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# Characterization of the effects of auxin on root vascular development in *DRAMAQUEEN* mutant *Arabidopsis*

Metropolia University of Applied Sciences

Bachelor of Social Services and Healthcare

Degree program in biomedical laboratory science

Thesis

22.02.2024

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| Author | Liubov Maria Khaikin |
| Title | Characterization of the effects of auxin on root vascular development in *DRAMAQUEEN* mutant *Arabidopsis* |
| Number of pages | 28 |
| Date | 22.02.2024 |
| Degree | Bachelor of Social Services and Healthcare |
| Degree program | Degree program in biomedical laboratory science |
| Supervisor | Merja Ojala |

The vascular tissue is crucial for transporting water and nutrients between the different parts of the plant. It consists of two main tissues, xylem and phloem. Xylem consists of protoxylem and metaxylem, with protoxylem developing first in the newest parts of the plant. Vascular development is regulated by plant hormones, such as auxin, cytokinin and abscisic acid, which interact with each other. *DRAMAQUEEN (DRQ)* is an unknown gene in *Arabidopsis* and other plants that might be involved in root vascular development. A partially functional *DRQ* mutant, *mDRQ,* was previously found to affect the root elongation rate and protoxylem phenotype, as well as increase cytokinin sensitivity in *Arabidopsis*, suggesting its role as a possible cytokinin inhibitor. In this thesis, three knockout *DRQ* mutant lines are checked for auxin sensitivity as part of a bigger research project aiming to unravel the role of *DRQ* in vascular development. The root length and xylem phenotype are compared to the naturally occurring genotype Col0 (wild type), and a mutant of *AHP6*, another cytokinin inhibitor, which displays some similarity to the *mDRQ* phenotype. The xylem anatomy was analyzed in the old, intermediate and differentiate zones of the *Arabidopsis thaliana* root.

Statistically significant differences were found in the protoxylem anatomy of the old and intermediate parts of the root, suggesting that DRAMAQUEEN affects protoxylem differentiation and is mostly expressed closer to the root beginning. *DRQ* mutants also displayed shorter roots than Col0 in all auxin concentrations, suggesting a possible higher auxin sensitivity. However, the inhibitory effect of exogenous auxin on the root elongation was similar between Col0 and the *DRQ* mutants.

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| Keywords | *Arabidopsis*, xylem, auxin, cytokinin, vascular development |

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| Tekijä | Liubov Maria Khaikin |
| Otsikko | Auksiinin vaikutuksen karakterisointi *DRAMAQUEEN*-mutantti *Arabidopsiksen* vaskulaarisen järjestelmän kehityksessä |
| Sivumäärä | 28 |
| Aika | 22.02.2024 |
| Tutkinto | Bioanalyytikko (AMK) |
| Tutkinto-ohjelma | Bioanalyytikon tutkinto-ohjelma |
| Ohjaaja | Merja Ojala |

Vaskulaarinen systeemi, eli johtosolukko, on ratkaisevan tärkeä veden ja ravinteiden kuljettamisessa kasvin eri osien välillä. Se koostuu kahdesta pääkudoksesta, ksyleemistä ja floeemista. Ksyleemi koostuu protoksyleemistä ja metaksyleemistä, joista protoksyleemi kehittyy ensimmäisenä kasvin uusimmissa osissa. Johtosolukon kehitystä säätelevät kasvihormonit, kuten auksiini, sytokiniini ja abskissihappo, jotka ovat vuorovaikutuksessa kunkin kanssa. *DRAMAQUEEN* (*DRQ*) on tuntematon geeni *Arabidopsiksessa* ja muissa kasveissa, joka saattaa olla osallisena juurien johtosolukon kehityksessä. Osittain toiminnallisen *DRQ*-mutantin, *mDRQ*:n, havaittiin aiemmin vaikuttavan juuren pidentymiseen ja protoksyleemin fenotyyppiin, sekä lisäävän sytokiniiniherkkyyttä *Arabidopsiksessa*, mikä viittaa sen rooliin mahdollisena sytokiniinin estäjänä. Tässä opinnäytetyössä kolmen knock-out *DRQ*-mutanttilinjan auksiiniherkkyys tarkistettiin osana suurempaa tutkimusprojektia, jonka tavoitteena on selvittää *DRQ*:n roolia johtosolukon kehityksessä. Juuren pituutta ja ksyleemin fenotyyppiä verrataan luonnollisesti esiintyvään fenotyyppiin Col0:aan (villi tyyppi) ja *AHP6*:n mutanttiin. AHP6 on toinen sytokiniinin estäjä, jonka mutantilla on samankaltainen fenotyyppi kuin *mDRQ:*lla. Ksyleemin anatomiaa analysoitiin *Arabidopsis thaliana* -juuren vanha-, keski- ja erilaistumisalueilla.

Tilastollisesti merkitseviä eroja oli havaittu juuren vanhan ja keskiosan protoksyleemianatomiassa, mikä viittaa siihen, että DRAMAQUEEN vaikuttaa protoksyleemin erilaistumiseen ja ilmentyy enimmäkseen lähempänä juuren alkua. *DRQ*-mutanttien juuret olivat myös lyhyempiä kuin Col0 kaikissa auksiinipitoisuuksissa, mikä viittaa mahdolliseen korkeampaan auksiiniherkkyyteen verrattuna villityypin kasveihin. Eksogeenisen auksiinin estävä vaikutus juuren pidentymiseen oli kuitenkin samankaltainen Col0:n ja *DRQ*-mutanttien välillä.

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| Avainsanat | *Arabidopsis*, ksyleemi, auksiini, sytokiniini, vaskulaarinen kehitys |

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## Central terms and abbreviations

Abscisic Acid / ABA – plant hormone that plays an important role in adaptation to stressful environmental conditions.

ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 / AHP6 – a cytokinin signalling inhibitor.

Auxin / IAA – plant hormone involved in numerous processes related to growth and development of different parts of the plant.

Cambium – meristem for secondary development.

Col0 – wild type, a naturally occurring *Arabidopsis* genotype.

Cytokinin / CK – plant hormones involved in cell divisions.

*DRAMAQUEEN / DRQ* – an unknown gene that could be involved in plant vascular development.

Meristem – actively dividing part of the plant.

Metaxylem / Mx – a type of xylem tissue, develops later than protoxylem.

Phloem – vascular tissue conducting photosynthesis products.

Procambium – primary cambium.

Protoxylem / Px – one of the xylem tissues, develops before metaxylem in the new parts of the plant.

Xylem – vascular tissue conducting water, consists of protoxylem and metaxylem.

# 1 Introduction

Unlike most animals, that develop into a pre-determined body plan, plants have the ability to continuously grow and develop new organs (Bishopp et al. 2011). This plasticity and self-organization allows maximal adaptability to a changing environment. Plants have zones with high mitotic activity and a stem cell niche, called meristems (De Rybel et al. 2015). The process of new organ development and patterning from actively dividing meristems is still being studied (De Rybel et. al 2015 & Bishopp et al. 2011).

Although the *Arabidopsis* genome is fully sequenced, the function of many genes is still unknown (Koornneef & Meinke 2010). The plants' vascular tissue supports the plant physically and transports water, hormones and nutrients between the different parts of the plant, therefore its development was the key event that allowed plants to grow bigger than mosses, and populate environments other than water. The vascular system consists of two main tissues, xylem and phloem, that transport water and photosynthesis products, respectively. Xylem and phloem differentiate from cambial tissue, which contains vascular stem cells. (De Rybel et al. 2015.) *DRAMAQUEEN* (*DRQ*) is an unknown gene found in *Arabidopsis* and other plants that was discovered by a genetic screening and may be involved in the vascular tissue patterning (Help-Rinta-Rahko & Ruonala & Hellmann & Helariutta 2015).

With *Arabidopsis thaliana* as model organism, this project focuses on studying the effects of the plant hormone auxin in different concentrations on the root xylem tissue development in *DRQ* mutants, compared to the naturally occurring phenotype Col0, also referred to as "wild type". It is a part of a bigger research project, titled "DRAMAQUEEN: a novel vascular regulator in *Arabidopsis* plants", that aims to unravel the role of the *DRQ* gene.

The project was done in the University of Helsinki, faculty of biological and environmental sciences, wood development research group during spring 2023. The group focuses on understanding the processes involved in the patterning of plants' vascular network. Understanding the molecular processes involved in plant development could eventually contribute to enhanced crop production. (Helsinki University.)

# 2 Plant vascular system development

## 2.1 *Arabidopsis thaliana*

Despite being considered a weed, *Arabidopsis thaliana*, also known as thale cress and mouse-ear cress, has proved to be an ideal model organism for research in plant biology (Kimball n.d.a.). The history of *Arabidopsis* research dates back to the early 1900s, and it was first suggested as a model organism for genetic studies in 1943 (Koornneef & Meinke 2010). The qualities that make *A. thaliana* a good model organism include its small size that allows it to be grown in laboratories, rapid life cycle (5-6 weeks from seed germination to the production of new seeds) and effective reproduction through self-pollination that produces up to 10 000 seeds per plant. *A. thaliana* has a small genome of $135 \times 10^6$ bps of DNA in 5 chromosomes (2n = 10), a total of 27 407 genes, making it good for genetic studies. Production of mutations and transgenic plants is easy using mutagenic treatment on seeds and *Agrobacterium tumefaciens* as foreign gene vector, respectively. (Kimball n.d.a.) The *Arabidopsis* genome was fully sequenced by the year 2000, making genetic studies even easier (Koornneef & Meinke 2010).

Plants can be classified as herbaceous or woody. Woody plants have stems that live for several years, growing each year in both height and width. Herbaceous plants may live for one (annual), two (biennial) or more years (perennial), but their stem dies every year and grows anew. (Streich 2007.) As an annual plant, the short life cycle is an advantage of *Arabidopsis* over the tree species used in the research group (*Betula* and *Populus*), that can take several years to flower and enable crossings (Helsinki University).

