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The influence of roots on the chemical properties of the apoplast and the rhizosphere

Vliv kořenů na chemické vlastnosti apoplastu a rhizosféry

Bachelor's thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Chemical properties of the apoplast and rhizosphere are crucial for plant development and its overall well-being. Apoplast includes space outside the plasma membrane and its content, such as gas, water, and solutes. Changes in apoplast properties significantly influence various processes. One of them is cellular growth – the pH-dependent extension of cell walls. The rhizosphere is the soil environment near and under the influence of roots. It is characterised by abiotic factors like the availability of nutrients and toxic compounds. It is also a space with a high representation of microbiome communities. Plants can significantly influence the chemical properties of both apoplast and rhizosphere to improve their growth conditions. This thesis summarises the most important mechanisms of plants' roots that change these chemical properties, focusing on the model plant *Arabidopsis thaliana*. It shows how and where these mechanisms intersect and in which way they influence each other. Emphasis is placed on the process of growth regulation and mechanisms of increasing nutrient availability.

Keywords: apoplast, rhizosphere, H^+ATPase, root growth, rhizodeposition, root exudates, nutrients, microbiome

Abstrakt

Chemické vlastnosti apoplastu a rhizosféry jsou zásadní pro vývoj a celkové zdraví rostliny. Apoplast zahrnuje prostor mimo plazmatickou membránu, včetně jeho obsahu jako je plyn, voda a rozpuštěné látky. Změny v jeho vlastnostech výrazně ovlivňují různé procesy. Jedním z nich je buněčný růst – prodloužení buněčných stěn závislé na pH. Rhizosféra je prostředí půdy v blízkosti a pod vlivem kořene. Je charakterizována abiotickými faktory, jako je přítomnost živin a toxických sloučenin. Také se jedná o prostor s vysokým zastoupením mikroorganismů. Rostliny mohou chemické vlastnosti apoplastu i rhizosféry výrazně ovlivňovat, aby zlepšily své podmínky pro růst. Tato práce shrnuje nejdůležitější mechanismy kořenů rostlin, které tyto chemické vlastnosti mění, se zaměřením na modelovou rostlinu *Arabidopsis thaliana*. Ukazuje, jak a kde jsou tyto mechanismy propojeny a jakým způsobem se vzájemně ovlivňují. Důraz je kladen na proces kořenového růstu a mechanismy zvyšování dostupnosti živin.

Klíčová slova: apoplast, rhizosféra, H^+ATPáza, růst kořene, rhizodepozice, kořenové exsudáty, živiny, mikrobiom

List of abbreviations

ABA	Abscisic acid
ABC	ATP binding cassette
ACC	1-amino-cyclopropane-1-carboxylic acid
ABI1	ABSCISIC ACID-INSENSITIVE 1
ABP1	AUXIN-BINDING PROTEIN 1
ABL1	ABP1-like proteins
AFB	AUXIN SIGNALING F-BOX
AHA	AUTOINHIBITED H^+ATPASE
ALMT	ALUMINIUM-ACTIVATED-MALATE TRANSPORTER
AMT1	AMMONIUM TRANSPORTER 1
ARF	AUXIN RESPONSE FACTOR
AUX1	AUXIN RESISTANT 1
BAK1	BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE
BNI	biological nitrification inhibitor
BIN2	BRASSINOSTEROID INSENSITIVE 2
bHLH104	BASIC HELIX-LOOP-HELIX 104
BR	brassinosteroid
BRI1	BRASSINOSTEROID-INSENSITIVE 1
CW	cell wall
EXPA	α-expansins
EXPLA	expansin-like A
EZ	elongation zone
FC	Fusicoccin
FER	FERONIA
FS	Fluorescein-5-(and-6)-Sulfonic Acid, Trisodium Salt
GFP	GREEN FLUORESCENT PROTEIN
IAA	Indole-3-acetic acid
ILR3	IAA-LEUCINE RESISTANT 3
MATE	MULTIDRUG AND TOXIC COMPOUND EXTRUSION
NPF	NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER
NRT2	NITRATE TRANSPORTER 2
PM	plasma membrane
PMF	proton motive force

PKS5	SOS2-LIKE PROTEIN KINASE 5
PIN	PIN-FORMED auxin transporter
PP2C.A	A-clade of protein phosphatases type 2C
PP2C.D	D-clade of protein phosphatases type 2C
PP2C	protein phosphatases type 2C
RALF1	RAPID ALKALINIZATION FACTOR 1
RLK	Receptor-Like Kinase
SAUR	SMALL AUXIN UP-RNA
SnRK2s	SNF1-RELATED PROTEIN KINASES 2
SOS	SALT OVERLY SENSITIVE
STOP1	SENSITIVE TO PROTON RHIZOTOXICITY 1
TMK1	TRANSMEMBRANE KINASE 1
TIR1	TRANSPORT INHIBITOR RESPONSE 1
TZ	transition zone

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1 Introduction

Plants constantly react to environmental stimuli and requirements for their growth. They can do this through roots and their ability to modify the chemical properties of their apoplast and rhizosphere. Although the rhizosphere can have a broad definition that also includes the apoplastic space, I will use the term rhizosphere to refer only to the soil close to and influenced by the root, separating these two spaces. The apoplast is the intercellular space and its content beyond the plasma membrane, which includes the cell wall. Its properties are a crucial factor for plants' growth. Cellular growth is described by the long-known acid growth theory, which is still debated and less understood in roots, as its initial research was focused on plants' organs above the ground. At the centre of the theory are H^+ATPases, which pump protons into the apoplastic space, resulting in the acidification necessary for cell walls to loosen. Their activity is regulated by auxin, a key phytohormone with a complex signalling pathway and mechanisms of action. Fluxes of protons also influence the chemical properties of the rhizosphere, although a crucial role plays a wide variety of compounds that the root exudes. Their exudation influences a variety of abiotic and biotic factors.

In my thesis, I describe prominent mechanisms of roots that influence the apoplast and its chemical properties, specifically its pH, to regulate root growth. I discuss how and where these mechanisms are connected. I have also included a chapter about root anatomy, as its understanding is necessary for the topics discussed. Except for acid growth theory and auxin, the action of H^+ATPases is controlled by other phytohormones like brassinosteroids or abscisic acid. I will also describe the root influence on nutrient availability and uptake, focusing on ions fluxes to the rhizosphere, exudation of carboxylates, phenolic compounds and mucilage. Of course, these are not the only compounds and signalling molecules utilised by plants. Plants also have the ability to change the rhizosphere properties for their purpose through the exudation of sugars, amino acids, or phytohormones, which will also be discussed. Another significant factor is root respiration and release of living cells into the soil. Apart from the effect on nutrition, I am going to describe the root influence on rhizosphere microbiome composition and toxic compounds.

2 Root anatomy

Looking at the root anatomy of different species of plants, we can observe slight or more significant variability. Roots have evolved independently in several clades of plants, gradually getting more complex and efficient in their functions. In vascular plants, they developed two times in lycophyte and euphyllophyte clades (Kenrick & Strullu-Derrien, 2014). Angiosperm clade, which is the focus of this work, can be divided into two groups, dicots and monocots. Their roots differ in anatomical properties, strategies for coordinating and balancing their root's absorption and transportation, mycorrhizal colonisation rate, or the ability of secondary growth, which is present only in dicots. However, differences also exist within these groups. A correlation between root form and its function is apparent (M. Zhou et al., 2022). Most visible is the different root system organisation. While one primary root and other lateral secondary roots are typically present in dicot plants like *Arabidopsis* (Dolan et al., 1993), cereal species like rice or maize, which belong in monocots, have fibrous root systems that consist of more root types, primarily of adventitious roots (Coudert et al., 2010).

2.1 Model plants

One of the widely used model plants is *Zea mays*, which has a history of genetic research and, among others, is being used as an example of a species with C4 photosynthesis. The next is *Oryza sativa*, which has been used for genetic, molecular and C3 photosynthesis studies. Both plants are classified as monocots from the Poaceae family and represent globally important agricultural crops, hence the interest in their root system (Müller & Grossniklaus, 2010). *Arabidopsis thaliana*, classified as the Eudicots clade and Brassicaceae family, is one of the most popular plant model organisms. Because of its small and simple genome, short life cycle, seed production and other characteristics as its complexity, it is used for research in developmental biology, plant stem-cell biology, pattern formation and more (Dolan et al., 1993; Müller & Grossniklaus, 2010).

2.2 Longitudinal zonation

Three zones can be distinguished when looking at the external morphology of the primary root tip. They are not strictly divided, but they overlap (Dolan et al., 1993). The meristematic zone (Dolan et al., 1993), or the division zone (Salvi et al., 2020), is on the root's distal end and is overlaid by the root cap. This zone is characterised by small cells, active cell division, and the present stem cell niche. In this order, the following zones are the elongation zone and the differentiation zone (Dolan et al., 1993; Salvi et al., 2020). The elongation zone (EZ) is where cells grow fast in length but keep almost the same width, with growth being focused primarily in one direction (Salvi et al., 2020; Verbelen et al., 2006). In the differentiation zone, cells stop growing, mature and differentiate to their final form (Dolan et al., 1993; Salvi et al., 2020). However, the transition zone (TZ) can also be distinguished, located between the meristematic zone and the zone where cells are rapidly starting to elongate (Di Mambro

et al., 2017; Pacifici et al., 2018; Verbelen et al., 2006). Formed postmitotic root cells from the meristem gain the ability to elongate intensively after they transverse through the transition zone (Verbelen et al., 2006) while losing their ability to divide (Pacifici et al., 2018). This boundary between cells that divide and cells that start to elongate and differentiate is called the developmental or transition boundary (Di Mambro et al., 2017; Salvi et al., 2020). Cell wall alterations (Di Mambro et al., 2017) and slow growth in length and width take place there (Verbelen et al., 2006), as well as the rearrangement of the actin cytoskeleton and filaments, preparing cells for rapid elongation (Arieti & Staiger, 2020). Cells on the distal side of the transition zone have retained the ability to divide, functioning as a reservoir of developmentally plastic cells. This allows the root to react to endogenous and exogenous stimuli and adapt its speed and direction of growth (Verbelen et al., 2006).

2.3 Radial axis

The root tip is covered by a root cap tissue divided into columella cells in the centre and the lateral root cap cells surrounding the columella and the root meristem (Dolan et al., 1993; Heimsch & Seago Jr., 2008). The root cap protects the root, has a secretory function and is part of the reception and transmission of environmental signals (Leitz et al., 2009; Cai et al., 2013). In comparison to the root itself, the size of the root cap is determined. While new cells are generated by the meristem, old peripheral cells are disposed of, thus creating a dynamic system of cell turnover (Fendrych et al., 2014). This system is ensured throughout the different taxa of plants by various mechanisms. Examples are the organised programmed cell death of the lateral root cap, resulting in detachment of the entire layer together with the columella cells, described in *Arabidopsis thaliana* (Fendrych et al., 2014), or changes in the cell wall composition documented in cereals or legumes (Durand et al., 2009; Mravec et al., 2017). During these processes, border-like cells or border cells are released into the rhizosphere (Durand et al., 2009; Fendrych et al., 2014; Mravec et al., 2017).

