Park JI et al., 2010)	Aa_G267910,Aa_G56660	AT3G03250.1,AT5G17310.2
140	RMF	AT3G61730	(Kim OK et al.,2010)	Aa_G33500	AT3G61730.1
141	FKP1	AT4G11820	(Ishiguro S et al., 2010)	Aa_G546090	AT4G11820.2
142	S6K1	AT3G08730	(Henriques R et al., 2010)	Aa_G24350,Aa_G271800	AT3G08730.1
143	CAM2	AT2G41110	(Landoni M et al., 2010)	NA_in_wb_table	unknown
144	PIRL1	AT5G05850	(Forsthoefel N R et al., 2010)	Aa_G285230,Aa_G546990	AT3G11330.1,AT5G05850.1
145	PIRL9	AT3G11330	(Forsthoefel N R et al., 2010)	Aa_G285230,Aa_G546990	AT3G11330.1,AT5G05850.1
146	IPMDH2 & IPMDH3	AT1G80560	(He Y et al., 2010)	Aa_G21440,Aa_G72800,Aa_G819160	AT1G80560.1
147	PGM2	AT1G70730	(Egli B et al., 2010)	Aa_G163410,Aa_G348270,Aa_G62630	AT1G23190.1,AT1G70730.3
148	PGM3	AT1G23190	(Egli B et al., 2010)	Aa_G163410,Aa_G348270,Aa_G62630	AT1G23190.1,AT1G70730.3
149	DRP2A	AT1G10290	(Backues SK et al.,2010)	Aa_G307870,Aa_G446410	AT1G10290.1,AT1G59610.1
150	DRP2B	AT1G59610	(Backues SK et al.,2010)	Aa_G307870,Aa_G446410	AT1G10290.1,AT1G59610.1
151	LOX3	AT1G17420	(Caldelari D et al.,2010)	Aa_G161200,Aa_G187120,Aa_G188700,Aa_G261750,Aa_G49590,Aa_G7180,Aa_G96590,Aa_G96620	AT1G17420.1,AT1G72520.1
152	LOX4	AT1G72520	(Caldelari D et al.,2010)	Aa_G161200,Aa_G187120,Aa_G188700,Aa_G261750,Aa_G49590,Aa_G7180,Aa_G96590,Aa_G96620	AT1G17420.1,AT1G72520.1
153	PIRL1	AT5G05850	(Forsthoefel NR et al., 2010)	Aa_G285230,Aa_G546990	AT3G11330.1,AT5G05850.1
154	PIRL9	AT3G11330	(Forsthoefel NR et al., 2010)	Aa_G285230,Aa_G546990	AT3G11330.1,AT5G05850.1
155	TIP5;1	AT3G47440	(Soto G et al., 2010)	Aa_G207430	AT3G47440.1

156	TiIP1;3	AT4G01470	(Soto G et al., 2010)	Aa_G347450	AT4G01470.1
157	PIP5K6	AT3G07960	(Zhao Y et al., 2010)	Aa_G121550,Aa_G258650,Aa_G272190,Aa_G324480,Aa_G371790, Aa_G483550,Aa_G88430,Aa_G43410,Aa_G549640	AT3G07960.1
158	LAP6	AT1G02050	(Kim SS et al., 2010)	Aa_G106130,Aa_G136110,Aa_G385130	AT1G02050.1
159	TKPR2	AT1G68540	(Grienenberger E et al., 2010)	Aa_G110410	AT1G68540.1
160	SEC22	AT1G11890	(El-Kasmi F et al., 2011)	Aa_G77460	AT1G11890.1
161	CHX21	AT2G31910	(Lu Y et al ., 2011)	Aa_G105290,Aa_G135500,Aa_G154710,Aa_G201100,Aa_G394500, Aa_G417370,Aa_G682960,Aa_G79420	AT2G31910.1
162	ATPS1	AT1G34355	(De Storme N et al., 2011)	Aa_G511210	AT1G34355.1
163	ATRABD2B	AT5G47200	(Peng J et al., 2011)	Aa_G352350	AT5G47200.1
164	ATRABD2C	AT4G17530	(Peng J et al., 2011)	Aa_G607290	AT4G17530.1
165	MGP4	AT4G01220	(Liu XL et al.,2011)	Aa_G232870,Aa_G53300,Aa_G46740	AT4G01220.1
166	eif3e/eif3h	AT2G39990	(Roy B et al., 2011)	Aa_G292270	AT2G39990.1
167	pad2-1	AT4G23100	(Zechmann B et al., 2011)	Aa_G301650,Aa_G301660,Aa_G301680,Aa_G301690	AT4G23100.1
168	POP2	AT3G22200	(Renault H et al., 2011)	Aa_G571290	AT3G22200.2
169	QRT1	AT5G55590	(Albert B et al., 2011)	Aa_G48490	AT5G55590.1
170	DPD1	AT5G26940	(Matsushima R et al., 2011)	Aa_G146620	AT5G26940.1
171	PSS1	AT1G15110	(Yamaoka Y et al., 2011)	Aa_G121050	AT1G15110.2
172	mpk4	AT4G01370	(Zeng Q et al., 2011)	Aa_G105750,Aa_G16900,Aa_G341460,Aa_G481470,Aa_G53210,Aa _G207660,Aa_G445430	AT4G01370.1
173	ACT12	AT3G46520	(Huang et al.1996)	Aa_G152880,Aa_G27970,Aa_G367680,Aa_G429520,Aa_G5290,Aa_ G537640,Aa_G576740,Aa_G67360,Aa_G99370	AT3G46520.1
174	ADF11	AT1G01750	(Ruzicka et al.2007)	NA	AT1G01750.1
175	ADF8	AT4G00680	(Ruzicka et al.2007)	Aa_G425530	AT4G00680.1
176	AGL18	AT3G57390	(Verelst et al.2007)	Aa_G461180	AT3G57390.1
177	AHA3	AT5G57350	(Robertson et al.2004)	Aa_G157270,Aa_G175370,Aa_G190130,Aa_G194510,Aa_G21590,A a_G241680,Aa_G307400,Aa_G409510,Aa_G41590,Aa_G490420,Aa_ G496820,Aa_G620370,Aa_G77390	AT5G57350.1
178	AMC/PEX13	AT3G07560	(Boisson-Dernier et al.2008)	NA	AT3G07560.1