Although *Arabidopsis* is a herbaceous plant, it has proved to be an excellent model for wood development too (Zhang & Elo & Helariutta 2011). Plant development can be divided into two separate growth stages, primary and secondary. During primary growth, plant tissues increase in length, an during secondary growth they increase in thickness. (Ye et al. 2021.) Xylem and phloem form from procambium during the primary growth. During secondary growth, the procambial cells divide and form the vascular cambium, a meristem for secondary development, that forms secondary phloem outwards and secondary xylem inwards. Wood is secondary xylem. Studies have uncovered the same mechanisms regulating the secondary growth in herbaceous and woody plants, making *Arabidopsis* suitable for studies of wood development. In *Arabidopsis* itself,

vascular secondary growth can only be observed in the older part of the root, the hypocotyl and inflorescence stem. The structure of the secondary tissues is similar to *Populus*. (Zhang et al. 2011.)

## 2.2 The vascular system of plants

The vascular tissues in plants transport water, nutrients, hormones and other molecules between the different parts of the plant. The vascular system contains two main tissues, xylem and phloem. Xylem transports water, while phloem transports hormones, photosynthesis metabolites and other molecules. (Augstein 2022; De Rybel et al. 2015.) Xylem consists of protoxylem (Px) and metaxylem (Mx). Generally, protoxylem develops first, and can later lose its functionality in some tissues and species, while in others it remains an important part to the plant's hydraulic function throughout its life (Rolland et al. 2015). The xylem's conductive cell type is called tracheary elements (TE) (De Rybel et al. 2015). Protoxylem's TE's function is transporting water in rapidly elongating tissues (Schuetz et al. 2014), whereas metaxylem contributes most to the water transport in older plants (Augstein 2022). Protoxylem and metaxylem usually have distinct locations in the root (figures 1 and 3), and can also be distinguished by their cell diameter and the characteristic pattern on their lignified secondary cell wall (SCW) (Augstein 2022; Růžička et al. 2015). Lignin is a phenolic organic polymer found in plant cell walls, that provides the transporting cells with stiffness and waterproofing, which allows the upward transportation of water (Britannica). The xylem SCW contains cellulose, hemicellulose and lignin, providing the SCW with strength, rigidity and impermeability to the polysaccharide components. At the same time, lignin is restricted to the SCW in order to preserve the plasticity of adjacent primary wall domains. (Schuetz et al. 2014.) The lignified secondary wall thickenings in protoxylem display an annular or helical pattern, whereas the metaxylem SCW has a pitted or reticulated lignification pattern. Metaxylem is also usually larger in diameter. (Augstein 2022; Růžička et al. 2015.) (Figure 3).

### 2.2.1 Root anatomy

Most modern plants have three main tissue types: the outer epidermis, the vascular tissues and the ground tissues. The vascular cylinder, also known as stele, is located in the center. The ground tissues are the tissues between the epidermis and the vascular tissue. (De Rybel et al. 2015.)

In a wild type *Arabidopsis* root, two marginally localized protoxylem poles, each consisting of a single cell, are connected by a metaxylem axis. It is surrounded by procambium - undifferentiated vascular tissue precursors. 90 degrees from the xylem axis, two phloem poles are located. This pattern of symmetry on two planes 90 degrees to each other is referred to as the bisymmetric pattern. (Bishopp et al. 2011.) (Figure 1).

Surrounding the vascular cylinder is the pericycle (Figure 1). It is a heterogeneous tissue, with cells adjacent to the xylem pole versus the phloem pole having differences in size, structure and gene expression. The pericycle is required for transportation of ions, such as boron, into the xylem (known as xylem loading), as well as the initiation of secondary roots, also called lateral roots (Figure 2). (Beeckman & De Smet 2014.)

The innermost cortical layer is the endodermis, surrounding the pericycle (Figure 1). The root endodermis controls the nutrient transport from the soil to the vascular tissue by forming diffusional barriers. It also prevents the backflow of nutrients. (Doblas et al. 2017.)

The cortex is located between the epidermis and the endodermis (Figure 1). One of its functions is mediating the transportation of water and nutrients absorbed through the epidermis into the vascular tissue. The cortex cells also store starch and secondary metabolites, such as latex, resin, essential oils and tannins. (Huijin et al. 2022.)

Finally, the outermost layer covering the plat is the epidermis (Figure 1). The epidermis has many functions, including mechanical protection from injury, infection and water loss, as well as absorption of water and nutrients and substance secretion (Britannica).

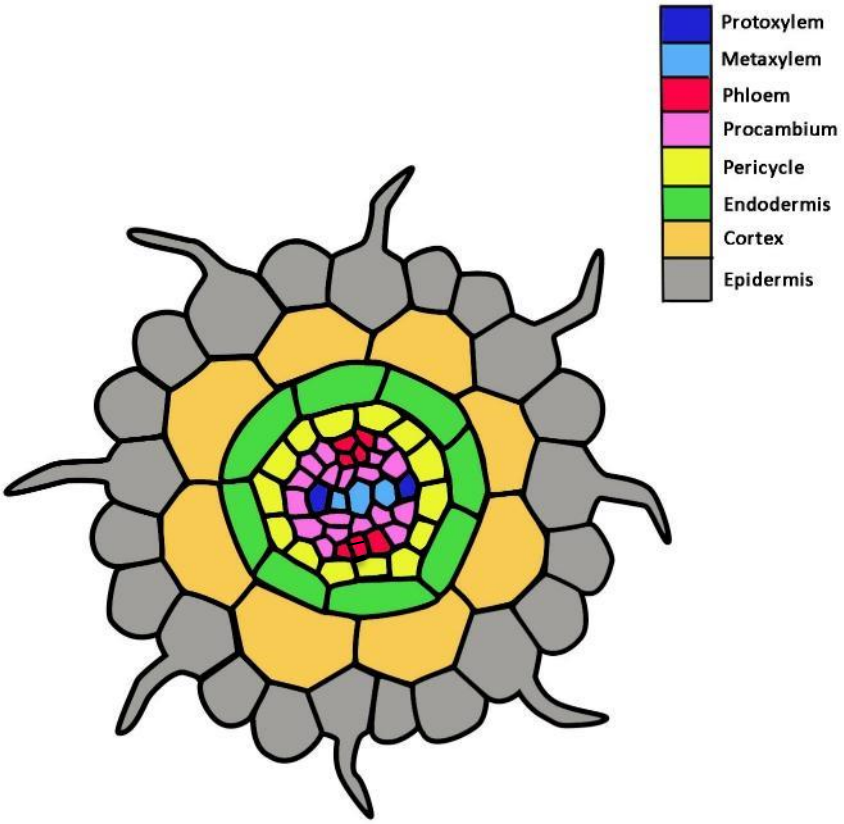


Figure 1. Illustration of an *Arabidopsis* root horizontal section. A central xylem axis is surrounded by procambium, with phloem poles on both sides in a bisymmetric pattern. Two protoxylem poles are connected by a metaxylem bridge, consisting of two cells of outer metaxylem and one inner metaxylem cell (central). Surrounding the vascular cylinder are the pericycle, endodermis, cortex and epidermis tissues. (Bouché.) Adapted from Bioicons.com.

### 2.2.2 Vascular tissue anatomy in old, intermediate and differentiate parts of the root

The root vascular system anatomy depends on the area of the root, as the different root areas display different levels of maturation. The most mature part of the root is located next to the transition to the stem (in young plants a primary stem is called hypocotyl). The root tip is the youngest part of the root. It includes the elongation zone, where cells grow in length, as well as a meristem, referred to as the root apical meristem, an actively diving part of the root. (Augstein 2022.) (Figure 2).

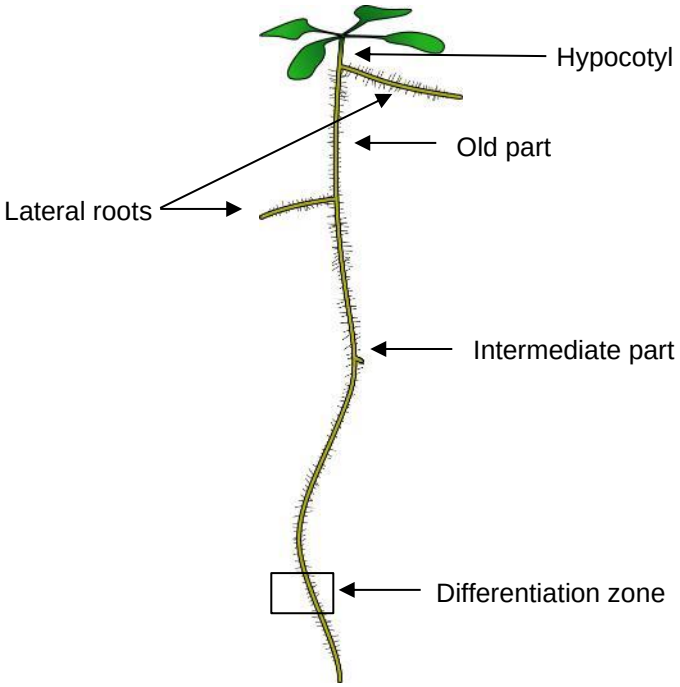


Figure 2. An illustration of a young *Arabidopsis* plant, with the approximate locations of the old, intermediate and differentiation zones. Two lateral roots can also be seen in the image. The hypocotyl is the primary stem connecting the root to the primary leaves, called cotyledons. (von Wangenheim.)

Two protoxylem poles begin to differentiate as the first water-conducting tissue in the differentiation zone close to the root tip. Then, two poles of outer metaxylem form, followed by an inner metaxylem pole that differentiates in the old part closer to the root beginning. The inner metaxylem is larger in diameter than the outer metaxylem. (Augstein 2022.) (Figure 3).