Transverse sections at different distances up from the root cap show that roots comprise specialised concentric layers (Di Mambro et al., 2017; Dolan et al., 1993). The epidermis is the outermost layer of the root that is in direct contact with the soil (Dolan et al., 1993). This layer is composed of two types of cells, hair-forming cells trichoblasts and non-hair cells atrichoblasts. The relative position to the adjacent cells of the next layer of the cortex determines which one of these types will form. Although the number of cells in the layer of the epidermis is conservative, it can vary along the length of the root due to factors of the environment (Dolan et al., 1994). The third and innermost cortical layer is the endodermis (Dolan et al., 1993), where the endodermal apoplastic diffusion barrier is present in the form of Casparian strips. These structures, formed from lignin typical for other plant cell types or lignin-like polymers, are part of the endodermal cell walls, forming a ring (Naseer et al., 2012). Under the endodermis is the pericycle, which gives rise to lateral roots (Dolan et

al., 1993; Dubrovsky et al., 2000). It also forms the outer layer of the diarch central cylinder, which is made from a stele consisting of vascular tissues, xylem and phloem elements. The mature root can go through the process of secondary thickening when the vascular cambium between the primary xylem and phloem forms an oval ring and produces secondary xylem and phloem (Dolan et al., 1993).

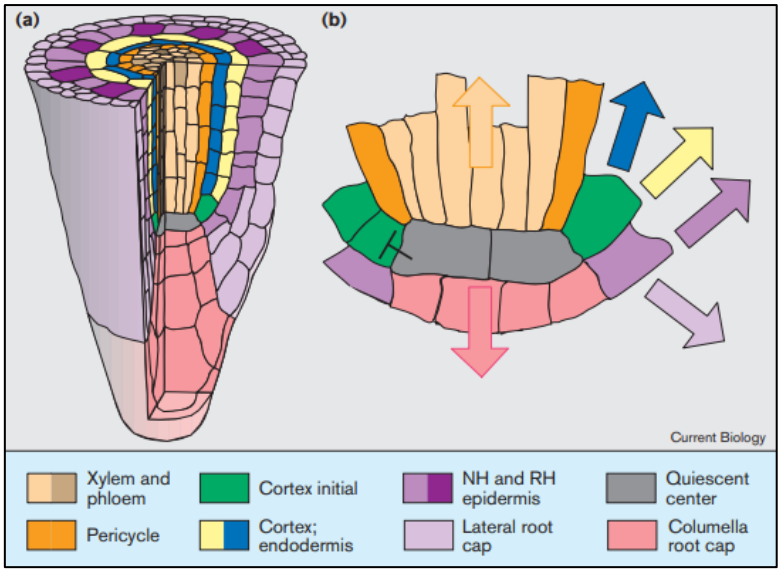

Figure 1 Schema of the cross-section of the root tip. **(a)** Radial layers of cell types. **(b)** Root initials and their division direction, adapted from (Benfey & Scheres, 2000).

The medial longitudinal section shows how cells forming different functional types of tissues are formed from the apical root meristem activity (Figure 1) (Dolan et al., 1993; Heimsch & Seago Jr., 2008). The root apical meristem is a reservoir of undifferentiated cells whose function is to sustain growth and differentiation (Baum et al., 2002). The meristem in *Arabidopsis thaliana* has a closed organisation and is arranged into three tiers of stem cells. The lower tier is formed by initials that give rise to the cells of the root cap and epidermis. They form two types of root cap tissues, the central columella and lateral root cap (Dolan et al., 1993; Heimsch & Seago Jr., 2008). The middle tier contains the initials of the cortex and endodermis and four inactive central quiescent cells. The upper tier is contiguous with cells of the stele. All tissues of the central cylinder are derived from initials from this tier of cells (Dolan et al., 1993). Stem cells divide anticlinally and asymmetrically so that one of the daughter cells can give rise to the relevant tissue, and the second cell retains the character of the initials (Baum et al., 2002). The lateral root's organisation of layers and overall structure is similar to the primary root, although variability in the number of cell files forming each tissue is greater here (Dolan et al., 1993). They arise endogenously from the pericycle in the differentiation zone. Their growth is initiated by the periclinal and anticlinal divisions of the pericycle layer cells and their subsequent formation of the lateral root primordium and meristem (Dolan et al., 1993; Dubrovsky et al., 2000).

2.4 Apoplast

Apoplast can be defined as the intercellular space outside the plasmalemma. Therefore, it includes all the compartments of plant tissues beyond it. It constitutes the space and components of the cell walls, the xylem and the rhizoplane, including the cuticle and the mucilage on the outer side of the root surface (Mcnear, 2013; Sattelmacher, 2001). It also includes gas, water and the enzymatic and nonenzymatic components like solutes and proteins, which are present in the xylem and intercellular spaces (Barbez et al., 2017; Mcnear, 2013; Planes et al., 2015; Sattelmacher, 2001; Y. Yu et al., 2023).

The apoplast thus represents an internal environment with several distinct essential functions. Throughout different processes in plants and their apoplast, dynamic changes happen in the apoplastic contents. Changes in the pH, presence of minerals, hormones, ions and therefore the ionic properties, as well as in the proteins and activity of the enzymes and their binding properties, occur there. If not disrupted, their coordination ensures the correct functioning of processes that include the apoplast (Almeida & Huber, 1999; Planes et al., 2015; Y. Yu et al., 2023). Also through these changes, the apoplast plays a key role in root-developmental processes, like its elongation, differentiation and gravitropic growth (Barbez et al., 2017; Caesar et al., 2011; Großeholz et al., 2022). Changes in the apoplast chemical properties are crucial in plants' many signalling pathways (L. Li et al., 2021; Miao et al., 2021; Y. Yu et al., 2023).

2.4.1 Rhizosphere

An important part of the apoplast is the interface between the root and the environment, called the rhizoplane, which includes the rhizodermis, the mucilage and the cuticle located on the root surface (Mcnear, 2013; Sattelmacher, 2001). The cuticle is present only on the very first layer of the root cap of young primary roots and emerging lateral roots, protecting the meristem. Its structure is analogous to the leaf cuticle, consisting of lipid components (Berhin et al., 2019). The root cap cells also secrete a substance rich in polysaccharides called mucilage (Diehl et al., 2023; Durand et al., 2009), which has numerous beneficial functions. For instance, it helps the root penetrate the soil by reducing friction (Mckenzie et al., 2013) or helps facilitate the water flow, supporting water uptake (Ahmed et al., 2014; Zarebanadkouki et al., 2019). The Rhizoplane is considered a part of the rhizosphere (Mcnear, 2013). Now, the rhizosphere can have a broad definition. It can be divided into three zones determined according to their relative proximity to and the level of influence of the root. The endorhizosphere refers to the inner zone, which includes a part of the cortex and the endodermis. The rhizoplane represents the medial zone, and the outer zone is defined as the ektorhizosphere, covering the space from the rhizoplane into the bulk soil. The rhizosphere cannot be strictly spatially defined due to the root complexity (Mcnear, 2013). From this point on, the rhizosphere refers only to the outer zone.

3 Root growth

The apoplast pH plays a crucial role in the plant's root growth, thus being of interest in studying plant regulatory mechanisms (Barbez et al., 2017). An essential role in the growth modulation plays proton pumping, with H^+ATPases being the main force of the proton extrusion into the extracellular space. Their function also establishes the membrane potential, which can be used by other ion channels (Falhof et al., 2016; Palmgren & Nissen, 2011). The proton pump action is modified by various signalling molecules (Ren et al., 2018), hormones (Barbez et al., 2017), and kinases (Haruta et al., 2014) that directly or indirectly affect their phosphorylation status. These molecules influence together forms a complex regulatory system. At the centre of the root growth regulation stands the long-debated acid growth theory.

3.1 Acid growth theory

After numerous attempts to discover the unknown growth-promoting substance in plants, Frits Went, using excised *Avena sativa* coleoptile tips on agar blocks, which obtained this substance through diffusion, captured a chemical signal that induced the coleoptiles' bending. This signal was later, through other analyses, named and identified as the hormone auxin, indole-3-acetic acid (IAA) (Abel & Theologis, 2010).

Foundations of the acid growth theory were laid on the discoveries by Rayle & Cleland (1970) and Hager et al. (1971), who proposed that the hormone auxin activates the membrane-bound ATPase, which leads to higher proton concentration in the cell wall, activation of the cell loosening proteins and ultimately resulting in the acidification of the apoplast and growth. Since then, it has been shown that auxin indeed plays a key part in acid growth with its diverse effects and functions, like in the process of the apoplastic pH regulation by the regulation of H^+ATPases action (Barbez et al., 2017; Di Mambro et al., 2017; Hager et al., 1971; Salvi et al., 2020). However, the auxin effect differs, which is prominently shown at the root bending. It also works in a concentration-dependent manner (Barbez et al., 2017). Because of these different functional mechanisms between plant organs and even species, the model of the acid growth theory is still evolving (Arsuffi & Braybrook, 2018). A crucial step in the acid growth theory is the protons extrusion that causes the apoplast acidification, which in turn drives cell growth (Arsuffi & Braybrook, 2018; Barbez et al., 2017; L. Li et al., 2021). Essential for this process is the acidic activation of proteins that cause the cell wall to loosen (Cosgrove, 2016; Pacifici et al., 2018).

3.1.1 Cell wall and its remodelling

The cell wall remodelling is enabled, besides other catalysts like xyloglucan endotransglucosylase/ hydrolases (Y.-B. Liu et al., 2007), through cell wall proteins named expansins that catalyse stress relaxation and loosening of the cell wall, allowing cell extension and enlargement without a lytic

activity, allowed by reversibly disturbing the noncovalent bonds and slippage (S. McQueen-Mason & Cosgrove, 1994; S. J. McQueen-Mason & Cosgrove, 1995) between cellulose microfibrils and matrix polysaccharides (S. J. McQueen-Mason & Cosgrove, 1995). Expansin proteins represent a vast group with diverse functions and different representations across plant species that can be phylogenetically divided into four main families. The first two that constitute the greater part are the α-expansins (EXPA) and β-expansins, while the smaller families are expansin-like A and expansin-like B. The EXPA family is the largest among them, having an important role in cell wall loosening (Sampedro & Cosgrove, 2005) at the root and its acidic growth (Pacifici et al., 2018), one of the factors that influence EXPA's function being changes of the pH of the apoplast (S. J. McQueen-Mason & Cosgrove, 1995; Pacifici et al., 2018). Expansins cause cell wall loosening, thus enabling cell elongation and differentiation (S. J. McQueen-Mason & Cosgrove, 1995; Pacifici et al., 2018). If the function of the EXPA proteins is impaired, cell wall loosening and the differentiation processes are disturbed, resulting in a change in the transition zone position (Pacifici et al., 2018).