Table S8. A selection of genes involved in the root hair development in *A. thaliana*. *A. thaliana* gene isoforms and orthologs in *A. alpina* are provided by Dr. Hequan Sun.

			sequenced genes via sanger sequencing		
			AaTTG1	AaGL1	AaGL3
more hairs	pep1-1	1264	Aattg1-3		
	pep1-1	788	not Aattg1	not Aagl1	Aagl3-3
	Pajares	65			
	Pajares	149			
	pep1-1	1159			
	pep1-1	1611			
	pep1-1	1839			
			AaCPC1	AaCPC2	
less hairs	pep1-1	1101	not Aacpc1	not Aacpc2	
	pj	1176	not Aacpc1	not Aacpc2	
	pep1-1	616			
	pj	1379	not Aacpc1	not Aacpc2	
	pj	1304			
	pep1-1	1034	not Aacpc1	not Aacpc2	
			AaRHD2		
only bulge	pj	1062	not Aarhd2		
	pep1-1	366			
	pep1-1	1276			
			AaRHD1	AaTIP1	
swollen	pj	1221	not Aarhd1		
	pep1-1	1127			
	pep1-1	433			
	pep1-1	503	not Aarhd1	not Aatip1	
	pep1-1	1186			
	pep1-1	235/244			
burst	pep1-1	1185			
	pj	1308			
	pep1-1	1085			
			AaTRH1	AaSCN1	
multiple hairs	pj	1384	not Aatrh1	Aascn1-1	

			AaRHD2		
short	pep1-1	397	not Aarhd2		
	pep1-1	223			
	pep1-1	381	not Aarhd2		
	pj	1245			
	pep1-1	1250			
	pep1-1	395	not Aarhd2		
	pep1-1	512			
	pep1-1	552			
	pep1-1	101			
	pep1-1	1070			
	pep1-1	1137			
	pep1-1	960			
	pep1-1	1051/1091			
	pep1-1	1390			
	pep1-1	1397			
	pj	1193/1183	not Aarhd2		
branched	pj	1725			
	pep1-1	216			
	pep1-1	197			

Table S9. Result of sequenced candidate genes in selected root hair mutants via Sanger sequencing.

phenotype			affected gene	mutation found
more hairs	pep1-1	1264	Aattg1-3	G (45) to D
	pep1-1	788	Aagl3-3	W (27) to *
	Pajares	65	Aagl3-4	SSC, exon6, G (3642) to A
	Pajares	149	Aagl3-5	Q (370) to *
	pep1-1	1159	Aagl3-6	S (123) to F
	pep1-1	1611	Aagl3-7	W (116) to *
	pep1-1	1839	Aagl3-8	D (265) to N
less hairs	pep1-1	1101		
	pj	1176		
	pep1-1	616		
	pj	1379		
	pj	1304		
	pep1-1	1034		
only bulge	pj	1062		
	pep1-1	366	Aacap1	E (465) to K
	pep1-1	1276	Aalrx2	P (588) to T
swollen	pj	1221		
	pep1-1	1127		
	pep1-1	433		
	pep1-1	503		
	pep1-1	1186		
	pep1-1	235/244		
burst	pep1-1	1185	Aakjk-1	T (854) to M
	pj	1308	Aakjk-2	G (248) to R
	pep1-1	1085	Aakjk-3	W (843) to *
multiple hairs	pj	1384	Aascn1-1	Q (46) to *
short	pep1-1	397	Aakeule-1	G (487) to R
	pep1-1	223	Aaact8-1	D (159) to N
	pep1-1	381	Aaact8-2	T (204) to I
	pj	1245	Aathe1-1	G (614) to R
	pep1-1	1250	Aaanx1-1	D (813) to N
	pep1-1	395		
	pep1-1	512		
	pep1-1	552	Aacsld6-1	E (289) to K
	pep1-1	101	Aapfn1-1	G (64) to E
	pep1-1	1070		
	pep1-1	1137	Aaspirrig-5	Q (2107) to *
	pep1-1	960	Aaspirrig-6	Q (1976) to *
	pep1-1	1051/1091	Aacow1-1	Q (505) to *
	pep1-1	1390	Aacow1-2	SSC, exon6, G(1849) to A
	pep1-1	1397	Aacow1-3	P (293) to L
	pj	1193/1183	Aacow1-4	SSC, exon5, G(1679) to A
branched	pj	1725	Aaark1-1	W (979) to *
	pep1-1	216	Aaark1-2	Q (899) to *
	pep1-1	197		

Table S10. Identified root hair mutant specific alleles.