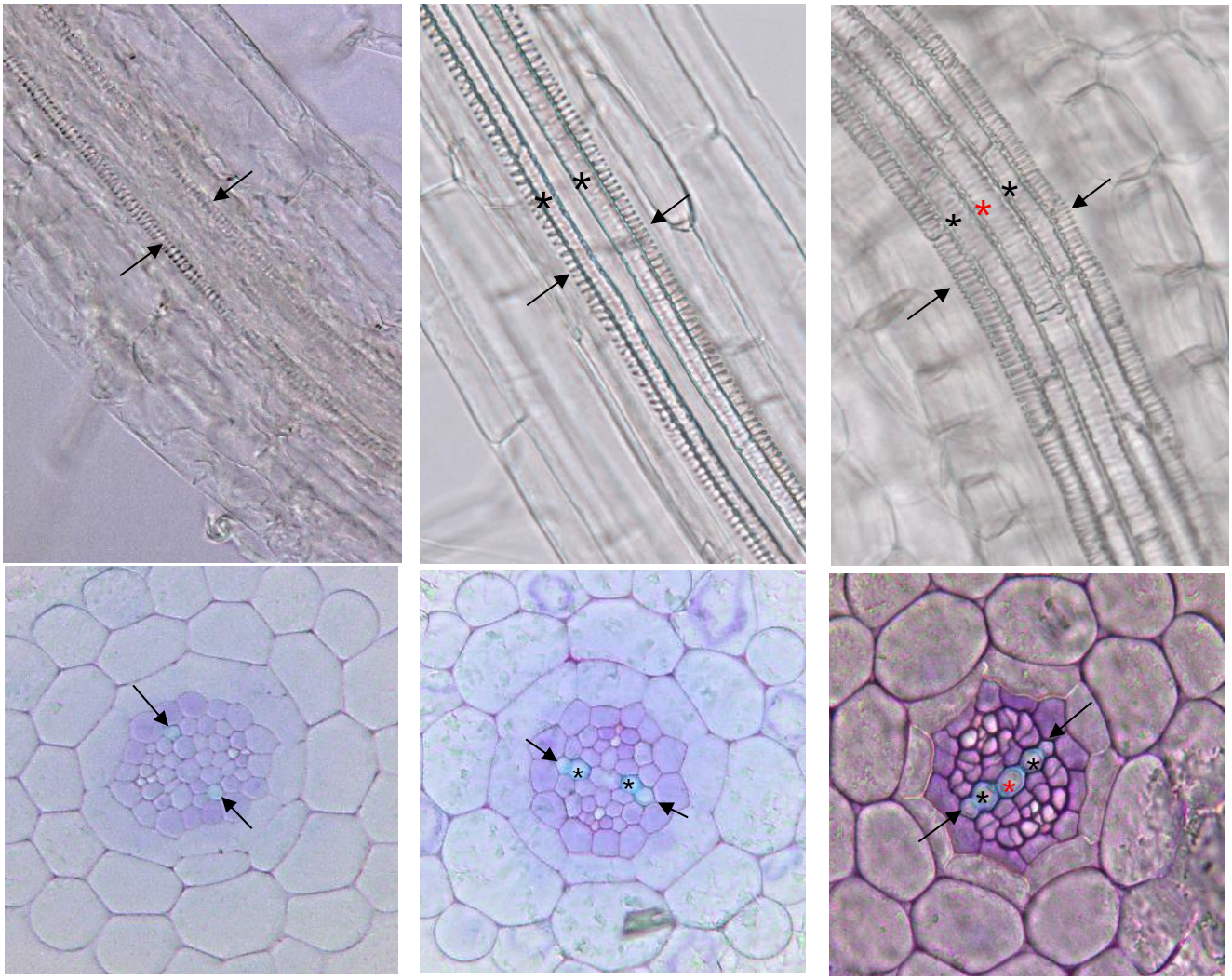


Figure 3. Microscope images of a wild type *A. thaliana* root in x20 magnification (above), and horizontal sections of the same areas, stained with toluidine blue, in x40 magnification (below). (A) Differentiation zone; (B) Intermediate area; (C) Old part of the root. Protoxylem is marked by arrows, outer metaxylem by black asterisks, inner metaxylem by red asterisks. Protoxylem cells display a spiral pattern on their lignified secondary cell wall, whereas the metaxylem SCW pattern is pitted. In toluidine staining, the xylem appears blue and the SCW can be seen. The inner metaxylem is larger in diameter than the outer metaxylem.

## 2.3 Auxins and cytokinins

Auxins and cytokinins are groups of plant hormones that take part in numerous processes. They can be naturally occurring or made synthetically. The most important auxin is indole-3-acetic acid (IAA). It plays a role in many processes, including embryonic development, root development, leaf formation, fruit development, phototropism or growth of shoots towards the light source, and gravitropism, or growing of the shoots upwards and roots downwards. (Kimball n.d.b.) IAA is synthesized in apical meristems, young

leaves and developing stems. It can be transported by two different mechanisms: non-polar transport through the phloem, or polar transport into cells by diffusion or influx transporters in the plasma membrane, and out of cells with the help of efflux transporters called PIN-FORMED proteins (PIN-proteins). (Ha & Morrow & Algiers n.d.a.)

Cytokinins are derivatives of purine adenine. Cytokinins are essential for certain plant processes, such as mitosis, chloroplast development, differentiation of the root tissues and shoot meristem, stimulation of branching, leaf formation and leaf senescence. For example, it is essential for seed germination, as it involves rapid cell division. Cytokinins are synthesized in young structures where cell division takes place, like embryos, root tips and fruits, as well as wounded tissues. They are transported through the xylem. (Ha & Morrow & Algiers n.d.b.)

Auxin and cytokinin have been found to have a mutual inhibitory interaction loop that plays a central role in the patterning of the *Arabidopsis* vascular cylinder. In particular, it determines the protoxylem vs procambium cell fate. The xylem axis cells display a high level of auxin signaling, but low cytokinin response, whereas procambium cells have a high cytokinin, but low auxin response. (Bishopp et al. 2011; Vaughan-Hirsch et al. 2018.) Mähönen et al. (2006) found that in plants with compromised cytokinin signaling, such as the *woodenleg (wol)* mutant, all vascular cells differentiate as protoxylem, therefore cytokinin is essential for promoting vascular cell types other than protoxylem. On the other hand, low auxin signaling results in no protoxylem differentiation (Bishopp et al 2011).

The auxin signaling gene MONOPTEROS (MP), also known as AUXIN RESPONSE FACTOR 5 (ARF5), is central to the regulation of cell divisions that establish the stele, and the subsequent tissue patterning. Perception of auxin through MP activates the transcription of downstream factors, like the transcription factor TARGET OF MONOPTEROS 5 (TMO5). TMO5 and its homologues form heterodimers with proteins from the LONESOME HIGHWAY (LHW) family. TMO5 and LHW have been shown to affect the size of the vascular bundle, as loss of function in either of the two results in plants with only few vascular cells, while overexpression causes a significant increase in the vascular cell number. (De Rybel et al. 2015 ; Vaughan-Hirsch et al. 2018.) The TMO5-LHW heterodimers activate the enzyme LONELY GUY 4 (LOG4) and its closest homologue LOG3, which cause an increased cytokinin synthesis (De Rybel et al. 2015). Cytokinin promotes cell divisions in the neighbouring procambial cells, as well as the radial transportation of auxin via PINs. In the procambial cells, cytokinin promotes the expression

and subcellular localization of the PIN proteins PIN1 and PIN7, which remove the auxin from the procambial cells into the protoxylem precursor meristematic cells. There, auxin promotes protoxylem specification. (Bishopp et al. 2011; Vaughan-Hirsch et al. 2018.) (Figure 4).

In addition, Bishopp et al. (2011) found that high auxin promotes the transcription of ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6), an inhibitor of cytokinin signaling. AHP6 is expressed in the protoxylem cells and the adjacent pericycle cells, where high auxin signaling is present. This shows that cytokinin has a negative effect on the formation of protoxylem. Thus, auxin inhibits cytokinin signaling. Mähönen et al. (2006) found that a mutated version of the *AHP6* gene, *ahp6-1*, results in a loss of protoxylem phenotype. (Figure 4.)

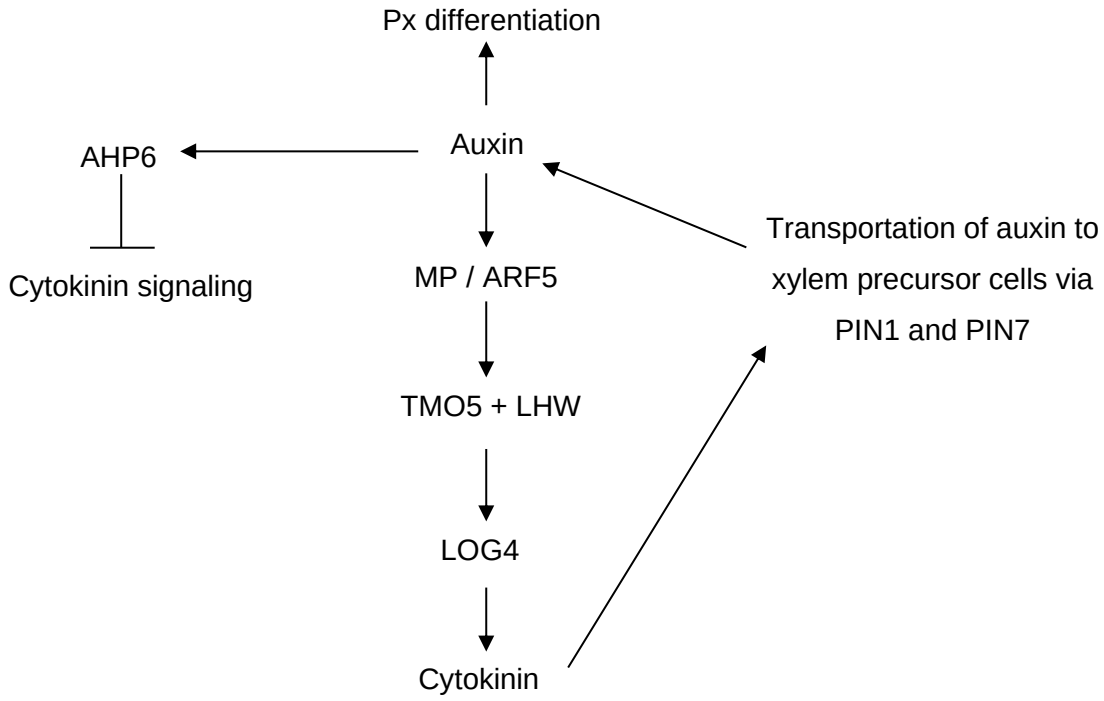


Figure 4. a scheme of the auxin-cytokinin inhibitory loop interaction in the root. By activating a chain of reactions, auxin induces cytokinin synthesis. Cytokinin promotes the radial transportation of auxin where it initiates protoxylem specification. At the same time, auxin promotes the production of AHP6, which inhibits cytokinin signaling.