An important aspect of studying the expansin action is the architecture of the primary cell wall. Formerly used models described the CW as cellular microfibrils linked with high contact by the key hemicellulose xyloglucan, together forming a load-bearing network (Pauly et al., 1999). More recent studies refute this concept by revealing that xyloglucan plays a smaller role in CW mechanics and is connected to cellulose only in small regions (Park & Cosgrove, 2012b, 2012a). This newer model shows that the strength of the CW is dependent on the xyloglucan presence and that it is important for the expansins action, but its deficiency has a minor influence on the plant development, its function being taken over by other matrix components (Park & Cosgrove, 2012b; T. Wang et al., 2013).

Highly specific and limited cellulose domains, functioning as biomechanical hotspots rich in xyloglucan, were determined as targets of the expansins binding in *Arabidopsis thaliana* (Park & Cosgrove, 2012a; T. Wang et al., 2013). The cellulose microfibrils in these regions are in close contact. The cellulose bound by the expansins has an altered conformation, as opposed to the bulk cellulose. The binding weakens the noncovalent bonds of cellulose and matrix polysaccharides, resulting in the loosening of the CW (T. Wang et al., 2013). Acidic pH influences the cell wall polysaccharides' conformational and chemical properties, resulting in calcium crosslinks disruption in homogalacturonan, inducing other dynamical and structural changes such as weakening the interaction between cellulose and pectin or increase in hydration of the CW components, which facilitate the polysaccharide slippage as a consequence of the cell loosening (Phyo et al., 2019).

3.2 H^+ATPases

Plasma membrane H^+ATPase present in fungi and plants belongs to the P-type ATPases superfamily of ions and lipid integral membrane protein pumps, specifically to the P3A-ATPases subfamily and

subgroup, defined by transport of one H^+per one ATP hydrolysis (Palmgren & Nissen, 2011). It is characterised by the cycle of phosphorylation and dephosphorylation, when the covalent phosphorylated intermediate is formed by the reaction of the γ-phosphate of ATP with the pump aspartic acid residue, causing conformational changes and thus driving transport of the protons (Focht et al., 2017; Pedersen et al., 2007). The H^+ATPase is an electrogenic pump, which translocates the H^+protons from the cell without a counter transport. This forms a steep transmembrane chemical proton gradient (ΔpH) (Falhof et al., 2016; Palmgren & Nissen, 2011) and establishes the electrical gradient (ΔΨ) (Falhof et al., 2016). That results in extracellular acidification and negative membrane potential on the inside of the cell. These processes energise the plasma membrane, providing the electrochemical energy for other solute secondary transport in the plant PM and driving cell growth (Falhof et al., 2016; Planes et al., 2015). This electrochemical gradient is commonly referred to as the proton motive force (PMF), generated and maintained by the plant's primary transporters (Andersen et al., 2023). The PMF impairment negatively affects the root growth (Haruta & Sussman, 2012).

3.2.1 AHA isoforms

The number of P3A-ATPases present across the plant species differs. There were identified 46 genes of P-type ATPases in *Arabidopsis thaliana*, with 11 of them encoding isoforms of the plasma membrane H^+ATPases, denoted AUTOINHIBITED H^+ATPASE 1 (AHA1) to AHA11, which can be further divided, depending on their sequence order and intron positions, into five clusters (Baxter et al., 2003). Predominant isoforms expressed in the root are AHA1 and AHA2, with overlapping and complex functions (Haruta et al., 2010; Haruta & Sussman, 2012). Loss-of-function mutation of both AHA1 and AHA2 isoforms is lethal for the plant embryo (Haruta et al., 2010), indicating its crucial role in ensuring the electrochemical gradient of the plasma membrane through the plant development for the formation of the embryo. On the contrary, a copy of one of the isoforms is enough to sufficiently maintain the protonmotive force, suggesting redundancy in these genes' function (Haruta et al., 2010). Double heterozygous mutants of these proton pumps exhibit larger meristem and whole roots compared to the wild type and impaired acidification ability (Pacifici et al., 2018). In knockout mutants of *aha1* and *aha2*, changes in various gene expressions occur, pointing to their complex influence on plant processes. Expression of genes involved in nutrient stresses was altered in *aha2* mutants, whilst in *aha1*, the genes were related to the metabolism of lipids. AHA2 has a role in maintaining the membrane potential and ΔpH, which form the proton motive force. This is supported by the fact that the phenotype changes in *aha2* happened in conditions closely connected to the PMF (Haruta & Sussman, 2012). Mutants *aha1* and *aha2*, when exposed to various stresses, demonstrate overall reduced catalytic activity in roots. This impaired protonmotive force in *aha2* manifests in resistance to soil toxic compounds. Hence, the mutant plants grow better than wild-type in such conditions. There

are no visible growth alterations in phenotype in ideal conditions. Differences in response to the various stresses between these two isoforms can be attributed to their expression within the root, where AHA2 is located more in the epidermal cells than AHA1 (Großeholz et al., 2022; Haruta et al., 2010).

These isoforms are present together with the AHA11 in all tissues. Except these three, another five AHA paralogs are expressed in the *Arabidopsis* roots, specifically AHA 3, 4, 7, 8 and 10 (Ueno et al., 2005), with AHA7 being another dominant H^+ATPase, highly expressed in root epidermal cells and root hairs, during their emergence and growth at their tip (Hoffmann et al., 2019). The root hair growth is regulated by both AHA7 and AHA2, utilising their different levels of ATP hydrolytic activity depending on the extracellular pH. In acidic conditions, when the pH is lower than 6.0, the AHA7 pump activity stops. The activity of AHA2 is high even in a more acidic environment, with optimum at 6.4 and still functioning at 4.5. This points to the AHA7 being responsible when the AHA2 is absent (Hoffmann et al., 2019). Thus, different AHAs can and do vary in their function and expression across plant tissues (Baxter et al., 2003; Haruta et al., 2010; Hoffmann et al., 2019) and development (Haruta et al., 2010).

3.2.2 Structure and mechanism

The structure of the H^+ATPase AHA2 of *Arabidopsis thaliana* was described by Pedersen et al. (2007) using an X-ray crystallography, model later improved by Focht et al. (2017). The protein pump consists of a transmembrane region of 10 α-helices, denoted M1 to M10, and a cytoplasmic region separated into three domains, namely the phosphorylation domain P, the nucleotide-binding domain N, and the actuator domain A, structures present also in other P3-type ATPases (Toyoshima & Nomura, 2002) supporting the assumption of conserved architecture throughout the subfamilies (Pedersen et al., 2007). The P3-type ATPases transmembrane region is always formed by the flexible transport domain of six core helices (Palmgren & Nissen, 2011; Toyoshima & Nomura, 2002). In addition, it can also have a rigid support domain with a specialised function, which is present in the H^+ATPase, consisting of four helices (Palmgren & Nissen, 2011; Toyoshima & Nomura, 2002). However, a fourth cytoplasmic domain, the autoinhibitory or regulatory domain R, is often present (Palmgren & Nissen, 2011). The R domain function is to inhibit the proton pump, apparently by wrapping around the A domain and stopping its rotation, thereby preventing entry of the protons to the binding site of the transmembrane region (Pedersen et al., 2007). Posttranslational phosphorylation and dephosphorylation of multiple sites, induced by various factors, modulate the proton pump activity, providing a flexible system (Haruta et al., 2014; Piette et al., 2011; Spartz et al., 2014). The penultimate Thr residue, crucial in the proton pump function, is located at the C-terminal region of the autoinhibitory domain. Its phosphorylation activates the H^+ATPase and creates a binding site for the 14-3-3 proteins. Their

binding to the residue stabilises the proton pump active state, representing a mechanism of regulation (Jahn et al., 1997; Piette et al., 2011).

3.3 Auxin

It was discovered that auxin acts through two different signalling pathways (L. Li et al., 2021). The first, which is longer studied and more understood, is referred to as the canonical TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX, shortly TIR1/AFB signalling pathway, which acts through transcriptional regulation (Gray et al., 2001). While auxin promotes apoplast acidification in the hypocotyl (Fendrych et al., 2016), its signalling pathway activation in the root, on the contrary, results in the apoplast alkalinisation (L. Li et al., 2021), thus working in a tissue-dependent manner. However, the *Arabidopsis thaliana* root reaction to the application and removal of the phytohormone auxin results in rapid growth rate modulation, which excludes more time-consuming processes like transcription and translation (Fendrych et al., 2018). Monshausen et al. (2011) speculated about a pathway independent of the TIR1/AFB signalling when describing the rapid mediation of root surface pH. Treatment with 0.1-1 µM IAA resulted in the immediate root surface alkalinisation at the EZ, which then continued to the meristematic region, accompanied by a decrease of cytosolic pH, signalling proton fluxes across the PM activated by auxin (Monshausen et al., 2011). This rapid response was later clarified when the non-transcriptional signalling branch of the canonical pathway was proposed and described (Dubey et al., 2023; Fendrych et al., 2018; Serre et al., 2021) in processes like reversible inhibition of root growth (Fendrych et al., 2018), or in the apoplast alkalinisation of roots (L. Li et al., 2021). An important role in this rapid response pathway has the auxin receptor AFB1, which is essential for membrane depolarisation and growth inhibition (Dubey et al., 2023; Serre et al., 2021). The importance of the intracellular signalling of TIR1 together with AFB1 in the regulation of the apoplast alkalisation and growth inhibition response is supported by the fact that *the tir1-10* and *afb1-3* mutants, as well as the *tir1 afb2 afb3* triple mutant, were resistant to the auxin effect (L. Li et al., 2021).

The second auxin signalling mechanism is the TRANSMEMBRANE KINASE1-mediated (TMK1) non-canonical auxin pathway, which directly regulates the activation of the H^+ATPases through the control of the phosphorylation status of the penultimate Thr residue (L. Li et al., 2021; Lin et al., 2021). TMK1 is an auxin cell surface receptor, the activation of which results in apoplastic acidification. Supporting this is the fact that the *tmk1* mutant is not able to induce the residue phosphorylation as efficiently (L. Li et al., 2021). Important molecular actors and the auxin coreceptors in this signalisation are the AUXIN-BINDING PROTEIN 1 (ABP1) primarily localised to the ER but also present in the apoplast (Friml et al., 2022; Y. Yu et al., 2023) and in this process recently described ABP1-like proteins, present exclusively in the apoplast, ABL1 and ABL2 (Y. Yu et al., 2023). ABP1 specifically binds auxin at a preferable acidic pH of 5.5, which is typical for the apoplast. This receptor is required to activate the

TMK1 signalling at the plasma membrane, thus inducing response to the auxin, as proteins phosphorylation and H^+ATPase activation. *abp1* mutants show several defects in auxin-related processes, supporting its role in its binding (Friml et al., 2022). The ABL1 and ABL2 also work in an auxin-dependent manner, interacting with the TMK1 extracellular domain, with their function overlapping but still distinct from the ABP1. The phenotypes of all three coreceptors' possible mutants suggest that the two ABL1-like proteins play a bigger role in the auxin signalling, with ABP1 having a minor function (Y. Yu et al., 2023).