AT3G02980	AT3G02990	/	AT3G03010	///	AtKJK (AT3G03050)		AT3G03060	AT3G03070	AT3G03080
Aa_G111880	Aa_G111870		Aa_G111860	Aa_G111790	AaKJK (Aa_G111780)		Aa_G111770	Aa_G111760	Aa_G111750
				Aa_G111800					
				Aa_G111810					
				Aa_G111820					
				Aa_G111830					
				Aa_G111840					
				Aa_G111850					
AT4G34555	AT4G34560		AT4G34570		AtCOW1 (AT4G34580)	/	AT4G34590	AT4G34600	AT4G34610
Aa_G614650	Aa_G614640	Aa_G547730	Aa_G547720	Aa_G547710	AaCOW1 (Aa_G547700)		Aa_G547690	Aa_G503090	Aa_G503100
AT3G14040	AT3G07860	AT3G07870		AtSCN1 (AT3G07880)		AT3G07890	AT3G07900	AT3G07910	
Aa_G767420	Aa_G767410	Aa_G765540		AaSCN1 (Aa_G258560)		Aa_G258570	Aa_G258580	Aa_G258590	
AT1G66100	AT1G03050	AT1G03055		AtSPIRRIG (AT1G03060)		AT1G03070	AT1G03080	AT1G03090	
Aa_G228400	Aa_G228390	Aa_G228380		AaSPIRRIG (Aa_G228370)		Aa_G228360	Aa_G228350	Aa_G228340	Aa_G228330
AT3G54840	AT3G54850	AT3G54860		AtARK1 (AT3G54870)		AT3G54880	AT3G54890	AT3G54900	
Aa_G381350	Aa_G381360	Aa_G381370		AaARK1 (Aa_G381380)		Aa_G381390	Aa_G381400	Aa_G565560	

Table S11. Synteny for root hair morphology genes. The left and right borders correspond to the flanking genes and present synteny. Gray boxes = homologous to *A. thaliana*, green boxes = non homologous to *A. thaliana*, black boxes = new gene in *A. alpina* and / = *A. thaliana* gene absent in *A. alpina*.

AtPP2A		Ct 1	Ct2	Ct 3	Average Ct	SD
6A	6A_3	25.121	24.934	24.831	24.96	0.15
	6A_4	24.616	24.720	24.583	24.64	0.07
	6A_5	23.987	23.986	24.011	23.99	0.01
1	1_4	24.741	24.778	24.689	24.74	0.04
	1_5	24.102	23.988	24.126	24.07	0.07
	1_6	26.012	26.250	25.934	26.07	0.16
2	2_3	24.413	24.575	24.294	24.43	0.14
	2_4	26.126	26.222	26.103	26.15	0.06
	2_5	24.928	25.067	24.960	24.99	0.07

AaSCN1-3		Ct 1	Ct2	Ct 3	Average Ct	SD	ΔCt	SD		$\Delta\Delta$Ct	2^-$\Delta\Delta$Ct	Ave. fold change	SEM	t-test	
6A	6A_3	26.740	26.741	26.682	26.72	0.03	1.76	0.15	0.27	1.49	0.3565	2.53	2.15		
	6A_4	22.081	22.048	22.294	22.14	0.13	-2.50	0.15		-2.77	6.8194				
	6A_5	25.668	25.451	25.522	25.55	0.11	1.55	0.11		1.28	0.4113				
1	1_4	23.839	23.574	23.616	23.68	0.14	-1.06	0.15		-1.33	2.5149	1.65	0.44	0.71	ns
	1_5	23.985	23.789	23.866	23.88	0.10	-0.19	0.12		-0.46	1.3783				
	1_6	26.038	25.972	26.721	26.24	0.41	0.18	0.45		-0.09	1.0662				
2	2_3	22.653	22.676	22.628	22.65	0.02	-1.77	0.14		-2.05	4.1292	5.30	1.36	0.34	ns
	2_4	24.588	24.436	24.513	24.51	0.08	-1.64	0.10		-1.91	3.7543				
	2_5	22.371	22.130	22.256	22.25	0.12	-2.73	0.14		-3.00	8.0210				

Table S12. qRTPCR result of 35S::*AaSCN1*-CDS in *Atscn1-3*. 6A, presents line expressing *AaSCN1*-CDS wild type copy. 1 and 2, preset lines expressing *Aascn1*-CDS mutated version.