## 2.4 Abscisic acid

Carotenoids are tetrateprene pigments present in plants, animals, fungi, archaea, algae and photosynthetic bacteria. In plants, they are essential for photosynthesis, and also act as antioxidants, photo-protectors, color attractants, and precursors of hormones in non-photosynthetic organs. (Maoka 2019.) One such hormone is abscisic acid (ABA), which is synthesized from the carotenoid zeaxanthin and transported via the vascular system. ABA is responsible for the plant's adaptation to stressful and potentially harmful environmental conditions, such as drought, cold temperatures, shorter day lengths and exposure to salt or salinated soil. For example, ABA enforces seed and bud dormancy to prevent premature germination and sprouting, and protects the plant from dehydration by reducing water loss and the damage it causes. (Ha & Morrow & Algiers n.d.c.)

Abscisic acid plays a role in xylem development as well by upregulating the microRNAs miR165 and miR166. They are produced in the endodermis, but move into the stele, where they downregulate mRNAs of class III homeodomain leucine-zipper (HD-ZIP III) transcription factors, which determine the protoxylem versus metaxylem cell identity. (Brookbank & Patel & Gazzarrini & Nambara 2021.) Cells with high dose of HD-ZIP III develop as metaxylem, whereas lower levels of HD-ZIP III develop as protoxylem. Auxin, on the other hand, promotes the expression of HD-ZIP III. (Ursache et al. 2014.)

## 2.5 *DRAMAQUEEN* gene

*DRAMAQUEEN (DRQ)* is a gene found in many plants throughout the plant kingdom, including *Arabidopsis*, yet its exact function is unknown. It was discovered during a genetic screen using a cytokinin signaling desensitized mutant background. A *DRAMAQUEEN* mutant, *mDRQ*, was found to have increased sensitivity to cytokinin signaling. It is expressed in the vascular tissue of roots, leaves, flowers, trichromes, pollen and silique abscission zones. It has also been found to be induced by wounding. The *mDRQ* mutant was shown to affect the root elongation rate, protoxylem phenotype, rosette sizes, etc. (Help-Rinta-Rahko et al. 2015.) Nuorti (2017) found that *drq* mutant had a protoxylem distribution that was statistically different from the wild type genotype, Col0. Since the mutant displayed increased cytokinin sensitivity, DRQ is thought to be an inhibitor of cytokinin signaling (Nuorti 2017).

# 3 Purpose, goal and research questions

The goal of this project is to characterize the effects of auxin on the root vascular tissue primary development in *DRAMAQUEEN* mutants, focusing on xylem anatomy. First information about the gene was published in 2015 (Help-Rinta-Rahko et al. 2015), then a pro-gradu project explored the gene further (Nuorti 2017). Nuorti (2017) investigated a partially functional mutant version of *DRQ,* that was found to increase cytokinin sensitivity, and suggested further research using a CRISPR knockout mutation. Such mutation is being used in a bigger project titled “DRAMAQUEEN: a novel vascular regulator in *Arabidopsis* plants”, which this thesis is also a part of. The final goal of it is to unravel the role of the *DRAMAQUEEN* gene in the vascular development of *Arabidopsis. DRQ* mutant plants are being treated with different hormones (cytokinin, auxin and abscisic acid) to study whether these hormones have an effect on *DRQ* mutant Arabidopsis plants.

The research group studies wood development, focusing on vascular tissue development in particular. *A. thaliana, Betula spp* and *Populus spp* are used as model organisms (Helsinki University). In the past, it unraveled the function of AHP6 as a cytokinin signaling inhibitor, as well as the phenotype of its mutant *ahp6-1* (Mähönen et al. 2006). The group has also found the inhibitory feedback loop between the hormones cytokinin and auxin, which regulates vascular tissue cell differentiation (Bishopp et al. 2011). Understanding the plant genes and their respective phenotypes may be eventually be used in biotechnological fields, like agriculture, to enhance the production of crops such as sweet potato and cassava using genetic engineering (Helsinki University). For example, *DRAMAQUEEN* mutants showed extra metaxylem vessels, therefore it displays enhanced water conduction. This phenotype could eventually be used, for example, for enhanced growth in drought conditions.

The research questions in this project were:

1. Does auxin affect the primary root elongation of *DRAMAQUEEN* mutant *Arabidopsis* roots?

2. Does auxin affect the root primary xylem development in *DRAMAQUEEN* mutant *Arabidopsis?*

# 4 Implementation of the thesis

## 4.1 Methods and materials

The *drq* mutants were produced using CRISPR-Cas9. New seeds were produced from three *drq* mutant lines: *cdrq1*-1B, *cdrq1*-3A and *cdrq1*-4E. The different lines have the same mutation in different alleles. The mutation in a knockout mutation, where a stop codon appears too early, producing an incomplete protein and therefore loss of function. The plants were grown in a greenhouse in long day conditions until new seeds were ready for collection.

For the experiment, the plants were grown in vitro in ½ Murashige & Skoog (MS) medium, 50 ml medium per petri plate. The medium contained MS salts (2,5 g/l), 2-(N-morpholino)ethanesulfonic acid (MES) monohydrate (0,5 g/l) and saccharose (1 g/l), mixed with $H_2O$ dd (double deionized), (ad desired volume) on a magnetic stirrer, after which KOH was added until pH reached 5,7-5,8. Finally, plant agar (10 g/l) was added, and the medium was autoclaved for sterilization. The plant agar was added last due to it dissolving in a high temperature, usually 100° C or higher. Agar solidifies fast at room temperature, therefore after the autoclaving, the medium was stored in a +60° C until the moment of use. One liter of medium was made for each experiment, 50 ml per plate × 18 plates. The remaining 100 ml were used to make plates for other uses. In experiment I, which served partly as technical practice, only six successful plating attempts were used for the experiment and the rest were discarded. The different concentrations of IAA were added to the medium before preparing the petri dishes. The concentrations used were 0 µM (control), 0,1 µM, 0,25 µM, 1 µM, 2,5 µM and 10 µM IAA, prepared from a 10 mM stock solution.

Fixation of samples was done with 4 % paraformaldehyde (PFA) in 1 × phosphate-buffered saline (PBS buffer), with addition of 10 µl/ml Triton X-100. Triton is a detergent and makes the membrane more porous, allowing the PFA to enter, replacing the gases, and preserve the structures. Vacuum treatment was used for one hour for the fixation process, as it improves the diffusion.

ClearSee solution was used to store the plants and later as mounting liquid for microscopy. ClearSee consists of xylitol (10 % weight/volume), sodium deoxycholate (15 %

w/v), urea (25 % w/v) + water to final volume, mixed on a magnetic stirrer until homogenous and clear, and stored in room temperature. The solution was prepared several times during the project, in volumes varying according to need.

Leica historesin embedding kit was used for plastic sectioning. The kit contained basic resin (glycol methacrylate monomer), activator powder bags (benzoyl peroxide) and a hardener liquid (derivate of barbituric acid). Solution A was made according to the kit's instructions. It contained 1 bag of the kit's Activator powder per 50 ml of basic resin and was stored in + 4° C in the dark.

The plants were stained with basic fuchsin (0,2 g/ml in ClearSee). Fuchsin stains lignified cells and is therefore effective for marking the xylem. The horizontal sections were stained with toluidine blue (0,05 % in 0,1 M sodium phosphate buffer). Toluidine blue is a polychromatic dye, as it reacts differently with different components, resulting in different colors within the same sample. For example, when the dye reacts with carboxylated polysaccharides, a pinkish purple color will appear, and a blue color will appear when the dye reacts to lignin (Mitra & Loqué 2014). For this reason the procambium appears pink or purple and xylem appears blue in toluidine staining.

Statistical significance tests were performed to compare between the mutants and Col0 phenotypes in the same auxin concentrations, using an individual sample t-test with 95 % confidence interval in MedCalc and SPSS. Average root length for all plants of the same genotype and condition in all three experiments was calculated using the weighted mean function in SPSS ("weight cases").

## 4.2 Experiment

### 4.2.1 Plant growth conditions

For all experiments, *A. thaliana* seeds were disinfected in a laminar hood with EtOH 100 % for 5 min, then EtOH 70 % for 5 min, followed by washing twice in sterile $H_2O$ dd for 2 min each time. This process ensures that the seeds are clean from possible bacterial or fungal infections. Then, the seeds were plated in ½ MS medium containing the different concentrations of IAA. The genotypes used in each condition and plate were Col0 (wild type), *cdrq1-3A* and *ahp6-1* in experiment I, and Col0, *cdrq1-1B*, *cdrq1-3A*, and *cdrq1-4E* in experiments II and III. Col0 genotype grown without auxin treatment served as the control group.

Plants were grown vertically in a growing cabinet in long day conditions (16 h light at 23° C and 8 h dark at 18° C) and 70% humidity. Experiment I served as a “test experiment”, in order to learn the required techniques. Not all the plating attempts were successful. For this reason, there was only one plate per condition, each plate containing approximately 15 plants of each genotype. In experiments II and III, there were 3 plates per condition to provide maximally reliable results. Damage, contamination, or other factors that can affect the growth rates may occur. In this case, the effect is diminished by having multiple plates of the same condition and calculating the average results of all plates. (Figure 5).