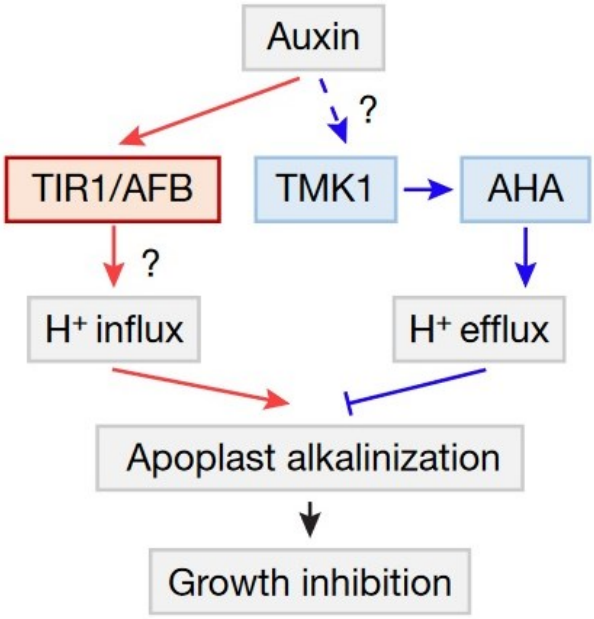

Figure 2 Modulation of the root apoplastic pH and growth by the antagonistic function of two auxin signalling pathways. TIR1/AFB (red) pathway induces the proton influx. TMK1 (blue) pathway activates AHAs and induces proton efflux, adapted from (L. Li et al., 2021).

L. Li et al. (2021) described how these two auxin signalling pathways work together regarding root growth modulation through the change of the apoplastic pH (Figure 2). Using the HPTS pH-sensitive dye revealed that the apoplastic pH decreases from the TZ to the elongation zone. Treatment with IAA triggers a rapid increase of the extracellular pH of cells independent of their position due to the net cellular H^+influx. This is simultaneously accompanied by root growth inhibition. Hence, manipulating apoplastic pH through medium change reveals that alkalization inhibits root growth while re-acidification promotes it. Application of auxin induces phosphorylation of activation sites of the H^+ATPases using the TMK1 signalling, which should lead to acidification. However, instead, the result is its alkalisation, pointing to the existence of an antagonistic system that causes this outcome. This system is the mentioned non-transcriptional signalling branch of the canonical pathway (L. Li et al., 2021). Fusicoccin (FC) is a fungal phytotoxin (Ballio et al., 1964), which induces the PM H^+ATPases activation by binding to and stabilising the proton pump and 14-3-3 protein complex (Jahn et al., 1997;

Olivari et al., 1998). The FC application resulted in rapid acidification and promotion of growth, while FC and auxin used simultaneously and consecutively caused pH response in respect of their ratio. This observation shows that the activation of H^+ATPases counteracts the auxin-induced apoplast alkalisation. Thus, the TIR1/AFB1 receptors, more dominant as the result of auxin application resulted in the alkalisation, work antagonistically to the TMK1-mediated H^+ export, together forming a mechanism allowing flexible mediation of the root growth (L. Li et al., 2021).

3.3.1 SAUR and PP2C.D interaction

SMALL AUXIN UP-RNA (SAUR) proteins represent a group of early auxin-induced genes with a cell growth-promoting function (Abel & Theologis, 2010; Nagpal et al., 2022; Spartz et al., 2014). The process of cell growth and expansion is modulated by the SAUR proteins' ability to inhibit through direct interaction the D-clade of protein phosphatases type 2C (PP2C.D) (Ren et al., 2018; Spartz et al., 2014), both showing a functional redundancy (Yin et al., 2020). The PP2C.D subfamily is responsible for the negative regulation of PM H^+ATPases phosphorylation status (Ren et al., 2018; Spartz et al., 2014; Wong et al., 2021). Its members differ in their localisation within the cell and its compartments, and their abundance increases in places with ongoing growth processes (Ren et al., 2018). PP2C.D2, PP2C.D5 and PP2C.D6 are three exclusively PM localised members with a significant role, as they are the primary effectors of PM localised SAUR proteins in the cell expansion (Ren et al., 2018). This antagonistic mechanism was described using several SAUR and PP2C.D group members. Overexpression of SAUR19 results in the proton pump's increased activity through stimulation of the C-terminal autoinhibitory domain phosphorylation. SAUR proteins inhibit the PP2C.D phosphatases by binding to them, therefore preventing their negative regulation of H^+ATPases. This results in a shift towards proton pumps' phosphorylated and active state. The SAUR-PP2C.D interaction thus represents a mechanism that allows modulation of the AHA phosphorylation status and, therefore, the cells' expansion (Spartz et al., 2014). Overexpression of PP2C.D5 results in reduced cell expansion. However, the overexpression of SAUR19 fused with the GREEN FLUORESCENT PROTEIN (GFP) overpowers this effect, providing more evidence of their antagonistic function (Ren et al., 2018). Induction of the GFP-SAUR19 expression results in longer root EZ epidermal cells (Barbez et al., 2017). Another example is the SAUR15, described to facilitate the formation of the lateral and adventitious roots of *Arabidopsis thaliana* (Yin et al., 2020), or the PP2C.D1 interaction with the AHA2 and dephosphorylation of the Thr-947 (Spartz et al., 2014).

SAUR and PP2C.D antagonistic mechanism was also described in hypocotyls (Fendrych et al., 2016) or in the stomatal movements regulation, where SAUR inhibits the PP2C.D phosphatases by affecting the K+ transport channels and altering the PM H^+ATPase activity (Wong et al., 2021). SAUR proteins and two PP2C.D members are upregulated by high temperature, representing another way of

regulation, preventing overgrowth and overexpansion (Ren et al., 2018). SAUR genes' expression increases due to auxin presence, providing another auxin link with PM H^+ATPases (Ren et al., 2018; Spartz et al., 2014). Auxin induces the expression of the SAUR genes, which peaks 30 minutes after the treatment. At the same time, it also slowly induces the expression of two members of the PP2C.D genes subfamily. So, the effect is delayed compared to the SAUR gene (Ren et al., 2018). AUXIN RESPONSE FACTORS ARF6,8 and ARF7,19 bind to the SAUR15 promotor, inducing its expression (Yin et al., 2020). Except for the inhibition of PP2C.D phosphatases to drive the cell expansion, SAUR15 also promotes the accumulation of auxin, potentially by positively influencing its biosynthesis, resulting in an increased concentration of free auxin, which induces signal transduction. These two mechanisms thus form a positive feedback loop that can trigger lateral root and adventitious root formation (Yin et al., 2020).

3.3.2 Root surface pH

We can also observe the pH of the root surface, which differs between root zones and opposing root flanks during the vertical growth and the gravitropic response (Monshausen et al., 2011; Serre et al., 2023; Staal et al., 2011). Monshausen et al. (2011) visualised the surface pH of roots exposed to a pH-sensitive compound, fluorescein-dextran, using confocal microscopy with computer vision-based image analysis. It showed dynamical changes along the root during the vertical growth and gravistimulation, supporting the acid growth theory (Monshausen et al., 2011). They manifested as complex periodic fluctuations between extracellular alkaline or acidic states, as alkalic bursts which travelled shootward along the root or as changes of pH between the root opposite flanks. Root surface pH was also increased against the medium pH, indicating an influx of protons. This differs from the acid growth theory and prediction of steady-state acidification (Monshausen et al., 2011). Serre et al. (2023) described similar pH changes at the transition zone, proposing it to be a mechanism for correcting growth regarding environmental conditions. The uninvasive microelectrode ion flux estimation technique showed that from the more acidic root apex of *Arabidopsis thaliana*, the pH gradually increases to its maximum at the apical part of the TZ, where was also measured the highest H^+ influx, forming an alkaline domain (Staal et al., 2011). From this site, the pH decreases rapidly to the lowest point, at the basal part of the fast elongation zone. Treatment with ethylene precursor 1-amino-cyclopropane-1-carboxylic acid (ACC) inhibits this elongation process by stopping the H^+ efflux. It alkalinises the apoplast, hence worsening conditions suitable for expansin action. Auxin influx transporter AUXIN RESISTANT 1 (AUX1) mutant *aux1-22* is not sensitive to ACC, suggesting that auxin is necessary for the cell's growth inhibition. A study of other auxin transport mutants shows that ethylene influences the auxin concentrations and, therefore, modulates the H^+ATPases activity (Staal et al., 2011).

Supporting these results, a similar pH pattern was recently described using visualisation and quantification methods (Serre et al., 2023). The root surface and rhizosphere pH were visualised using Fluorescein-5-(and-6)-Sulfonic Acid, Trisodium Salt, a pH-sensitive fluorescent dye (Figure 3). These methods highlighted two relatively acidic pH zones and one alkaline zone (Serre et al., 2023). The pH from the acidic region at the root tip increases shootward and peaks at the transition zone, forming an alkalic domain that exceeds the beginning of the elongation zone. After that, the pH rapidly drops, forming an acidic domain, starting at the late elongation zone and continuing through the whole differentiation zone (Serre et al., 2023). The thought that the domains are determined by different AHA ATPases abundance was not confirmed, as their decrease in the alkaline domain was not recorded at the transition zone. The application of the FC resulted in decreased pH of the acidic domains but did not affect the alkaline domain. While the modulation of AHA activity influences the root's overall pH and growth, the alkaline domain cannot be prevented from forming, showing that the proton pumps are not the primary determinant of pH zonation (Serre et al., 2023).