AaRAN3		Ct 1	Ct2	Ct 3	Average Ct	SD													
Aattg1-2	1577_1	29.090	29.155	28.976	29.07	0.09													
	1577_2	29.552	29.342	29.468	29.45	0.11													
	1577_3	27.075	27.214	27.252	27.18	0.09													
35S AaGL3 in Aattg1-2	1_1	29.432	29.714	29.501	29.55	0.15													
	1_2	30.777	30.427	30.794	30.67	0.21													
	1_3	31.095	30.792	30.520	30.80	0.29													
AaGL3: DC 167/168		Ct 1	Ct2	Ct 3	Average Ct	SD	ΔCt	SD		$\Delta\Delta$Ct	$2^{\wedge}\Delta\Delta$Ct	Ave. fold change	SEM	Fold change ranks	Sum of ranks	Count	U statistic	Critical value	Significant
Aattg1-2	1577_1	39.054	37.772	35.252	37.36	1.93	8.29	1.94	7.20	1.09	0.47	1.13	0.34	1	4	3	-2	0	*
	1577_2	35.381	35.800	36.765	35.98	0.71	6.53	0.72		-0.67	1.59			3					
	1577_3	33.239	34.879	33.748	33.96	0.84	6.77	0.84		-0.42	1.34			2					
35S AaGL3 in Aattg1-2	1_1	33.835	35.135	36.506	35.16	1.34	5.61	1.34		-1.59	3.00	8.06	4.55	4	15	3	9		ns
	1_2	35.894	Undeterm	35.810	35.85	0.06	5.19	0.22		-2.01	4.03			5					
	1_3	33.722	33.899	34.074	33.90	0.18	3.10	0.34		-4.10	17.15			6					

Table S13. qRTPCR result of 35S::*AaGL3*-CDS in *Aattg1-2* (Chopra et al., 2014). *AaGL3* is 8 fold more expressed in 35S::*AaGL3* in *Aattg1* compared to the background.

AaRAN3		Ct 1	Ct2	Ct 3	Average Ct	SD													
Aattg1-2	1577_1	29.090	29.155	28.976	29.07	0.09													
	1577_2	29.552	29.342	29.468	29.45	0.11													
	1577_3	27.075	27.214	27.252	27.18	0.09													
pep1	pep_1	28.038	27.961	27.712	27.90	0.17													
	pep_2	28.272	28.424	28.519	28.40	0.12													
	pep_3	28.403	28.774	28.191	28.46	0.30													
AaGL3: DC 167/168		Ct 1	Ct2	Ct 3	Average Ct	SD	ΔCt	SD		$\Delta\Delta$Ct	$2^{\wedge}\Delta\Delta$Ct	Ave. fold change	SEM	Fold change ranks	Sum of ranks	Count	U statistic	Critical value	Significant
Aattg1-2	1577_1	39.054	37.772	35.252	37.36	1.93	8.29	1.94	7.20	1.09	0.47	1.13	0.34	2	7	3	1	0	ns
	1577_2	35.381	35.800	36.765	35.98	0.71	6.53	0.72		-0.67	1.59			5					
	1577_3	33.239	34.879	33.748	33.96	0.84	6.77	0.84		-0.42	1.34			3					
pep1	pep_1	34.739	33.910	35.187	34.61	0.65	6.71	0.67		-0.49	1.40	1.13	0.41	4	11	3	5		ns
	pep_2	34.853	34.968	34.762	34.86	0.10	6.46	0.16		-0.74	1.67			6					
	pep_3	35.013	39.649	37.216	37.29	2.32	8.84	2.34		1.64	0.32			1					

Table S14. *GL3* expression in *ttg1* mutant is the same as wild type in *A. alpina*. Aattg1-2 (Chopra et al., 2014) is used. The wild type background is *pep1-1*.

AaRAN3		Ct 1	Ct2	Ct 3	Average Ct	SD
1794	1794_1	28.883	29.833	28.667	29.13	0.62
	1794_2	29.498	29.172	28.726	29.13	0.39
	1794_3	28.006	28.666	28.777	28.48	0.42
35S::AaGL2	11_1	33.333	Undeterm	34.200	33.77	0.61
	11_2	30.971	30.630	31.122	30.91	0.25
	11_3	Undeterm	31.832	33.811	32.82	1.40

AaGL2_JS 23/24		Ct 1	Ct2	Ct 3	Average Ct	SD	ΔCt	SD		$\Delta\Delta$Ct	2^-$\Delta\Delta$Ct	Ave. fold change	SEM	Fold change ranks	Sum of ranks	Count	U statistic	Critical value	Significant
1794	1794_1	33.454	38.501	Undeterm	35.98	3.57	6.85	3.62	5.15	1.70	0.31	1.47	0.80	1	6	3	0	0	*
	1794_2	32.857	35.469	Undeterm	34.16	1.85	5.03	1.89		-0.11	1.08			2					
	1794_3	31.958	32.602	31.557	32.04	0.53	3.56	0.67		-1.59	3.01			3					
35S::AaGL2	11_1	Undeterm	33.700	31.001	32.35	1.91	-1.42	2.00		-6.56	94.45	38.34	28.24	6	15	3	9		ns
	11_2	Undeterm	Undeterm	33.826	33.83	#DIV/0!	2.92	#DIV/0!		-2.23	4.68			4					
	11_3	33.940	33.960	34.034	33.98	0.05	1.16	1.40		-3.99	15.88			5					

Table S15. qRTPCR result of 35S::*AaGL2*-CDS in *Aagl2-1*. *AaGL2* is 8 fold more expressed in 35S::*AaGL2* compared to the mutant background.