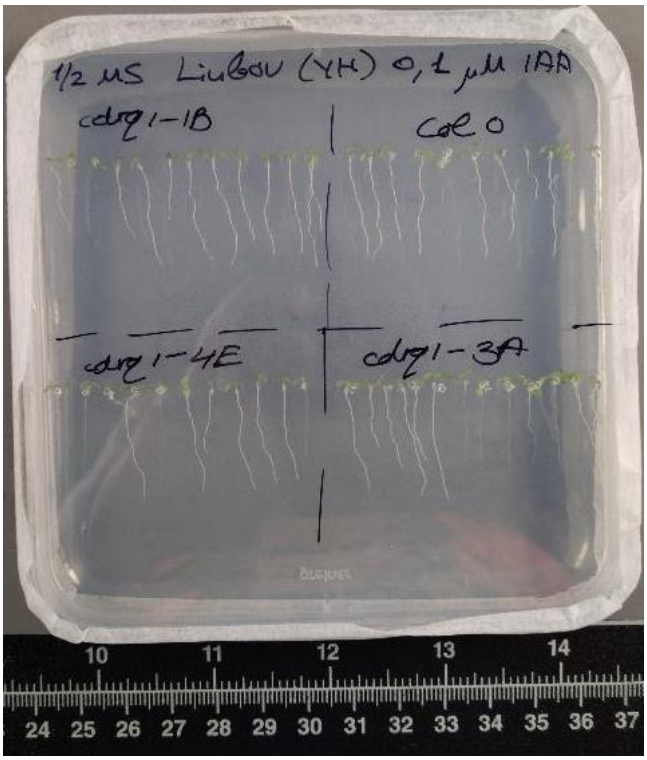


Figure 5. An example of a petri plate from experiment III with the plants still in it, showing Col0 plants and three *drq* mutants grown in 0,1 µM IAA in ½ MS medium. Nearby is a scale bar in centimeters and inches.

There were approximately 10-15 seeds of each genotype sown on every plate. The genotypes were placed differently in the three plates: in each plate there are different gradients of humidity, which might affect the growth. Therefore, covering all the possible positions is used to avoid the potential influence of the placement on the growth. (Figure 6).

| ½ MS | |
|---|---|
| Col0 | *cdrq1-3A* |
| *cdrq1-1B* | *cdrq1-4E* |

| ½ MS | |
|---|---|
| *cdrq1-3A* | *Cdrq1-1B* |
| *cdrq1-4E* | Col0 |

| ½ MS | |
|---|---|
| *cdrq1-1B* | *cdrq1-4E* |
| Col0 | *cdrq1-3A* |

Figure 6. An example of genotype placement in the three plates of the same condition, in order to avoid the effect of the moisture gradient in the plates. This was done for every condition in experiments II and III.

### 4.2.2 Root length analysis

For all three experiments, all the plates containing seven-day old plants were photographed next to a measuring scale (showing centimeters and inches) with a cellphone camera. One such photograph is figure 5. The root lengths were measured using the image analysis software Fiji by ImageJ. Using the scale bar in the photo, the number of pixels per centimeter was established in Fiji for each image separately. This step allowed the accurate measurement of roots. Using the segmented line tool in Fiji, the shape of each root was traced, and the final length was converted from pixels to centimeters. The images were saved on a USB drive.

A maximum of 15 root lengths were measured per each genotype in each plate, or less if there were less than 15 germinated seeds in the genotype. So a total of maximum 45 roots were measured in each plate in experiment I, and a maximum of 60 roots per plate in experiments II and III. Then, the mean root length of each genotype and condition was calculated.

### 4.2.3 Xylem morphology analysis

Xylem morphology was analyzed using microscopy. Whole seven-day old plants were transported into wells by gently pulling them out of the medium, and fixed with 4 % PFA in 1 × PBS for 1 hour in vacuum. After that, the plants were washed with PBS twice with gentle agitation and transferred to ClearSee solution. The plants were stored in the wells in ClearSee at +4° C. Before microscopy, the whole plants were stained with basic fuchsin in the wells for 30 minutes with gentle agitation, then washed twice with ClearSee for 30 minutes each time. Plants were put on a microscope slide and mounted with water (experiment I) or Clearsee solution (experiments II and III), after

which a cover slip was added. They were pictured with Leica DMLB optical microscope using × 20 magnification. In each plant, the old, intermediate and differentiate areas were pictured. The old area was pictured in the beginning of the root, close to the transition from the hypocotyl to the root. The intermediate area was pictured in the approximate physical middle area of the root, and the differentiation zone close to the root apical meristem, where the vascular tissue begins to differentiate. Five plants were pictured per each genotype in each condition, for each of the three experiments. The photographs were saved on a USB drive. The number of protoxylem, outer metaxylem and inner metaxylem vessels in each of the three different root zones was quantified by counting.

For the plastic sectioning (experiment III only), seven-day old plants were collected from the growing medium into wells, where they were fixed with 4 % PFA in 1 × PBS for one hour in vacuum, after which the plants were washed with PBS twice on a shaker. The plants were stored in PBS in +4° C until dehydration in ethanol gradient. The plants were first dehydrated in 10 % EtOH, then 30 % EtOH and 50 % EtOH for 30 minutes each time, and later in 70 % EtOH overnight. The next day, dehydration continued with 30 minutes in 96 % EtOH and finally 30 minutes in 100 % EtOH twice. After dehydration, the plants were incubated in 100% EtOH and Leica Historesin solution A in 1:1 proportion for one hour, in +4° C in the dark, after which the samples were incubated in solution A overnight in the fridge and dark.

Roots separated from the cotyledons and hypocotyls were placed in plastic chambers and covered with a mix of solution A and Leica Historesin hardener in 15:1 proportion. The chambers were then covered and left to polymerize overnight. Polymerization can only occur without air. After polymerization, embedded samples were stacked and embedded again in solution A + hardener in 15:1 proportion and left to polymerize. Finally, blocks were made by putting the stacked samples into molds, covering with solution A and hardener (15:1) and a plastic slip to create absence of air, and leaving to polymerize overnight. The blocks were sectioned on a Leica Jung Biocut 2035 microtome in a 5-10 µm thickness and left to straighten in warm water for a few seconds, after which they were collected onto a microscope slide, 2-3 sections per slide. The microscope slides were dried on a hot plate, after which they were stained with toluidine blue (0,05 % in 0,1 M sodium phosphate buffer) by sinking the slide into the dye for a few seconds, then rinsing twice in $H_2O$ dd. The sectioned plants were mounted with water and a cover slip and photographed with Leica DMLB microscope in × 40 magnification and

saved on a USB drive. The pictures were used for better understanding of the anatomy. Longitudal microscopy can be challenging for this purpose, as the tissues may layer on each other and make analysis difficult.

### 4.3 Data analysis

The individual root lengths were copied from Fiji to Excel and organized into tables. First, the average root length was calculated for each genotype in each condition for the experiments separately. Then, the mean root length of all plants of the same genotype and condition in all three experiments was calculated using the previously achieved means. Since the sample sizes were unequal, the weighted mean function was used in the statistical software SPSS, which also calculated the standard deviation. The results were organized into a table and a graph in Excel (Figure 8).

A statistical significance test was performed to compare between the root lengths of mutant and Col0 phenotypes in the same auxin concentration, using an individual sample t-test with 95 % confidence interval in MedCalc. The test used the total sample size and the previously calculated means of the means and standard deviations.

Xylem morphology was analyzed by manually counting the number of protoxylem, outer metaxylem and inner metaxylem cells in five plants per each genotype and condition in each of the three experiments. In every plant the phenotype was analyzed for the old, intermediate and differentiate part of the root. Thus, a total of 210 root phenotypes were analyzed, each of them in three areas.

A total percentage of phenotypical defects was calculated for each genotype and condition, separately for each xylem cell type and each of the three root zones. Defect percentages were statistically compared between the mutants and Col0 for plants grown in the same conditions using an individual sample t-test in SPSS, 95 % confidence interval. Data was sorted into tables and graphs in excel (Figures 9, 10 and 11).

## 5 Results

### 5.1 Root length

Root measurements show that in all auxin concentrations, mutants of both *drq* and *ahp6-1* displayed shorter root lengths in comparison to Col0 (Figure 7). The difference

in average root length was found to be statistically significant, using a t-test with a confidence interval of 95%. Furthermore, auxin treatment appears to inhibit the primary root elongation in Col0 and *drq* mutants. As seen in figure 7, the roots of the plants grown in 10 µM IAA were significantly shorter than those grown in control conditions.