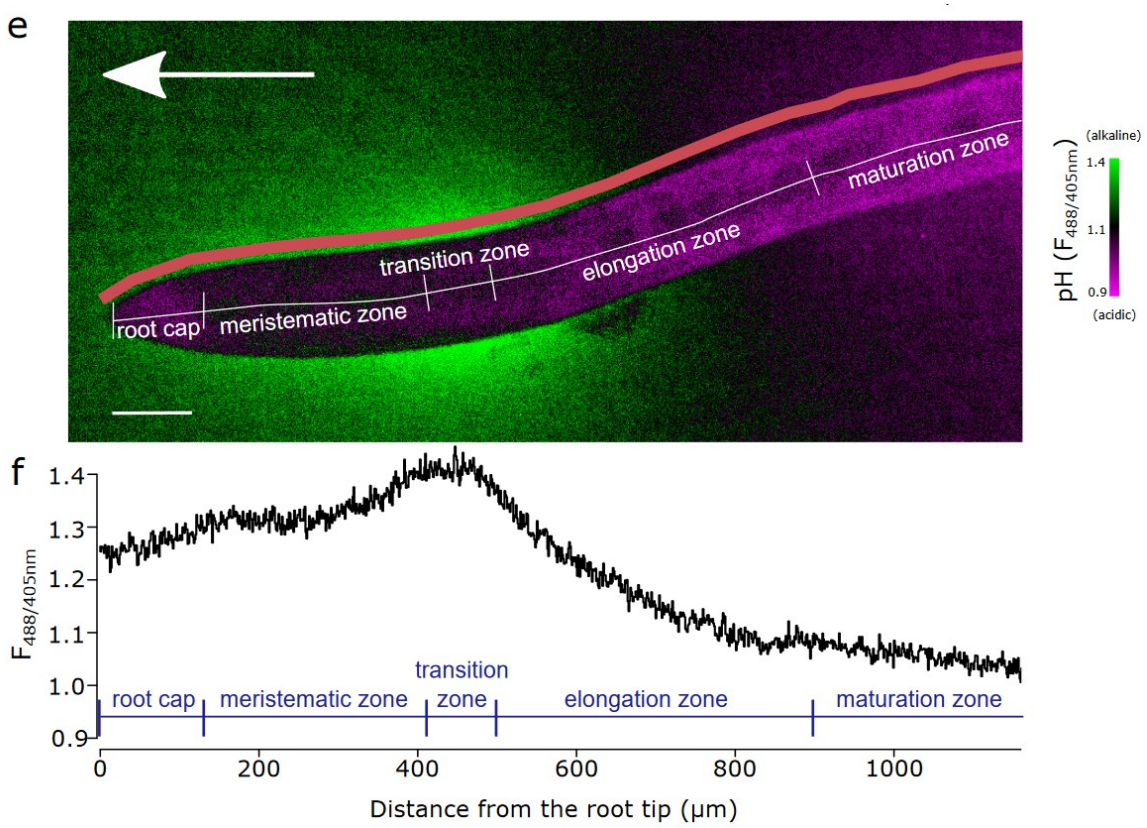

Figure 3 The root surface alkalic and acidic domains, visualised by the pH-sensitive Fluorescein-5-(and-6)-Sulfonic Acid, Trisodium salt (FS) dye. **(e)** Fluorescence of the FS at the $F_{488/405}$ excitation ratio. **(f)** The $F_{488/405}$ excitation ratio at longitudinal root zones, adapted from (Serre et al., 2023).

The establishment of the root surface pH and the TZ alkalic domain was shown to be influenced by auxin transport, redistribution and its influence on the AHAs activity (Monshausen et al., 2011; Serre et al., 2023; Staal et al., 2011). Treatment with 0.1-1 µM IAA results in the immediate surface alkalinisation at the EZ, which then continues to the meristematic region and is accompanied by a

decrease in cytosolic pH (Monshausen et al., 2011). With the increase of auxin level, the alkaline zone can expand to the point when the acidic domain disappears, pointing to the alkalic domain as a hotspot of the auxin response (Serre et al., 2023). A key player in this auxin action was revealed to be the auxin influx carrier AUX1 (Dindas et al., 2018; Monshausen et al., 2011; Serre et al., 2023). The processes that take place after the auxin application, like the membrane depolarisation, H^+ influx and extracellular alkalisation of the root hairs in the differentiation zone of *Arabidopsis thaliana*, are impaired in the AUX1 loss of function mutant (Dindas et al., 2018). The pH fluctuations along the root are significantly weakened, although still present in the *aux1* mutant (Monshausen et al., 2011). The root surface pH is influenced by the fact that the *aux1* cannot fully activate the proton pumps (Serre et al., 2023). The alkaline domain at the transition zone is absent, with a more linear acidification gradient along the root surface showing a predominantly acidic character (Monshausen et al., 2011; Serre et al., 2023). The application of FC causes surface acidification except at the TZ, where a small alkalic domain is established (Serre et al., 2023). These discoveries show that this alkalic domain establishment and root pH surface pattern depend on the AUX1-mediated auxin influx (Serre et al., 2023).

Significant pH changes are also present during the root gravitropic response. Upon gravistimulation, the surface pH of the lower flank of the root increases, becoming more alkalised, while the upper flank becomes acidic, the alkaline character of the TZ domain disappearing (Monshausen et al., 2011; Serre et al., 2023). This pH gradient is established 5 minutes upon stimulation, correlating with the bending initiation (Serre et al., 2023). Auxin accumulates at the lower flank of the root, alkalising the apoplast, stopping its growth and causing its bending (Barbez et al., 2017). The pH changes are missing in the *aux1* mutant, which does not react to the gravistimulation (Serre et al., 2023).

3.3.3 Concentration and time-dependent effect of auxin on root growth

Although the general root reaction to auxin application is alkalisation (L. Li et al., 2021; Monshausen et al., 2011), discoveries made regarding the auxin effect on the apoplastic pH during cell growth and gravitropic response suggest that it works besides the tissue-dependent manner (Fendrych et al., 2016; L. Li et al., 2021) also in a concentration and time-dependent manner (Barbez et al., 2017; Evans et al., 1994). The short-term effect of exposure to low picomolar auxin levels is root apoplast acidification and rapid growth promotion (Barbez et al., 2017; Evans et al., 1994; Vanderhoef & Dute, 1981). This rapid growth effect is transient, as in a long-term slower and steady-state growth rate is established (Vanderhoef & Dute, 1981). On the other hand, auxin concentrations above the nanomolar level and long exposure cause growth inhibition (Barbez et al., 2017; Evans et al., 1994). When looking at the epidermal cells at the start of the elongation zone, low endogenous auxin concentration and nuclear

auxin signalling are essential for the apoplast acidification and the beginning of elongation, after which the pH returns to its original value. Impairment of the auxin signalling pathway disrupts these processes, as well as exposure to an alkalic medium, resulting in shorter roots (Barbez et al., 2017).

However, roots treated with high levels of auxin react with the transient alkalisation phase followed by the acidification phase. This effect was described after putting *Arabidopsis* seedlings on medium with 250nM IAA. After two hours upon treatment, marking the end of the alkalisation, the pH decreases again to a similar value as the mock-treated seedlings. Longer exposure to high-level auxin later results in even more significant acidification than the control. This transient biphasic effect is also present after an increase in the endogenous auxin levels. This effect causes root growth inhibition, which occurs after the alkalisation phase and lasts even after the acidification (Barbez et al., 2017). It was suggested that the PM-localized receptor like kinase FERONIA (FER) is needed in this inhibition process, as it is disturbed in the *fer4* mutant (Barbez et al., 2017). This was proved incorrect, as it was shown that the auxin-induced growth inhibition is not dependent on FER. However, signalling by FER and its peptide ligand RAPID ALKALINIZATION FACTOR 1 (RALF1) contributes to it (L. Li et al., 2022). The *fer4* response to auxin might result from disturbed internalisation of PIN-FORMED auxin transporters (PINs), which FER controls (Barbez et al., 2017; L. Li et al., 2022; M. Yu et al., 2020). RALF1 and auxin signalling pathways, which are independent of each other, converge. RALF1 has a biphasic action. Within 1 minute of its 10µM application, RALF1 alkalinise root apoplast, reversibly inhibiting primary root growth. This alkalinisation results from rapid H^+ influx mediated by FER. The second phase of the RALF1 action is 1 hour delayed. RALF1 induces the upregulation of auxin biosynthesis through the canonical TIR1/AFB pathway, thus contributing to sustained growth inhibition. This shows that RALF1 ensures plant root response to immediate environmental stimuli and, in the long term, influences growth and development (L. Li et al., 2022). FERONIA also has a role in the gravitropic response. *Fer4*-mutant shows higher pH and less precise direction of growth regarding the gravitropic vector, although the root growth itself is not impaired. This shows the importance of the transient alkalisation at the beginning of the gravitropic response process. The role of H^+ATPases in these processes was not tested in this case (Barbez et al., 2017).

The root surface's and extracellular environment's pH visualisation showed that they differ (Barbez et al., 2017; Monshausen et al., 2011; Serre et al., 2023), suggesting that the plant is capable of regulating these spaces separately. While the apoplastic space at the transition zone exhibits an acidic character, with pH gradually decreasing through the longitudinal zones from the root tip to the EZ (Barbez et al., 2017; Großeholz et al., 2022), the root surface TZ is characterised by its alkalic domain, with relatively acidic domains being at the surface of root meristematic zone and the late EZ and differentiation zone (Serre et al., 2023). All these discoveries show that auxin roles and influences on the root form a highly complex and dynamic regulatory system, challenging to study for its complicated

effects, dependent except the auxin concentration also on the time scale of its effects (Barbez et al., 2017).

3.4 Brassinosteroids

The H^+ATPases activity is also modulated by the phytohormones brassinosteroids (BRs) and the canonical BRs signalling, influencing the root growth through the slow response pathway, including the gene expression regulation (Großeholz et al., 2022; Kim et al., 2009), and through the fast BR response, resulting in the proton pumps upregulation (Caesar et al., 2011; Minami et al., 2019). The genomic pathway influences processes like the rate of growth or cell shape, regulating overall root meristem establishment (Fridman et al., 2021). The fast response pathway of BR signalisation primarily occurs within the cell PM (Caesar et al., 2011; M. Li et al., 2022). Treatment with 0.1 nM to 10 nM of brassinolide results in apoplastic acidification and PM hyperpolarisation at the epidermal cells of the EZ, influencing the cell elongation and expansion (Caesar et al., 2011; Großeholz et al., 2022). This is achieved by the rapid upregulation of the H^+ATPases activity through the induction of the penultimate Thr residue phosphorylation and the 14-3-3 protein binding to the proton pump (M. Li et al., 2022, p. 15; Minami et al., 2019). Without specifics of the mechanism, it was proposed that this proton pump activation is induced through the AHAs BR-dependent direct interaction with the BRASSINOSTEROID-INSENSITIVE 1 (BRI1) (Caesar et al., 2011). Later, another study described an essential role in this process of the SAUR proteins, specifically the SAUR9 and SAUR19, but under the control of the BRI1 and BRASSINOSTEROID INSENSITIVE 2 (BIN2) signalling pathway and without the direct BRI1-AHA interaction. The application of brassinolide causes the SAUR accumulation, which inhibits the PP2C.D phosphatases, enhances the binding of the 14-3-3 proteins to AHAs, as well as positively affects the transcription of KAT1, the K^+channel, and the EXPLA2, the member of the expansin-like A family, inducing the cell wall loosening and water uptake (Minami et al., 2019).

More recent discoveries also showed SAUR as the essential part of the proton pump activation, but this time upstream of the BRI1 (M. Li et al., 2022). SAUR15, also having the role in the auxin-induced lateral roots growth (Yin et al., 2020), interacts in a BR-dependent manner with BRI1, enhancing its phosphorylation, thus inducing the formation of the complex with BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE (BAK1) (M. Li et al., 2022). This SAUR15 and BRI1 interaction then promotes the direct BRI1 interaction and activation of the H^+ATPases, independent of the BIN2-mediated signalling pathway (M. Li et al., 2022). Using a pH indicator, the *bri1-301* and *bri1-701* mutants show increased apoplast pH, compared to the WT, and decreased levels of AHA phosphorylation (M. Li et al., 2022). The BR signalling is also connected to the FERONIA action, as shown by the already-mentioned regulation of the BRI1 levels at the PM (M. Yu et al., 2020) and the RALF1 peptide (Dressano et al., 2017). The BR regulates gene expression of RLKs that influence the

elongation of the cells, including FERONIA, showing that it has a role in the BR signalling (Guo et al., 2009). BRs are also involved in the inhibition of cell expansion induced by the *Arabidopsis* root-specific RALF1 and that with BAK1, which is necessary for the induction of the RALF1-responsive genes (Dressano et al., 2017).