AaRAN3		Ct 1	Ct2	Ct 3	Average Ct	SD
1794	1794_1	28.883	29.833	28.667	29.13	0.62
	1794_2	29.498	29.172	28.726	29.13	0.39
	1794_3	28.006	28.666	28.777	28.48	0.42
pep1	pep_1	26.819	27.240	27.076	27.05	0.21
	pep_2	28.650	27.507	27.542	27.90	0.65
	pep_3	28.218	28.288	27.602	28.04	0.38

AaGL2_Patrizia		Ct 1	Ct2	Ct 3	Average Ct	SD	ΔCt	SD		$\Delta\Delta$Ct	2^-$\Delta\Delta$Ct	Ave. fold change	SEM	Fold change ranks	Sum of ranks	Count	U statistic	Critical value	Significant
1794	1794_1	33.454	38.501	Undeterm	35.98	3.57	6.85	3.62	5.15	1.70	0.31	1.47	0.80	1	9	3	3	0	ns
	1794_2	32.857	35.469	Undeterm	34.16	1.85	5.03	1.89		-0.11	1.08			2					
	1794_3	31.958	32.602	31.557	32.04	0.53	3.56	0.67		-1.59	3.01			6					
pep1	pep_1	32.668	31.782	30.681	31.71	1.00	4.67	1.02		-0.48	1.40	1.74	0.19	3	12	3	6		ns
	pep_2	31.242	33.497	31.333	32.02	1.28	4.12	1.43		-1.02	2.03			5					
	pep_3	32.310	32.950	31.756	32.34	0.60	4.30	0.71		-0.84	1.79			4					

Table S16. *GL2* expression in *gl2* mutant is the same as wild type in *A. alpina*. The wild type background is *pep1-1*.

	pep1	Aagl3_1 1422/1822	Aagl3_2 1191/1911	Aagl3_3 788	Aagl3_6 1159	Aagl3_7 1611	Aattg1_3 1264	Aagl2_1 1794/1517
1	69	70	82	39	78	46	82	59
2	43	81	66	51	52	45	77	60
3	31	82	76	41	40	41	110	127
4	39	62	100	43	48	51	103	60
5	27	86	95	46	86	41	88	47
6	31	114	90	63	63	40	55	50
7	33	92	70	64	60	39	41	66
8	40	86	32	43	59	41	64	47
9	42	77	60	51	42	44	69	56
10	34	94	45	84	49	38	66	32
11	25	43	63	44		42	86	44
12	34	50	36	59		54	69	43
13	53	64	49	33		38	63	52
14	41	59	59	48		46	54	61
15	42	45	56	43		41	71	44
16	30	48	62	34		46	94	60
17	50	52	48	45		55	74	41
18	41	49	72	64		42	49	44
19	36	59	66			40	57	46
20	23	61	50			43	53	66
21	26	50	49			42	24	38
22		50	49			29	60	51
23		42	46			65	76	67
24		48	68			49		41
25		57	57			50		46
Ave	37.62	64.84	61.84	49.72	57.70	44.32	68.91	53.92
SD	10.70	19.17	17.31	12.77	14.94	7.03	19.78	17.94
t-tes		0.00	0.00	0.00	0.00	0.01	0.00	0.00
		***	***	***	***	*	***	***

Table S17. Root hair counting result in patterning mutants in *A. alpina*. The wild type background is *pep1-1*. *Aattg1*, *Aagl3* and *Aagl2* mutants have significantly higher number of the root hairs compared to the wild type. N=10 to N=25.