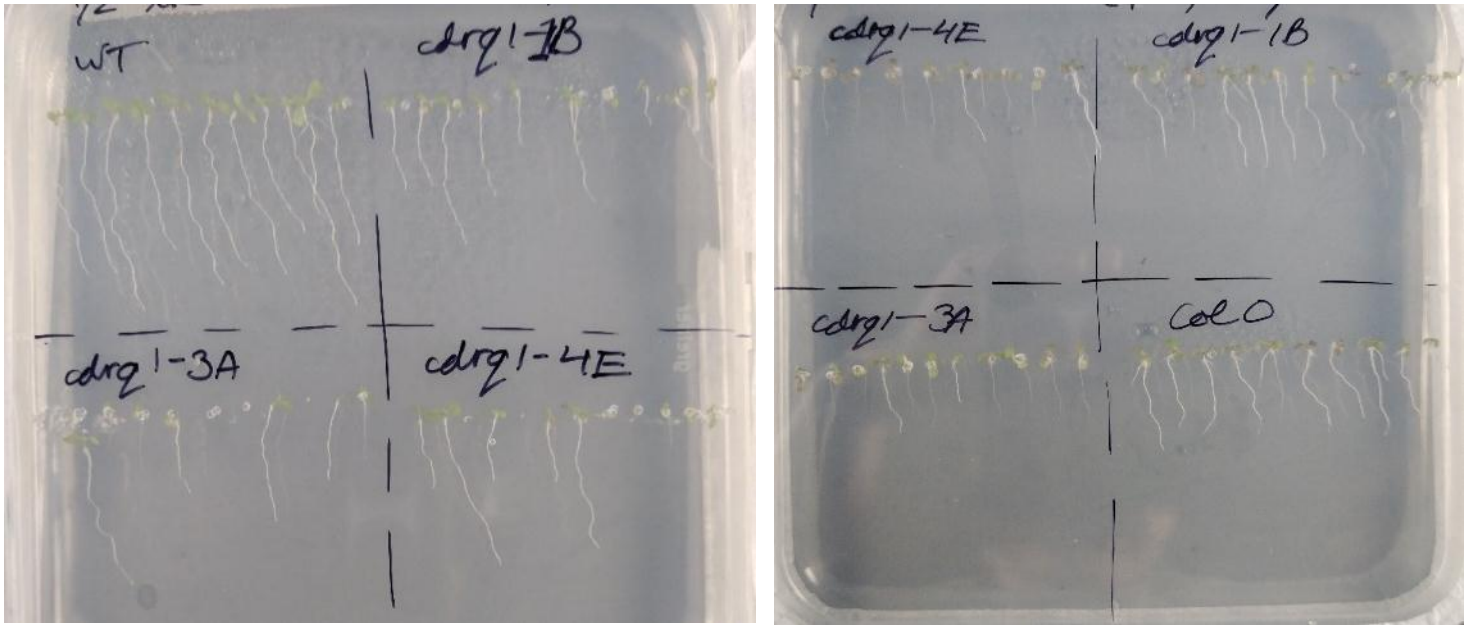


Figure 7. An example of two plates with ½ MS medium in control conditions (left) and 10 µM IAA (right). Even on macroscopic level, the average root length of all three *drq* mutant lines is notably shorter than the root length of Col0 plants (also marked as "WT" for "wild type"). Moreover, plants grown in 10 µM auxin are significantly shorter compared to plants grown in control conditions.

The root length shows a stable decline as the IAA concentration increases (Figure 8). The average root length in control conditions was 2,498 cm, 2,196 cm, 2,199 cm and 2,155 cm for Col0, *cdrq1-3A, cdrq1-1B* and *cdrq1-4E*, respectively, compared to 0,968 cm, 0,853 cm, 0,870 cm and 0,746 cm for Col0, *cdrq1-3A, cdrq1-1B* and *cdrq1-4E*, respectively, grown with 10 µM of exogenous IAA in the medium. The difference in the root lengths was statistically significant between the mutants and the wild type, but not between the mutant lines themselves. The length decrease pattern was similar between all *drq* mutants and Col0.

*ahp6-1* mutants, however, displayed a notable increase in root length at the 0,25 µM IAA concentration (1,809 cm), after an initial decline between 0 µM IAA (1,844 cm) and 0,1 µM IAA (1,381 cm). The root length declines again to 1,014 cm at the 1 µM IAA concentration, followed by small increase to 1,086 cm at the 2,5 µM concentration, and a final decline till 0,724 cm at the 10 µM IAA concentration (Figure 8).

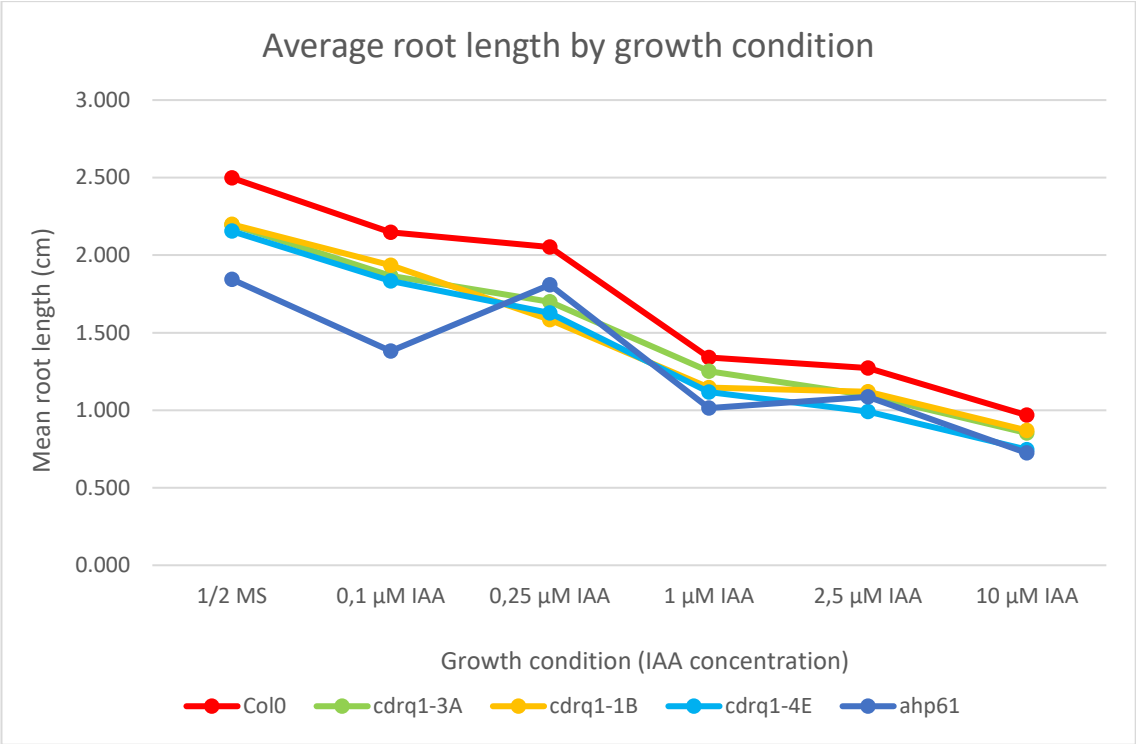


Figure 8. The graph displays the average root lengths of all the genotypes used in the experiment as a function of the IAA concentration in the growing medium. All three *drq* mutant lines show a decline pattern in root length as the auxin concentration increases, similar to Col0. *ahp6-1* shows a markedly different pattern.

## 5.2 Xylem anatomy analysis

Defects in the xylem morphology were observed in all genotypes and conditions, in all three xylem cell types (protoxylem, outer metaxylem and inner metaxylem), as shown in figures 9-11. In *ahp6-1* a partial loss of protoxylem was observed, often displaying only one protoxylem cell instead of the typical two. In *drq* mutants, the phenotypes included partial loss of protoxylem, extra outer metaxylem and inner metaxylem with improper level of development (immature in the old part, too mature in the intermediate part).

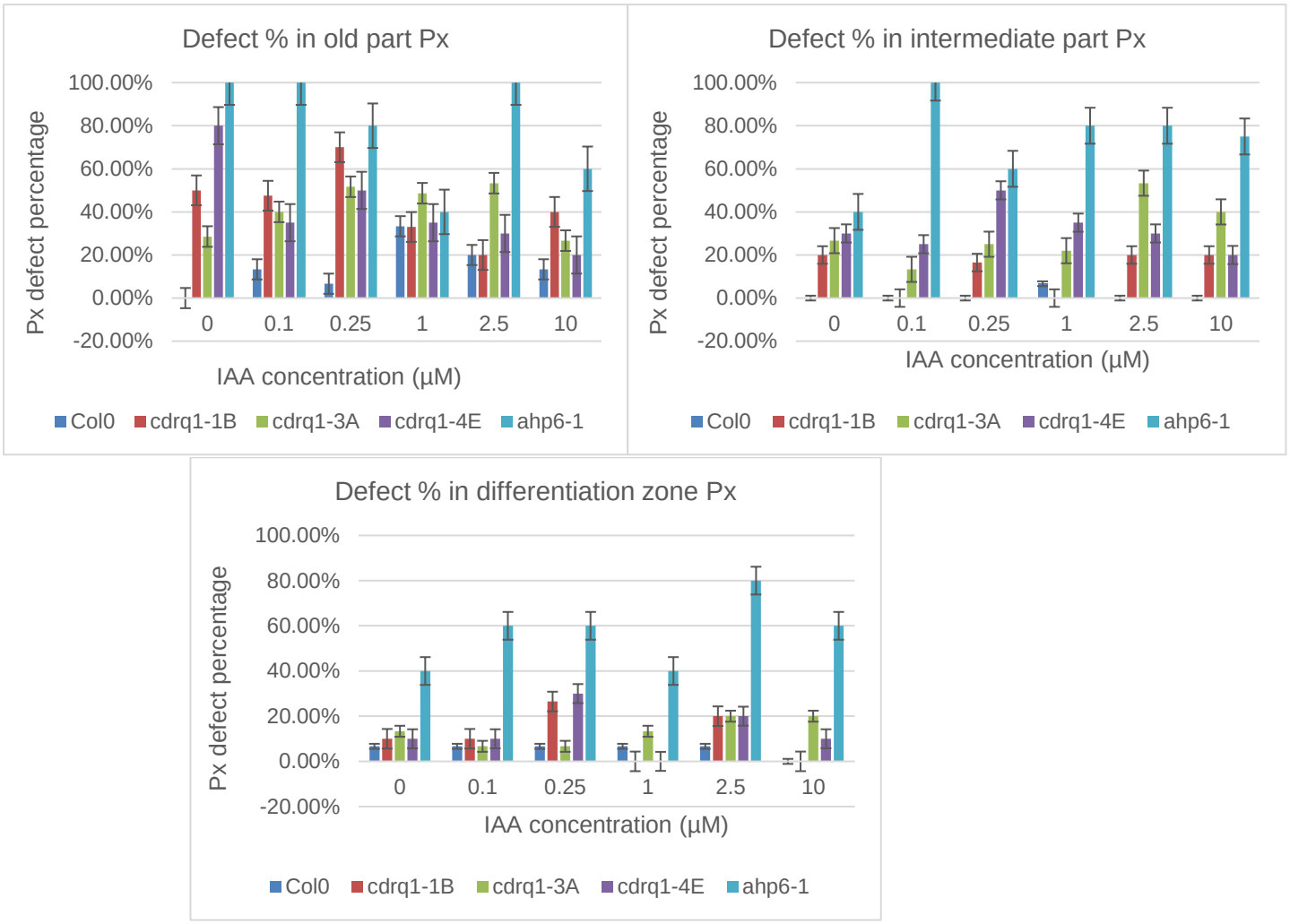


Figure 9. Graphs that show the average defect percentage in the protoxylem in the old, intermediate and differentiation zones of the root in all of the genotypes and growing conditions used in the experiment.