3.5 Abscisic acid

Abscisic acid (ABA) is another phytohormone that can influence the AHA's penultimate Thr phosphorylation status and regulate their action (Hayashi et al., 2014; Miao et al., 2021). A key role in ABA signalling has negative regulators from the A-clade protein phosphatases type 2C (PP2Cs) (Miao et al., 2021; Planes et al., 2015). Perception of ABA with its receptors results in the inactivation of PP2Cs and activation of downstream signalling factors, including the SNF1-RELATED PROTEIN KINASES 2 (SnRK2s) positive regulators, which induce the ABA-transcriptional response (Planes et al., 2015). Miao et al. (2021) showed that the PP2C.A can directly interact with the proton pumps and induce their downregulation. This interaction was described for the ABSCISIC ACID-INSENSITIVE 1 (ABI1), one of the genes of the PP2C.A inhibitors and an essential part of the ABA signalling pathway. ABI1 directly interacts with the C-terminus of the AHA2, inducing the Thr^{947} dephosphorylation, thus decreasing the H^{+}efflux (Miao et al., 2021). Inhibition of the proton pumps and decreased H^{+}extrusion occurs after application of higher concentration (10 μM)/ 30 μM) of ABA to the roots (Hayashi et al., 2014; Miao et al., 2021; Planes et al., 2015). Low concentrations (0.1 μM) have the opposite effect, inducing the H^{+}ATPase phosphorylation and activation by inhibiting the ABI1, resulting in a longer root phenotype. Hence, the ABA influence appears to work in a concentration-dependent manner (Miao et al., 2021), as also shown in auxin (Barbez et al., 2017). Treatment with ABA also influences the efflux of other ions into the apoplast. Upon its application occurs Cl^{-} and K^{+} efflux. Their cytosolic level decreases, influencing the cell turgor and negatively affecting its growth (Planes et al., 2015). Another mechanism that regulates plant growth through ABA includes FERONIA, which mediates the ABA interaction with the growth-regulating peptide RALF1. The ABA-insensitive 2 phosphatase can directly interact with the FER, inducing its dephosphorylation (J. Chen et al., 2016).

Other phytohormones, like brassinosteroids (Deng et al., 2022) or auxin (Luo et al., 2023), also affect the ABA signalling. The crosstalk of these hormones plays a key role in the regulation of root growth and architecture. ABA and BR interaction helps the plant survive drought stress by controlling stomatal closure and regulating primary root growth. The BRI1 and BAK1 work as negative regulators of the primary growth inhibition induced by ABA to obtain water from deeper levels of soil (Deng et al., 2022). Recently, it was also described that ABI4 negatively influences the cell cycle progression by repression of the cyclin-dependent kinases B2;2/B1;1 expression and auxin biosynthesis, hence inhibiting the primary root growth (Luo et al., 2023).

4 Nutrition

Roots influence the rhizosphere's chemical properties through many mechanisms. These significantly affect nutrients' availability, both directly and indirectly. Rhizodeposition has a prominent role in influencing these properties. Plants exude various compounds like carboxylates and phenolics (McKay Fletcher et al., 2021; Rosenkranz et al., 2021), secrete mucilage (Zarebanadkouki et al., 2019), or release border cells and border-like cells (Durand et al., 2009; Mravec et al., 2017). Through these processes, plants also influence other factors, like the rhizosphere microbiome's composition (Lidoy et al., 2023; Stringlis et al., 2018), toxic compounds availability (Cai et al., 2013), or carbon sequestration (Ahmed et al., 2018; Odell et al., 2008). Chemical properties of the rhizosphere are also influenced by fluxes of ions between the root-soil interface (Santi & Schmidt, 2009).

4.1 Ions fluxes

Transport of ions and membrane potential changes not only accompany the process of root growth but are also an essential mechanism in its maintenance. It increases nutrient availability and has a role in response to abiotic stresses like the ones caused by toxic compounds (Degenhardt et al., 1998; Z. Yang et al., 2019) or nutrient deficiencies (Ligaba et al., 2004; Santi & Schmidt, 2009). At the centre are the H^+fluxes (Degenhardt et al., 1998), mainly mediated by the activity of H^+ATPases (Z. Yang et al., 2019), which function and mechanisms of regulation were described in the chapter Root growth above. But also other transporters participate in proton translocation, like the $Na^+/\ H^+$antiporters (Quintero et al., 2011) or $Cl^-/\ 2H^+$symporters (Felle, 1994), hence regulating the salt intake. Together, the proton fluxes change the pH and the H^+gradient, thus changing the PMF, which is the main force behind the transport of nutrients (Andersen et al., 2023; Haynes, 1990). Essential is the balance of cations and anions uptake, which influences H^+ and OH^- extrusion and, therefore, the acidification or alkalinisation of the rhizosphere and apoplast (Haynes, 1990). Nutrient uptake significantly influences rhizosphere pH (Custos et al., 2020). The rhizosphere's pH determines which nutrients and toxic compounds are available in the soil (Chaignon et al., 2009; Chowra et al., 2017).

4.1.1 Root acidification and its role in response to Pi, Fe and N deficiencies

Plants can be exposed to soil conditions with inorganic phosphate and iron deficiencies. In both cases occurs the process of H^+ efflux into the rhizosphere (Santi & Schmidt, 2009; Yuan et al., 2017). The low Pi signal induces H^+ATPases expression and their posttranslational activation (Yuan et al., 2017). It accompanies the main mechanism against Pi deficiency, the exudation of organic acids (Mora-Macías et al., 2017). Concomitant with a citrate exudation is enhanced H^+ATPases activity and H^+ efflux, which might help with the subsequent release of Pi from molecules, which are unavailable for the plant (Ligaba et al., 2004). Transport to plant then drive the Pi/nH^+ symporters (Preuss et al., 2011). A more prominent mechanism is proton efflux in the response to Fe deficiency. This way of increasing the Fe

availability and uptake is referred to as Strategy I, as opposed to Strategy II, which only utilises molecules with chelating activity phytosiderophores (Marschner & Römheld, 1994). The former of these two land plant strategies is present in both monocots and dicots, including *Arabidopsis thaliana* (Santi & Schmidt, 2009), while the latter in grasses like *Zea mays* and *Oryza sativa* (Ishimaru et al., 2006; Marschner & Römheld, 1994). Fe deficiency induces activation of H^+ATPases at the PM of root surface cells, specifically AHA2 and AHA7. That results in proton extrusion, acidification of the rhizosphere, and the promotion of root hair growth (Santi & Schmidt, 2009). This decrease of pH influences the Fe solubility. It helps the PM localised ferric chelate reductase reduce in the soil mainly present Fe^{3+} form bound in compounds to Fe^{2+}, which is available for uptake (Marschner & Römheld, 1994; Schwertmann, 1991; Waters et al., 2002). Other players participating in rhizosphere acidification are the basic loop-helix-loop transcription factors like bHLH104 and IAA-LEUCINE RESISTANT 3 (ILR3) in *Arabidopsis*, which modulate the plant's overall response to Fe deficiency (J. Zhang et al., 2015). More recently, it was shown that a role in response to Fe deficiency has, through inducing H^+ATPases, the SAUR23 gene. Its expression is higher under the Fe stress (Y. Sun et al., 2020). However, in this Fe stress response, various root exudates are also heavily utilised together with the proton efflux (Marschner & Römheld, 1994; Sisó-Terraza et al., 2016a).

Two main forms of inorganic nitrogen that plants utilise are ammonium $NH_4^+$and nitrate NO_3^-, both with different mechanisms of transport and assimilation by the plant. Through the translocation of protons, their transporters influence the rhizosphere pH level. Uptake of NH_4^+ through its transporters like AMMONIUM TRANSPORTER 1, shortly AMT1, which is NH_4^+/H^+ symporter (Neuhäuser et al., 2007; Ortiz-Ramirez et al., 2011), have a limited influence on the rhizosphere pH (Custos et al., 2020). On the other hand, NO_3^- uptake induces significant rhizosphere alkalinisation (Custos et al., 2020). Among its transporters are NITRATE TRANSPORTER 2, shortly NRT2 (Lupini et al., 2016), and NRT1, later renamed to NPF, NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (Léran et al., 2014; Parker & Newstead, 2014), both NO_3^-/H^+ symporters. Nitrogen is a nutrient whose uptake significantly influences the rhizosphere's pH (Custos et al., 2020). It is accompanied by and influences fluxes of other ions (Martinez & Cerdá, 1989), including essential nutrients like K^+ (Xia et al., 2015).

4.1.2 Rhizosphere pH determines the availability of toxic compounds

Aluminium is one of the most significant toxic compounds in the soil. Its toxicity is dependent on soil acidity, which increases Al solubility. Its toxic forms are starting to be present at a pH level below 5.5. Among the most important forms belongs $Al(H_2O)_6^{3+}$, or shortly Al^{3+} (Chowra et al., 2017; Kinraide, 1991). Plants use multiple mechanisms to reduce Al^{3+} effect and increase their own tolerance. One is the increase of rhizosphere pH, thus mitigating the Al toxicity. Using *alr-104*, a mutant with increased resistance to Al, and comparing it to the WT, the mutant plant maintains after exposure to Al^{3+} higher

rhizosphere pH at the root tip by 0.15 unit. Even though the specific mechanism has not been discovered, this change results from increased H^+influx (Degenhardt et al., 1998). But the main tolerance strategy regarding the aluminium toxic forms is the exudation of organic acids into the rhizosphere, where they chelate Al^{3+} ions (J. Liu et al., 2009; Zhu et al., 2022).

Root proton fluxes also have a role in salt stress, alleviating the toxic effect of the Na^+ (Qiu et al., 2002; Quintero et al., 2011; Z. Yang et al., 2019). One of the forces behind the H^+ fluxes are H^+ATPases, whose activity is enhanced in response to the salt stress signal (Z. Yang et al., 2019). Salinisation activates the Salt Overly Sensitive (SOS) pathway, an essential salt tolerance mechanism (Qiu et al., 2002; Z. Yang et al., 2019). The secondary messenger Ca^{2+} modulates the activity of SOS2, the serine/threonine protein kinase, by regulating its binding to the 14-3-3 protein. The protein and SOS2 interaction results in kinase inhibition. Their binding is promoted by the SOS2 negative regulator, the SOS2-LIKE PROTEIN KINASE 5 (PKS5) (Z. Yang et al., 2019), which also inhibits the 14-3-3 protein binding to the H^+ATPases (Fuglsang et al., 2007). However, the salt signal induces the 14-3-3 protein and PKS5 interaction, which lets the SOS2 activated (Z. Yang et al., 2019). This results in enhanced proton pump activity, increasing the driving force for other transporters, like the SOS1 antiporter (Quintero et al., 2011; Z. Yang et al., 2019). The PM-located SOS1 antiporter is a part of the SOS pathway. SOS1 increases Na^+ level in apoplast and H^+ in the cell, or when located at the soil-cell interface, mediates Na^+ efflux into the rhizosphere (Miranda et al., 2017; Qiu et al., 2002; Quintero et al., 2011; Y. Wang et al., 2023). In response to the high salt concentration in soil is also involved the $Cl^-/\ 2H^+$symporter, which increases their cytosolic level (Felle, 1994).