Detailed name	Sequence 5' -> 3'	Reference
AaTTG1_FW_out	CAAATGTATGGACCGAATTATCAAG	designed by Divykriti Chopra
AaTTG1_RV_out	GCAATCAAGAATCTCTAGAACCAAG	designed by Divykriti Chopra
AaGL1_FW_out	TACAAACACACACTAGTGACAC	designed by Divykriti Chopra
AaGL1_RV_out	GTAGTCGTAGTACATCGTATTC	designed by Divykriti Chopra
AaGL3_FW_out	CCTATTTCGGCTATAAATTCAA	designed by Divykriti Chopra
AaGL3_RV_out	TAGAAAACCTAACCGGGTAAA	designed by Divykriti Chopra
AaCPC1_FW_out	CCTCTTCTTTCATCACTCTT	designed by Heike Wolff
AaCPC1_RV_out	GCGGTTAATGGGATCAAAT	designed by Heike Wolff
AaCPC2_FW_out	CACGAATCATAGCTACCAC	
AaCPC2_RV_out	CTAGGGTTTGGTACAGTGA	
AaRHD2-FW-out	CCCTGGTATATAATCTCCCA	
AaRHD2-RV-out	CAACATCAACCATTTCCCC	
AaRHD1-FW-out	CCAGAAACGAAAATGTGAC	
AaRHD1-RV-out	GGCTTGATTTGGTCTCTAC	
AaTIP1-FW-out	TGGCGGTTAGATTCCTAGT	
AaTIP1-RV-out	GTGGAGGCATTTACGATA	
AaTRH1-FW-out	ccaagaaatacgattgggc	
AaTRH1-RV-out	gcaaattcgagaaagaggt	
AaSCN1-FW-out	ACAGTGAATTATCCGTGAG	
AaSCN1-RV-out	GTACGAGATGGTGTCATGA	
AaRHD2-FW-out	CCCTGGTATATAATCTCCCA	
AaRHD2-RV-out	CAACATCAACCATTTCCCC	
AaEGL3-amiR1-s I	gaTAATATGTTCGGTAGTGGCGActctcttttgtattcca	
AaEGL3-amiR1-a II	agTCGCCACTACCGAACATATTAtcaaagagaatcaatga	
AaEGL3-amiR1*s III	agTCACCACTACCGATCATATTTtcacaggtcgtgatatg	
AaEGL3-amiR1*a IV	gaAAATATGATCGGTAGTGGTGActacatatatattccta	
AaEGL3-amiR2-s I	gaTCAGACTAAGTAATACCGCTCctctcttttgtattcca	
AaEGL3-amiR2-a II	agGAGCGGTATTACTTAGTCTGAtcaaagagaatcaatga	
AaEGL3-amiR2*s III	agGAACGGTATTACTAAGTCTGTtcacaggtcgtgatatg	
AaEGL3-amiR2*a IV	gaACAGACTTAGTAATACCGTTCctacatatatattccta	
AaGL1-amiR3-s I	gaTTTGCGAAAATGACGTTGCGTctctcttttgtattcca	
AaGL1-amiR3-a II	agACGCAACGTCATTTTCGCAAAtcaaagagaatcaatga	
AaGL1-amiR3*s III	agACACAACGTCATTATCGCAATtcacaggtcgtgatatg	
AaGL1-amiR3*a IV	gaATTGCGATAATGACGTTGTGTctacatatatattccta	
AaGL1-amiR8-s I	gaTTGATACGAAGTTCCTGCCTGctctcttttgtattcca	
AaGL1-amiR8-a II	agCAGGCAGGAACTTCGTATCAAtcaaagagaatcaatga	
AaGL1-amiR8*s III	agCAAGCAGGAACTTGGTATCATtcacaggtcgtgatatg	
AaGL1-amiR8*a IV	gaATGATACCAAGTTCCTGCTTGctacatatatattccta	
AaGL2-amiR6-s I	gaTCAATTGAATGGCACTATCGTctctcttttgtattcca	
AaGL2-amiR6-a II	agACGATAGTGCCATTCAATTGAtcaaagagaatcaatga	

126

AaGL2-amiR6*s III	agACAATAGTGCCATACAATTGTtcacaggtcgtgatatg	
AaGL2-amiR6*a IV	gaACAATTGTATGGCACTATTGTctacatatatattccta	
AaGL2-amiR14-s I	gaTCAATTGAATGGCACCCTCGActctcttttgtattcca	
AaGL2-amiR14-a II	agTCGAGGGTGCCATTCAATTGAtcaaagagaatcaatga	
AaGL2-amiR14*s III	agTCAAGGGTGCCATACAATTGTtcacaggtcgtgatatg	
AaGL2-amiR14*a IV	gaACAATTGTATGGCACCCTTGActacatatatattccta	
attB1-adapter	GGGGACAAGTTTGTACAAAAAAGCAGGCT	Received from Dr. Marc Jakoby
attB2-adapter	GGGGACCACTTTGTACAAGAAAGCTGGGT	Received from Dr. Marc Jakoby
Aascn1-3_SDM_FW	TGTTGGATCTTTAGGGAGATAAATGAGCGAAGCTTCTCT	
Aascn1-3_SDM_RV	AGAGAAGCTTCGCTCATTTATCTCCCTAAAGATCCAACA	
AaGL3-qPCR-FW	GGCAAAAAGTGCTGCCGTTAAG	
AaGL3-qPCR-RV	CTGTAGTACCGATCTCAACGAC	
AaGL2-qPCR-FW	GGAGAAAGAGCGTGCTGAAG	designed by Julian Schiffn
AaGL2-qPCR-RV	AACCAGAGGGAGGAAGAAGC	designed by Julian Schiffn
AaSCN1-qPCR-FW	TGGAAGGAACAGCTTCTTGGA	
AaSCN1-qPCR-RV	GGATTCCCATTCTCTGGAACC	
AaRAN3-qPCR-FW	CACAGGAAAAACCACATTCGT	designed by Divykriti Chopra
AaRAN3-qPCR-RV	CCATCCCTAAGACCACCAAAT	designed by Divykriti Chopra
AtPP2A-qPCR-FW	CAAGAGGTTCCACACGAAGGA	Frerigmann et al., 2016
AtPP2A-qPCR-RV	TGTAACCAGCACCACGAGGA	Frerigmann et al., 2016