*ahp6-1* mutants showed statistically significant defects in the protoxylem anatomy in all parts of the root. The most common defect was loss of protoxylem: only one of the two protoxylem polls was present. *drq* mutants showed an abnormal protoxylem distribution as well, albeit only some cases were found statistically significant, most of the in the old part of the *cdrq*-1 3A line roots. In the *drq* mutants, protoxylem was breaking and appearing again in numerous areas along the root. The *drq* mutants the defect percent is highest in the old part of the root, lower in the intermediate part and insignificantly low in the differentiation zone.

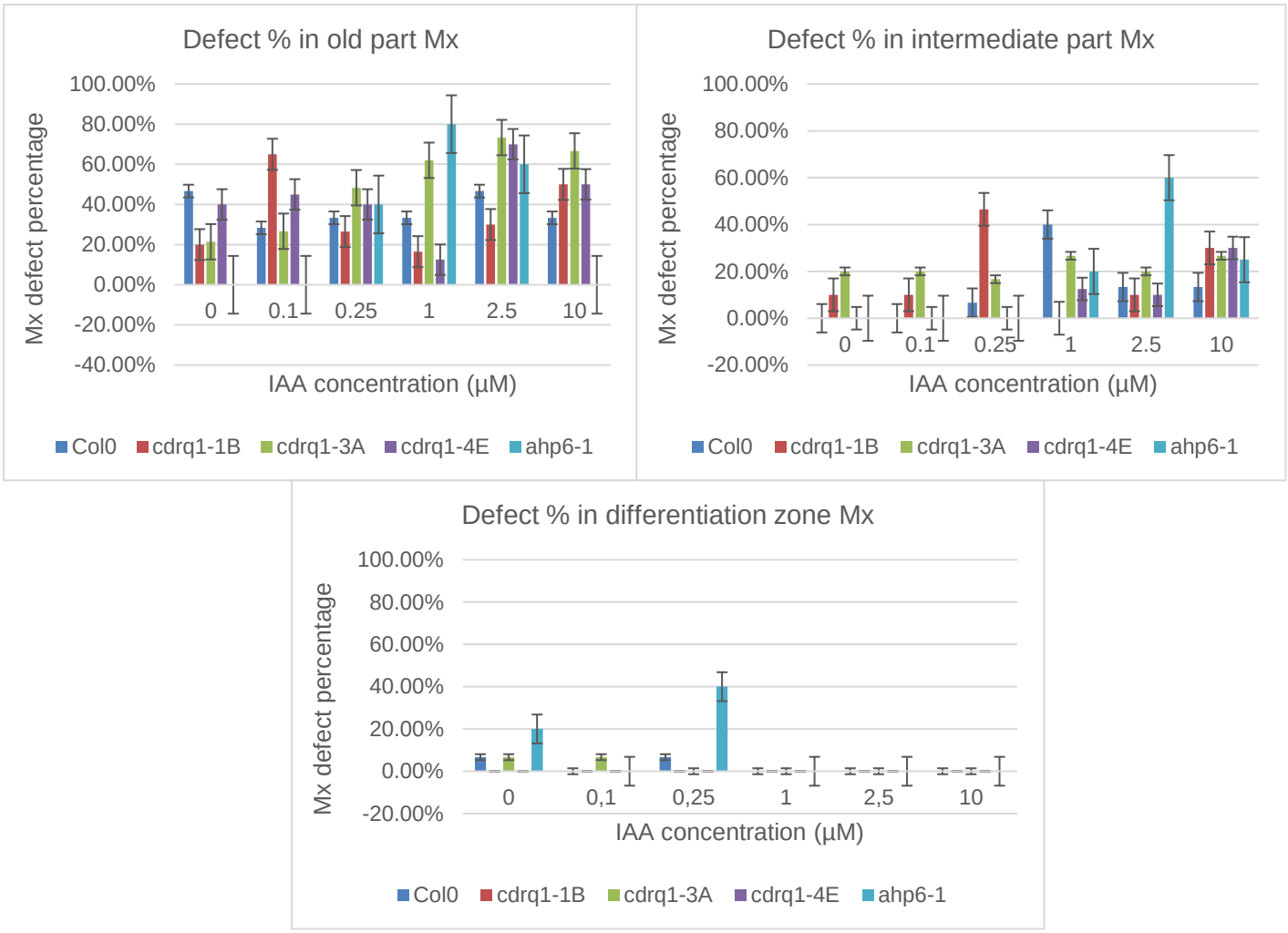


Figure 10. Graphs that show the average defect percent in the outer metaxylem in the old, intermediate and differentiation zones of the root in all of the genotypes and growing conditions used in the experiment.

Defects in the metaxylem phenotype were detected in both Col0 and *drq*, especially in the higher auxin percentages. The difference between Col0 defect percent and the mutant defect percentages were not found statistically significant. This would suggest that the defects were mostly the result of the exogenous auxin treatment, rather than the mutation. Some metaxylem defects were observed in the *ahp6-1* mutant phenotypes, mostly in the higher auxin concentrations (0,25 – 10 µM). The most common defect was an extra metaxylem vessel, full or partial.

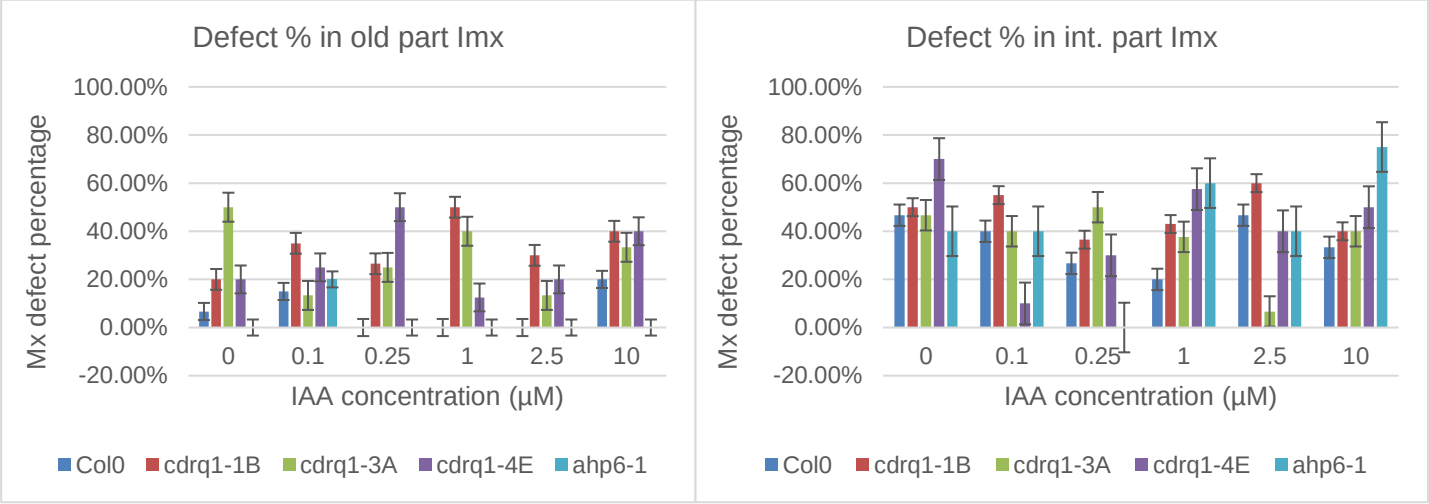


Figure 11. Graphs that show the average defect percent in the inner metaxylem in the old, intermediate and differentiation zones of the root in all of the genotypes and growing conditions used in the experiment. The differentiation zone is not included because the inner metaxylem is not yet differentiated in this zone.

Defects in the inner metaxylem were observed in the old part mostly in the *drq* mutants, and in the intermediate part in all of the genotypes. The most common defects were loss or partial loss of the inner metaxylem, as well as an incorrect level of maturation (immature in the old part, too mature in the intermediate part). Defect were more common in the intermediate part of the root.

# 6 Discussion

## 6.1 Result analysis

The roots were shorter at the higher the concentrations of exogenous IAA in the MS medium, pointing to an increased auxin sensitivity of the mutant. This result is consistent with multiple previous reports, that showed exogenous auxin to inhibit primary root elongation (Alarcón & Salguero & Lloret 2019). Furthermore, in control conditions, *ahp6*-1 and *drq* plants were statistically significantly shorter than Col0. This is also consistent with a previous report, that showed *mDRQ* to affect the root elongation (Help-Rinta-Rahko et al. 2015).

Previous results on the effect of *ahp6-1* mutation on the root length were not found. This result, however, could be explained by the fact that cytokinin normally inhibits root

elongation (Werner et al. 2010). Since the *AHP6* gene was not active in the mutant and therefore did not inhibit the cytokinin signaling, the negative impact of cytokinin on the root elongation is noticeable. This would explain the length of the root being shorter compared to Col0 in control conditions. However, the increase and decrease of the *ahp6-1* mutant root length in different IAA concentrations remains unclear, and more experiments would be needed in order to explain this result.

Possible reasons for inaccuracy in this part of the experiment were a relatively small number of samples and lack of repetition in the *ahp6-1* experiment, as it was only done once and had only one plate per condition. Therefore, any possible damage, misplacement or other similar factors could have affected the root growth. There were small fungal contaminations in 3 plates in experiment III. While no plants were collected nor measured from the contaminated areas, it is possible that the plants experienced stress that could have impacted the root growth rate. Furthermore, it created a smaller sample size in these plates. Another reason for smaller sample size was that some of the seeds were old and therefore did not germinate.