4.2 Root exudates

Plants' roots change the rhizosphere's chemical properties through the exudation of a wide variety of compounds. They change the pH of the rhizosphere (Jiang et al., 2023), increase the bioavailability of nutrients (Bolan et al., 1994; Maruyama et al., 2019), affect the root architecture (Mora-Macías et al., 2017), alleviate toxic compound effects (Zhu et al., 2022) and shape the root microbiome (Badri et al., 2013; Cosme et al., 2021; Jiang et al., 2023; Stringlis et al., 2018). Through these functions, they play an essential role. The composition of exudates changes in response to environmental factors and throughout plant developmental stages (Lopes et al., 2022, 2023; Maruyama et al., 2019). The compound composition is also species-specific (Zwetsloot et al., 2018).

4.2.1 Carboxylates

Exudates with significant influence are carboxylates, low molecular weight compounds, which have a role in nutrient acquisition and the mechanism of Al-tolerance (Bolan et al., 1994; Carvalhais et al., 2011; McKay Fletcher et al., 2021; Zhu et al., 2022). Most studied are organic acids malate and citrate, the most common exudates in these processes, and their transmembrane protein transporters.

ALUMINIUM-ACTIVATED-MALATE TRANSPORTER (ALMTs) are anion channels responsible mainly for the exudation of malate (J. Liu et al., 2009; Maruyama et al., 2019; Mora-Macías et al., 2017; Zhu et al., 2022), while MULTIDRUG AND TOXIC COMPOUND EXTRUSION (MATE) family of transporters exude mainly citrate (J. Liu et al., 2009). These families of transporters, present both in roots and to a lesser degree also in shoots, are activated and essential for organic acids exudation to the rhizosphere in response to the Al^{3+} stress and Pi deficiency (J. Liu et al., 2009; Maruyama et al., 2019). Although they are expressed in various plant tissues and cell organs, crucial for these two processes are transporters that are located at the PM of the root epidermis and, therefore, responsible for the exudation to soil (J. Liu et al., 2009; Maruyama et al., 2019; Mora-Macías et al., 2017). In *Arabidopsis thaliana*, 14 ALMT genes were described. The Al-stress induces the expression of AtALMT1, one of the genes crucial for the malate efflux (Hoekenga et al., 2006) and AtMATE gene, which is behind the citrate efflux (J. Liu et al., 2009). Both genes are primarily expressed in roots (Hoekenga et al., 2006; J. Liu et al., 2009). These two families of transporters are independent of each other, as shown in their single mutants, where the absence of one gene does not influence the function of the other. However, they have a common zinc finger transcription factor SENSITIVE TO PROTON RHIZOTOXICITY 1 (STOP1), which regulates their expression and activation and is required for the subsequent organic acids extrusion (J. Liu et al., 2009; Mora-Macías et al., 2017).

Carboxylates chelate toxic compounds like Al^{3+}, alleviating their negative effect (J. Liu et al., 2009; Zhu et al., 2022) and releasing other nutrients like phosphate from for the plant unavailable complexes (Bolan et al., 1994). The citrate extrusion in response to both Al toxicity and P deficiency occurs parallel with H^{+}ATPases upregulation (Q. Chen et al., 2013; Ligaba et al., 2004). Carboxylates' ability to increase the availability of P is lowest for monocarboxylic acids like acetate and highest for tricarboxylic acids like citrate (Bolan et al., 1994). Pi deficiency results in the activation of AtALMT3, located mainly at the PM of root hairs, and malate exudation. Out of all AtALMT genes, this transporter is significantly upregulated under this condition (Maruyama et al., 2019). Under the condition of low Pi, malate also plays a role in altering the root growth to increase the chance of finding a Pi source. AtALMT1 with STOP1 regulates the efflux of malate to the apoplast of meristematic cells, which leads there to Fe^{3+} accumulation. This activates a signalling cascade that changes root development by influencing root apical meristem, inhibiting primary root growth (Mora-Macías et al., 2017). Among organic acids, citrate is the most effective in major and trace elements mobilisation. However, oxalacetate has a higher effect on some elements such as Cu, Ni or Zn. The efficiency of organic acids changes with soil pH (Terzano et al., 2015). All these studies show the importance of carboxylates for the availability of nutrients.

4.2.2 Phenolic compounds

The plant utilises a variety of phenolic compounds with diverse functions. Their secretion helps with nutrient acquisition, mainly P, N, Fe and Mn (Rosenkranz et al., 2021; Sisó-Terraza et al., 2016a; Tomasi et al., 2008). They also participate in rhizosphere signalling by attracting symbionts and repelling pathogens, thus establishing a root microbiome (Badri et al., 2013; Lidoy et al., 2023; Stringlis et al., 2018). In *Arabidopsis thaliana*, the exudation of phenolic compounds is ensured by ABC (ATP binding cassette) transporters, specifically the ABCG37/PDR9 transporter. Under the condition of low Fe, its expression is upregulated (Fourcroy et al., 2014). It is mainly localised at the lateral root cap and root epidermis (Ito & Gray, 2006). Phenolics are used together with the extrusion of protons by the Strategy I plants to solubilise Fe (Marschner & Römheld, 1994; Sisó-Terraza et al., 2016a). The exudation of coumarinolignans, coumarin-phenolic-like compounds, and their precursors is highly increased in response to Fe deficiency. Major coumarin that contains catechol moiety and effectively solubilises Fe *in vitro* was shown to be fraxetin (Sisó-Terraza et al., 2016a). Non-catechol coumarins are less effective in chelating Fe, but despite that, they have significant roles (Rosenkranz et al., 2021; Sisó-Terraza et al., 2016a). Coumarinolignans and coumarins accumulation and secretion levels change with environmental pH (Rosenkranz et al., 2021; Sisó-Terraza et al., 2016a). Soil-grown *Arabidopsis thaliana* predominantly exudes the iron-mobilizing coumarin scopoletin in response to Fe deficiency (Rosenkranz et al., 2021). However, scopoletin also shapes the rhizobiom (Cosme et al., 2021; Stringlis et al., 2018). It negatively influences the growth of fungal pathogens because of its antimicrobial effect. The rhizobacteria that promote plant growth are tolerant to this scopoletin activity. This shifts the root microbiome composition to benefit the plant's health (Stringlis et al., 2018).

Phenolic compounds are, compared to other root exudates, the main modulators that increase soil microbiome diversity. Other compounds' exudation, like sugars or amino acids, also influences the diversity but to a lesser extent. Different compositions of exudates result in different representations of bacteria (Badri et al., 2013; Lopes et al., 2022). Coumarins induce the root colonisation of mutualistic bacteria by influencing the bacteria's flagellar biosynthesis, which could help the evasion of plant immunity (K. Yu et al., 2021). Coumarins, especially scopoletin, promote the initial steps of arbuscular mycorrhizal colonisation (Cosme et al., 2021). They also induce an allelopathic effect, negatively influencing the growth of other nearby plants (Wu et al., 2015). In *Lolium multiflorum*, this effect stimulates the abundance of beneficial bacteria in the rhizosphere, increasing their ability to survive stress. However, it also stimulates a few pathogenic bacteria. Coumarins indirectly influence the plants' metabolome through these changes in the rhizosphere's microbiome, which positively affect plant energy metabolism and antioxidant activity (Y. Yang et al., 2023). These discoveries show the significance of coumarins in the complex plant-mycorrhizal fungi and mutualist communication (Cosme et al., 2021; Y. Yang et al., 2023; K. Yu et al., 2021).

Flavonoids are other phenolic exudates with a broad range of functions. Their exudation increases the availability of elements in the rhizosphere, including Pi mobilisation (Terzano et al., 2015; Tomasi et al., 2008). Flavonoids, specifically quercetin and rutin, are most efficient in mobilising metals like Fe and Mn. They also have a synergistic effect with the organic acid citrate, which increases the effectivity of the elements' mobilisation, although it depends on the soil pH (Terzano et al., 2015). They are also crucial for the reutilization of Fe from the apoplast, as their removal results in the activation of the plant's answer to Fe deficiency (Jin et al., 2007). Flavonoids, as the already mentioned coumarins, have an allelopathy effect (H. Zhang et al., 2017). They also have a significant role in rhizosphere signalling and plant-microorganisms interaction, thus shaping the rhizosphere microbiome (Kudjordjie et al., 2021; Tian et al., 2021). *Arabidopsis thaliana* mutants with disrupted flavonoid pathways have a depleted root microbiome. The lack of flavonoids significantly affects the rhizosphere fungi communities and, to a lesser extent, the abundance and diversity of bacteria (Kudjordjie et al., 2021). While acacetin and rhamnetin inhibit the colonisation process by the *Gigaspora* in the tomato plant, quercetin promotes it (Scervino et al., 2005). A recent study using tomato plants shows a positive effect on *Rhizophagus irregularis* arbuscular mycorrhizal symbiosis and spore germination by low quercetin, rutin, and chrysin doses (Lidoy et al., 2023). Invasive plants use flavonoids to enhance the arbuscular mycorrhizal fungi colonisation and increase their biomass. That results in an improvement in their performance and helps during plant invasions (Pei et al., 2020; Tian et al., 2021). Exudation of flavonoids promotes interactions with N-fixing bacteria and, in legume plants, initiates nodule formation (Gough et al., 1997; Wasson et al., 2006). Phenolics, such as flavonoid sakuranetin (Subbarao et al., 2013), and other exudates like fatty alcohol 1,9-decanediol (L. Sun et al., 2016) belong among biological nitrification inhibitor (BNI) compounds. Exudation of BNIs decreases the loss of N from the soil (Subbarao et al., 2013; L. Sun et al., 2016).