Table S18. List of primers used in this study.

	amiRNA/sgRNA target site
amiR1-AaEGL3	TAATATGTTCGGTAGTGGCGA
amiR2-AaEGL3	TCAGACTAAGTAATACCGCTC
amiR3-AaGL1	TTTGCGAAAATGACGTTGCGT
amiR8-AaGL1	TTGATACGAAGTTCCTGCCTG
amiR5-AaCPC1	TATTGAAACTCCTAAGCGCTC
amiR9-AaCPC2	TTACCCCACGAAAACTCGCAA
amiR6-AaGL2	TCAATTGAATGGCACTATCGT
amiR14-AaGL2	TCAATTGAATGGCACCCTCGA
sgRNA-AaEGL3 (1)	CCGGTGGCTCTCAGGTCAGTAGA
sgRNA-AaEGL3 (2)	CCGCCGCCGCTCTATCCCCGGAG
sgRNA-AaGL1 (1)	tatgtccttactcacggcaaagg
sgRNA-AaGL1 (2)	ttgagccctaatgtgaagaaagg
sgRNA-AaCPC1	GGAAGCTGTGAATATGTCTGAGG
sgRNA-AaCPC2	CAGAGGTGACTAGTGTTGAGTGG

Table S19. Sequences of artificial microRNAs/sgRNA target sites used in this study. The PAM sequences are marked in red.

caaacacacgctcggacgcatattacacatgttcatacacttaatactcgctgttttgaattgatgtttttaggaatatatatgt

agGAACGCTTAGGAGATTCAATTtcacaggtcgtgatatgattcaattagcttccgactcattcatccaaataccga

gtcgccaaaattcaaactagactcgttaaatgaatgaatgatgcggtagacaaattggatcattgattctctttgaTATTG

AAACTCCTAAGCGCTCctctcttttgtattccaattttcttgattaatctttcctgcacaaaaacatgcttgatccactaa

gtgacatatatgctgccttcgtatatatagttctggtaaaattaacattttgggtttatctttatttaaggcatcgccatgGTT

GTTGTTGTTATGGTCTAGTTGTTGTTGTTATGGTCTAATTTAAATATGGTCTAAAGAAGAAGAAT

ATGGTCTAAAGAAGAAGAATcaaacacacgctcggacgcatattacacatgttcatacacttaatactcgctgtttt

gaattgatgtttttaggaatatatatgtagTTACGAGTTTTCGAGGGGTATtcacaggtcgtgatatgattcaattag

cttccgactcattcatccaaataccgagtcgccaaaattcaaactagactcgttaaatgaatgaatgatgcggtagacaaat

tggatcattgattctctttgaTTACCCCACGAAAACTCGCAActctcttttgtattccaattttcttgattaatctttcct

gcacaaaaacatgcttgatccactaagtgacatatatgctgccttcgtatatatagttctggtaaaattaacattttgggttta

tctttatttaaggcatcgccatg

Figure S1. Structure of artificial microRNA targeting *AaCP1* and *AaCPC2* simultaneously. Blue sequences present *amiRNA** and *amiRNA* for *AaCPC1* and *2* respectively. Yellow sequence is the 88 bp linker (Yan et al. 2012).

ACTAGTGAAGACAAACTAGCGGCCGCAGAATGATTAGGCATCGAACCTTCAAGAATTTGATTGAATAAAAC
ATCTTCATTCTTAAGATATGAAGATAATCTTCAAAAGGCCCCTGGGAATCTGAAAGAAGAGAAGCAGGCCC
ATTTATATGGGAAAGAACAATAGTATTTCTTATATAGGCCCATTTAAGTTGAAAACAATCTTCAAAAGTCCC
ACATCGCTTAGATAAGAAAACGAAGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGTG**GGAAGC**
TGTGAATATGTCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGCACCGAGTCGGTGCTTTTTTTCTAGACCCAGCTTTCTTGTACAAAGTTGGCATTACGCT**TTTTTTATT**
TTTGTTACATTTTCCACCTGTTTCTCTTCTTAAAACTTTTGTTTTCCCCAGGCGATAACGGTTTATGTTAGTTT
GAGAATGATTAGGCATCGAACCTTCAAGAATTTGATTGAATAAAACATCTTCATTCTTAAGATATGAAGATA
ATCTTCAAAAGGCCCCTGGGAATCTGAAAGAAGAGAAGCAGGCCCATTTATATGGGAAAGAACAATAGTA
TTTCTTATATAGGCCCATTTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGAAG
CTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGTG**CAGAGGTGACTAGTGTTGAG**GTTTTAGAGCTA
GAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTC
TAGACCCAGCTTTCTTGTACAAAGTTGGCATTACGCTGCGGCCGCTTACTTGTCTTCGGATCC

Figure S2. Structure of *U6p::sgRNA-AaCP1CPC2* cassette. Green sequences present *U6* promoter. Red sequences are target sequences for *AaCPC1* (first) and *AaCPC2* (second). Blue sequences present *U6 snRNA* (Hyun et al. 2015). Yellow sequence is an 84 bp linker (the sequence is provided by Dr. Mohsen Hajheidari).