A significant difference was observed in the root lengths between plates in the same experiment and same condition, most notably in the 10 µM IAA concentration. This could be caused by improper mixing of the auxin in the medium at the time of plating, which resulted in unequal auxin concentrations. The effects of this result were minimized by calculating the average root lengths from all plates and experiments.

A loss of protoxylem was observed in both the *ahp6-1* and *drq* mutants. These results were accurate in comparison to previous research. Mähönen et al. (2006) found a similar phenotype in *ahp6-1* mutants, and Help-Rinta-Rahko et al. (2015) and Nuorti (2017) described an altered protoxylem phenotype in *mDRQ* plants. AHP6 is known to be a cytokinin inhibitor (Mähönen et al. 2006), and *DRQ* is suggested to be a cytokinin inhibitor as well (Nuorti 2017). Since cytokinin normally inhibits protoxylem formation, and in these mutants the inhibiting proteins are not expressed, the negative effect of the cytokinin on the protoxylem differentiation is visible. Since auxin promotes protoxylem differentiation, auxin treatment could explain the partial restoration of the missing protoxylem polls.

There were no significant differences in the outer metaxylem phenotype between the *drq* mutants and the wild type, as similar phenotypes were observed in both. This suggests that metaxylem is not, or less affected by the mutation. The increased number of

metaxylem vessels could be due to the auxin treatment, since auxin induces xylem differentiation through HD-ZIP III transcription factors, high levels of which promote metaxylem formation. (Ursache et al. 2014). Metaxylem defects were uncommon in the *ahp6-1* mutants, only appearing in the 0,25, 1 and 2,5 µM IAA concentrations in the old part, 1, 2,5 and 10 µM IAA concentrations in the intermediate area and in the 0 and 0,25 µM IAA concentrations in the differentiation zone. This could be a case of normal natural variation, as only five ahp6-1 mutant plants were checked in each IAA concentration, therefore a more detailed experiment with larger sample size would be needed to provide accurate results in this mutant.

In the intermediate area, all genotypes displayed defects in the inner metaxylem. The large percentage of inner metaxylem defects is not necessarily due to the mutations. For example, the overdeveloped Imx phenotype was observed a total of 26 times, nine of which were in Col0, but only one of these nine in control conditions. Therefore, it could be an effect of the auxin treatment rather than the mutation. It could, for example, make the inner metaxylem differentiate closer to the root tip.

Possible mistake sources include poor quality pictures or pictures taken in the wrong area of the root. In longtitudal microscopy, the vascular tissues can layer on each other and be therefore hard to quantify correctly. The chance of this happening can be minimized by proper mounting. For most accurate results, pictures of the same root area should be taken at approximately the same spot in every plant. This was not always possible due to the reason described above. In addition, some pictures were taken too close to a lateral root. Those pictures may provide inaccurate results due to vascular tissue changes in those areas, as the xylem develops and extends from the primary root to the lateral root.

There was overstaining in some of the plants in experiment II due to fuchsin accumulating on the dish lid and dropping down onto the plants stores in ClearSee. Normally, fuchsin stains only the lignified cells. In the plastic sectioning, some of the roots were in the wrong angle or sectioned in the wrong zone (for example, too close to the root tip). These reasons made the vascular anatomy analysis impossible in some plants. Moreover, the toluidine blue washed off. This could be due to incorrect staining protocol, as it was later found out that the staining gives better results when done in higher temperatures. The affected samples were re-stained.

## 6.2 Reliability

Reliable research is conducted with accuracy, meticulousness, and integrity. These principles apply to every step of the research, from initial planning and data collection to conducting the research to analyzing and reporting the results. Data is collected from ethically sustainable and trustworthy sources. Methods used for the research are to be justified for the research in question. All necessary research permits must be acquired. Results are presented with transparency, openly and responsibly. Results are compared with previous research on the subject, in order to check if the findings are consistent. Quality is assured by unbiased peer review. (TENK 2021.)

The concepts validity and reliability are used in assessing the quality of a research, both quantitative and qualitative. Validity refers to the integrity of choosing the research methods and collecting the results. It includes the following aspects: whether the collected results cover the subject that was being investigated; whether the measurement methods are relevant for the subject; the relation of constructs and measurements between one another, in terms of correlation and prediction. Reliability refers to the consistency of results, whether achieved by conducting the same test multiple times, or by comparing the results to previous research results. (Taherdoost 2016.)

In this project, reliability was established through repetition of the same experiment using the same methods, having three plates per each growing condition in experiments II and III, as well as comparing the results to previous similar experiments, if such experiments were performed. All phenotype quantifications were double checked before calculating defect percentages. Positioning the genotypes differently in the plates aims to avoid the possible effect of humidity gradient in the plates on the root growth, which may create less accurate results.

The experiment with *ahp6-1* mutant was only done once and had only one plate in each condition. It was done to learn and practice the necessary techniques and methods for the other experiments, which is also important for achieving reliable results. The loss of protoxylem phenotype, however, was logical as it has already been shown before (Mähönen et al. 2006). The experiment included one *drq* mutant line, *cdrq1-3A*, which was compared to the later experiments and included in the statistical calculations. Validity was achieved by planning the experiment with a postdoctoral researcher, thus selecting the appropriate techniques for achieving the results.

The manuscript was reviewed by the supervisors and student opponents during seminars. Scholarly sources were used for gathering of information and comparing results. Most of the sources were no more than ten years old, albeit older articles were cited too. For example, the papers by Mähönen et al. (2006) and Bishopp et al. (2011) present important discoveries in the process of vascular tissue development that is still being studied, and are still widely referred to. Moreover, the experiment by Mähönen et al. (2006) has similarities with this project as both used *ahp6-1* mutants, so it was necessary to compare results. Other older articles provided general theoretical information that is still relevant, for example the classification of plants (Streich 2007) or the history of *Arabidopsis* in research (Koornneef & Meinke 2010).

## 6.3 Ethics

Other researchers' work is to be acknowledged and respected by citing their publications properly. Their work is to be given the recognition it deserves. The research process and its results are to be presented and analyzed with honesty. The members of the research team have a set of rights and responsibilities that are discussed and agreed upon by all participants before the start of the process. All necessary contracts and research permits are to be acquired. Data has to be acquired from sources where the researcher is granted official access. Data is protected and distributed only in a legal and ethical manner. (TENK 2021.)

This was established in this project using photographs taken during the project or images under the CC license with credit to their authors, crediting the information sources within the text and in the bibliography, and presenting the experiment methods and results openly and honestly. Manuscripts that were not openly accessible online were acquired with the author's permission and credited properly. A research permit was not required for this project, but a contract was signed between Metropolia University of Applied Sciences and the University of Helsinki discussing the project terms and publication rights. In addition, the manuscript is subject to plagiarism check in Turnit.

This project deals with transgenic plants. Aside from the original debate on whether it is ethical to genetically modify living organisms, another issue arises concerning the EU's strict regulations about genetically modified organisms (GMO). The regulations are some of the strictest in the world, and are aimed to protect the humans, animals and environment (European Parliament). In order to prevent the potential spread of mutant seeds into nature and interfering with the existing gene pool, strict regulations are taking place in the laboratory practices. For example, sticky mats are placed in room

where seeds are dealt with, so any possible seeds stuck on shoes will stay inside. The rooms are also carefully cleaned before leaving. All transgenic organisms are disposed of into a separate bag and are later autoclaved.

## 6.4 Conclusions

The root length analysis suggests that *DRAMAQUEEN* could have a role in primary root elongation, as mutants of this gene displayed a shorter root phenotype compared to Col0. High concentrations of auxin cause a decline in *drq* root length in a pattern similar to wild type, showing a similar or increased sensitivity.

*DRQ* is likely to affect the protoxylem anatomy in the roots, as the mutants showed partially missing protoxylem strands. The similarities in the protoxylem phenotype between *drq* mutants and *ahp6-1* mutants support the suggestion that DRQ may be a cytokinin inhibitor. An extra outer metaxylem vessel and defects in the inner metaxylem were observed as well, although they are more likely to be the result of the auxin treatment, as they were observed in all genotypes including Col0. Furthermore, since most protoxylem defects were detected in the old part of the root and almost none in the differentiate zone, it may suggest that *DRAMAQUEEN* expression is highest in older areas of the root and gradually decreases towards the tip.

## 6.5 Further research

Similar tests are being performed in the group studying the effects of other hormones, cytokinin and ABA, on the vascular development of *DRAMAQUEEN* mutants. Furthermore, the expression pattern of *DRAMAQUEEN* is measured using the reporter gene system GUS (β-glucuronidase), which helps locate the gene expression areas in the plant. More experiments can be done to investigate the possible role of the *DRAMAQUEEN* gene in phloem development, wounding, flowering and other biochemical processes in the plant where the gene was shown to be involved. In addition, a repeat experiment could be done for more reliable results on the effects of auxin treatment in *ahp6-1* mutants.

## 6.6 Professional growth

During the course of this project, I learned a lot of new theory about plant biology, and some of the techniques used in plant research. I got acquainted to a new sample type

and its handling. As a medical laboratory scientist, many of the techniques were generally similar to the ones used in medicine, but performed somewhat differently. For example, the histological sectioning used historesin instead of paraffin. Toluidine blue is used for human histological samples as well, but the protocol is different.

Otherwise, I got to practice the work of a laboratory technician, such as preparing solutions, sterile work, work in fume hoods and laminar hoods, fixing samples, staining, microscoping and sectioning on a microtome. I have practiced general meticulousness in laboratory work. I also learned more about statistics, and the use of SPSS in particular.

Moreover, I was introduced to an aspect of laboratory work that is not used as commonly in the medical field. Since samples are not from humans or animals, and thus carry no risk of infection or risk of affecting a diagnosis if samples are accidentally mixed, equipment typically made for single use, such as Pasteur pipettes, can be reused much more often compared to a medical lab, contributing to a more financially and environmentally sustainable work.

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