4.2.3 Mucilage

Mucilage is a high-molecular-weight gelatinous substance composed primarily of polysaccharides and, to a lesser extent, also proteins, lipids and minerals (Alizadeh Behbahani et al., 2017; Brax et al., 2019; Diehl et al., 2023; Durand et al., 2009). Its composition, properties and amount of exudation change in response to environmental factors, like the texture of soil (Ahmed et al., 2016; Knott et al., 2022; Rahim et al., 2024). The polysaccharides network of root mucilage is less cross-linked than seed mucilage, allowing flexibility and adaptability (Diehl et al., 2023). Mucilage is exuded from root cap cells, where the Golgi apparatus synthesises it (Iijima & Kono, 1992). Its exudation by *Arabidopsis thaliana* is studied predominantly in the context of seed development (Kulich et al., 2010; Western et al., 2000). However, recently, a model of mucilage extrusion from columella cells during their differentiation was proposed. During the transition from the fifth to the sixth columella layer, mucilage starts to accumulate in the

periplasmic space between the PM and CW. Partial degradation of the CW occurs in this layer. This allows the subsequent mucilage extrusion from the columella cell of the seventh layer to the space between the cell walls. From there, it is exuded to the rhizosphere due to turgor pressure (Maeda et al., 2019). Mucilage is also secreted by root border cells and border-like cells (Cai et al., 2013; Durand et al., 2009; Ropitaux et al., 2020). Mucilage has a wide range of functions at the root-soil interface. It's secretion and sloughed-off border cells cause low friction during the root penetration of the soil, functioning as a lubricant (Mckenzie et al., 2013). It is crucial for the soil aggregation and formation of rhizosheaths, clumps of soil attached to the root surface (Albalasmeh & Ghezzehei, 2014; Rahim et al., 2024). Mucilage properties change with the water level, increasing viscosity and concentration with drier soil. The intermediate concentration of 0.12 g dry mucilage g^{-1} water establishes the largest rhizosheath, maximally increasing the root surface and area of root influence (Rahim et al., 2024). Besides the uptake, mucilage properties also affect the flow of water and solutes (Ahmed et al., 2014, 2016; Diehl et al., 2023; Knott et al., 2022). Under drought conditions, mucilage keeps the rhizosphere wetter than the bulk soil and increases the connectivity of the liquid phase (Ahmed et al., 2014; Zarebanadkouki et al., 2019). These effects maintain diffusion transport, preventing deficiency and depletion of nutrients, as well as salt accumulation and stress (Zarebanadkouki et al., 2019).

Root mucilage polysaccharides comprise uronic acids with a high affinity to cations (Watanabe et al., 2008). This affinity increases plants' resistance to toxic compounds (Cai et al., 2013; Watanabe et al., 2008; M.-X. Zhou et al., 2020). Among them is Al, which mucilage binds and immobilises, mitigating its negative effect on root growth. Exposure to Al significantly enhances its secretion in a concentration and time-dependent manner (Cai et al., 2013; Geng et al., 2012). The Al-resistant cultivar of *Glycine may L* exudes larger amounts of mucilage with higher content of uronic acids (Cai et al., 2013). Removal of mucilage results in the inhibition of elongation and higher Al accumulation in the root, as well as a higher rate of root border cells' death (Cai et al., 2013; Geng et al., 2012). Root exudates, with mucilage among them, also serve as a carbon source (Ahmed et al., 2018). Mucilage appears to have a more prominent role for microorganisms under drought conditions. Besides serving as the C source, mucilage, through its ability to keep soil wetter, also provides protection (Ahmed et al., 2014, 2018; Zarebanadkouki et al., 2019). The mucilage composition also influences the structure of microbiota, which use it as their habitat (Pang et al., 2023).

4.3 Other rhizodeposits and forms of C deposition

The strategy I plants (Marschner & Römheld, 1994), like *Beta vulgaris*, exude flavins upon Fe deficiency. Flavins are other compounds that significantly help with Fe acquisition. They mine Fe from ferric oxide by reducing the Fe^{3+} to Fe^{2+}, working as a soluble redox shuttle (Sisó-Terraza et al., 2016b). Phytosiderophores, exuded by Strategy II plants (Marschner & Römheld, 1994), bind and form complex

with Fe^{3+}. This complex is then taken up by the plant without the step of Fe^{3+} reduction (Ishimaru et al., 2006; Marschner & Römheld, 1994). Efflux of phytosiderophores to the rhizosphere is ensured by the transporter of mugineic acid family phytosiderophores 1 (TOM1), localised in the PM of the root epidermis (Nozoye et al., 2011). However, several examples demonstrated that plants' mechanisms are more complex (Ishimaru et al., 2006; Marastoni et al., 2020). Exceptions exist, with rice plants being able to take up both phytosiderophores complexes and Fe^{2+} (Ishimaru et al., 2006). A different study on hydroponically-grown and Fe-deficiency tolerant grapevines, considered Strategy I plants, described the presence of phytosiderophore 3-hydroxymugenic acid among the exudates (Marastoni et al., 2020). Other significant exudates are amino acids and sugars (Carvalhais et al., 2011; Marastoni et al., 2020). Grapevines decrease their exudation to reduce microbial communities they compete with for the Fe while increasing the exudation of compounds that reduce Fe^{3+} (Marastoni et al., 2020).

Exuded sugar concentrations and forms contribute to the formation and composition of root-associated rhizosphere microbiome. Different sugars correlate with increased specific bacteria genera. At the early developmental stages, maize exudes glucose and sucrose, which positively influence bacterial communities. In the later stages, the plant utilises other sugar forms, such as trehalose, to support its growth (Lopes et al., 2022). Sugars' and amino acids' exudation patterns also change in response to other abiotic stresses like N and P deficiencies (Carvalhais et al., 2011). Amino acids, most prominently proline, play a role in osmotic stress. Exudation of proline and phytohormones is significantly higher under salt and heat conditions (Vives-Peris et al., 2017; Xie et al., 2020).

Phytohormones are other compounds which plants exude in various circumstances. As mentioned, phytohormones like salicylic acid, jasmonic acid, ABA and IAA are in large quantities exuded by citrus plants into the rhizosphere under abiotic stresses (Vives-Peris et al., 2017). Under conditions of low P, sugar beet root secretes salicylic acid to solubilise and increase its availability (Khorassani et al., 2011). Phytohormones like strigolactone shape the root microbiome, functioning as chemoattractants (Lopes et al., 2022, 2023; Yoneyama et al., 2007). Red clover plants utilise the exudation of strigolactone orobanchol, which stimulates arbuscular mycorrhizal symbiosis to increase P supply (Yoneyama et al., 2007). Jasmonic acid, ABA, and IAA presence in root exudates of maize significantly affect the composition of bacterial communities of the rhizosphere (Lopes et al., 2022, 2023). Exudation of IAA influences the microbiome at the early stages of maize development, while ABA shapes its composition at later stages (Lopes et al., 2023).

Depending on the species, plants release from the root surface border cells or border-like cells (Durand et al., 2009; Mravec et al., 2017). Legume plants like *Pisum sativum* and *Glycine max L* or grasses like maize release into the rhizosphere separate border cells (Cai et al., 2013; Canellas & Olivares, 2017; Mravec et al., 2017). They elongate and curve, and their cell wall becomes thinner before their release. To disrupt the adhesion between cells, the homogalacturonan of the middle

lamellae is dissolved (Mravec et al., 2017). Together with mucilage, they coat the root tip (Ropitaux et al., 2020). Border-like cells, which are released from the root tip by *Arabidopsis thaliana*, remain attached and thus are released as a sheet. Disruption of homogalacturonan biosynthesis and its absence results in the loss of this adhesion (Durand et al., 2009). Border cells, located between the root and its environment, have several roles in root-soil and microbiome interactions. Sloughed-off border cells and mucilage ensure low friction during root growth (Mckenzie et al., 2013). They also secrete mucilage in response to Al toxicity, participating in the mitigation of its negative effects (Cai et al., 2013; Geng et al., 2012). The release of border cells, especially by young roots with higher production, contributes to soil C deposition (Odell et al., 2008). They also influence the root microbiome. Border cells positively affect root colonisation by diazotrophic bacteria (Canellas & Olivares, 2017) while protecting plants from pathogens (Ropitaux et al., 2020; Tran et al., 2016). After exposure, pea root border cells release extracellular DNA that traps a pathogenic bacteria and immobilises it (Tran et al., 2016). Border cells and their exudates of *Glycine max L* can form root extracellular trap that inhibits zoospores' colonisation of root (Ropitaux et al., 2020).

A significant role in the C deposition and sequestration, except for root exudates (Ahmed et al., 2018) and border cells (Odell et al., 2008), also plays carbon dioxide. The root respiration and diffusion from the atmosphere elevate CO_2 levels in the rhizosphere (Kelting et al., 1998; Rosado-Porto et al., 2022). There, it serves as a C source for the microbiome and a factor that influences its activity (Rosado-Porto et al., 2022; Xu et al., 2019). High levels of CO_2 stimulate some of the bacterial genera, which results in a shift in the rhizosphere microbiome structure (Rosado-Porto et al., 2022). CO_2 acidifies the rhizosphere's pH through its dissolved form carbonic acid (H_2CO_3) that can dissociate to bicarbonate (HCO_{3-}) and H^+ (Kirk et al., 2019; Oh & Richter Jr., 2004). Its presence induces cations exchange and mineral dissolution. CO_2 can displace almost all base cations, thus having high acidifying potential. It also increases the concentration of toxic Al^{3+} (Oh & Richter Jr., 2004). Removal of CO_2 and thus H_2CO_3 from the soil increases pH by 0.7 units in the proximity to the rice root. CO_2 influence on pH might change nutrient availability (Kirk et al., 2019).

5 Conclusions

In my thesis, I summarised the most prominent mechanisms of plants' roots that modify the chemical properties of the apoplast and rhizosphere. In the context of root growth, I described key players that regulate H^+ATPases, which are the main force behind proton pumping. Among them are phytohormones auxin, brassinosteroids and abscisic acid. Less prominent roles also have other phytohormones like ethylene, which influences the proton pump activity via its influence on auxin concentrations. These hormones' signalling converges on the phosphorylation status of H^+ATPase, through which they influence the pH level of the apoplast and subsequent activation of cell wall loosening proteins. Auxin is the central phytohormone of the acid growth theory. In roots, It modulates pH through two antagonistic pathways, TIR1/AFB, involving transcriptional regulation and TMK1, which directly influence proton pump activity. Auxin was also shown to work in a concentration and time-dependent manner and define the root surface pH. These findings show that auxin creates a complex and dynamic system that allows the root to modulate the apoplast properties. The complexity of mechanisms regulating H^+ATPases action shows the need for further research.

I also described how roots influence the nutrient availability of the rhizosphere by ions fluxes between the soil and root, which also decrease the level of toxic compounds, thus alleviating stress. Proton pumping and root respiration change the rhizospheres' pH. However, the most significant impact on the chemical properties appears to have root exudates such as organic acids malate and citrate, phenolic compound scopoletin or mucilage. Their exudation notably affects the mobilisation of iron, inorganic phosphate or nitrogen, as well as decreases levels of aluminium toxic ions. Furthermore, these exudates can influence each other, having a positive synergic effect. Together with released border and border-like cells, they can also indirectly change nutrient availability with their influence on the microbiome. While attracting symbionts and helping to establish arbuscular mycorrhizal symbiosis, they also trap, immobilise and repel pathogens. The microbiome composition of the rhizosphere is of significant interest to recent studies. Research of root exudates and how they influence the microorganism communities might show novel ways to affect plants' growth positively.

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