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen –, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie –abgesehen von unten angegebenen Teilpublikationen –noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Martin Hülskamp betreut worden.

Teilpublikationen:

Die in Kapitel 3.1.3 dargelegten Daten wurden vor Abgabe dieser schriftlichen Arbeit zur Veröffentlichung als Wissenschaftsartikel eingereicht, welcher sich zurzeit noch im Review-Prozess befindet.

Köln, den 28. November 2018

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EDUCATION

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	Molecular Biology, PhD	
Sep 2004 - Mar 2007	**Tehran University**	**Tehran, Iran**
	Agriculture Biotechnology, MSc, GPA 17.81out of 20	
Sep 1999 - Jul 2003	**Shahed University**	**Tehran, Iran**
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RESEARCH PROJECTS

PhD Thesis   *Studying Trichome and root hair development in Arabis alpina*

MSc Thesis   *Studying transformation of chitinase and 1, 3-glucanase genes for improving resistance to verticilium in commercial cultivars of cotton **Score 19.76 out of 20***

MSc Seminar   *Application of Nanotechnology in Molecular Science of Agriculture, **Score 19 out of 20***

BSc Internship   *Preparation of genetic background for Iranian Olive cultivars, **Score 19.5 out of 20***

BSc Seminar   *Studying transformation of CryIAb gene for improving resistance to Heliothis obsolete in cotton, **Score 20 out of 20***

HONORS

IMPRS (International Max Planck Research School) fellowship for PhD education

Granted for free education in Master level at the excellence center of Tehran University

Granted for free education in Bachelor level at the excellence center of Shahed University

Ranked 1% top student (14 from 7563) among all attendance students in M.Sc. national exam (The number of accepted applicants: 70)

Ranked 1% top student (3329 from 398530) among all attendance students in B.Sc national Exam

132

WORK EXPRIENCES

| April 2014-June 2014 | **Max Planck Institute for Plant Breeding Research**   **Cologne, Germany** |

Training in the Molecular Genetics Lab

I have done all steps of molecular cloning to clone different fragments into a suitable binary vector, ready for plant transformation. I also did lots of plant transformation-floral dipping technique.

So I have a very good hand in the Lab works and techniques: digestion, band recovery, ligation, bacterial culture, plasmid extraction, chemical and electro bacterial transformation, PCR, primer design, bacterial cloning PCR …

Jun 2007 – March 2014   **Bonian Daneshpajouhan Institute**   **Tehran, Iran**

Biotechnology project manager

Conducted researches were mostly about applications of biotechnology in different industries

My main duties were carrying out field research about the problem of the client and make a definition for the project, prepare work break-down structure, conduct steps of timing project, resource allocation plan and project team building through negotiation with human resource of the company and driving of Project implementation. Finally, providing written reports for clients and company management

May 2004 - Jun 2007   **Agriculture Biotechnology Research**   **Karaj, Iran**
Institute of Iran (ABRII)

Research assistant in the Genomics and Tissue Culture Labs

I was mainly working on the production of transgenic cotton resistant to *Verticilium* wilt via *Agrobacterium* by using shoot apex and cotyledon pieces as our explants.

So I am completely familiar with the cell and tissue culture methods (embryogenesis and shoot apex), genetic transformation of plants, Hardening and survival of tissue culture plantlets under greenhouse conditions, Molecular analysis of transgenic plants, DNA Extraction, Southern blotting and Western blotting

OTHER CERTIFICATES

2004	**Certification as an assistant of Rice Transformation Workshop**	**Agricultural Biotechnology Research Institute of Iran**
2005	**Certification as a participant of 1st Iran-Russia Joint Seminar & Workshops on Nanotechnology**	**Iran University of Mines and Industry, Iran**
2005	**Certification as a participant of 4st National Biotechnology Congress of I. R. Iran**	**International Center for High Technology & Environmental Science**
2009	**Certification as a participant of 2nd National Congress on Application of Nanotechnology to Agriculture**	**Nano Technology Committee Ministry of jihad-e-agriculture**

PABLICATIONS

- Mapar, M. Ghasemzadeh, S. Salami, A. & Ghasemi, M. 2004. Bio safety of Transgenic Crops. 1st National Bio safety Congress. February 2004.
- Mapar, M. Abde Mishani, S. & Bihamta, M. R. 2009. Nanotechnology and Its Applications in Agriculture. The 6th National Biotechnology Congress of I. R. Iran. 13-15 August, 2009

LANGUAGES

- English   IELTS 7
- German   A2   4
- Persian (Farsi)   Native

IT SKILS

Windows & Office tools
- Computer Skills   Advanced

PERSONAL HOBBIES

- Painting – oil color and pastille
- Swimming
- Traveling – especially historical places