



UvA-DARE (Digital Academic Repository)

Going in the right direction

Cellular mechanisms underlying root halotropism

Korver, R.A.

Publication date

2019

Document Version

Other version

License

Other

[Link to publication](#)

Citation for published version (APA):

Korver, R. A. (2019). *Going in the right direction: Cellular mechanisms underlying root halotropism*.

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Chapter 1

General introduction

High soil salinity is an increasing problem for agriculture; it causes reduction in crop yield or in more extreme cases even loss of arable land. The use of inefficient irrigation together with the changing environment (e.g. drought and heat) has increased soil salinity worldwide (Smedema and Shiati, 2002; D'Odorico et al., 2013). For example, the altered water cycles and rising sea levels increase soil salinity in Europe (Daliakopoulos et al., 2016). High salt confronts plants with physiological stress, which consists of two main components. First, an increase in ions in the soil causes osmotic stress, limiting water uptake. Additionally, elevated levels of Na^+ inside the plant lead to ionic stress, which inhibits photosynthesis and other essential biochemical processes in plant cells. The cytosolic levels of Na^+ are highly dependent on sodium transport in the plant (Plett and Moller, 2010). Plant roots take up Na^+ from the soil through Na^+ transporters or channels in the membrane of epidermal cells. Next, after radial movement of the ions within the symplast and apoplast, Na^+ is loaded into the xylem. Na^+ is then transported in the xylem from root to shoot through the transpiration stream (de Boer and Volkov, 2003). Nonetheless, while much is known, some knowledge gaps concerning Na^+ movement in the plant remain (Britto and Kronzucker, 2015; Keisham et al., 2018). Strategies of plants to cope with high salinity vary. Most plants, including major crops, are glycophytes, which are sensitive to salt. However, specialized plants called halophytes thrive in the presence of salt. Halophyte adaptations include storage of high amounts of ions in centralised vacuoles of leaf cells and excretion of salt through salt glands. As such, halophytes might be used as genetic resources for the creation of more salt tolerant crops or even as crops themselves (Flowers and Colmer, 2015; Ventura et al., 2015). Nonetheless, studying differences in the salt tolerance of glycophytes, and especially in the model plant *Arabidopsis*, is essential to unravel the underlying cellular mechanisms of the plants salt response.

Unsurprisingly, high soil salinity alters the development of plants. For one, the growth of the roots comes to a complete stop for a short period, called the quiescence phase (Duan et al., 2013; Geng et al., 2013), after which growth recovers but never reaches the same growth rate as before the stress (Julkowska and Testerink, 2015). If the salt exposure of the root is asymmetrical, the root will bend away from the higher salt concentration in the soil, a phenomenon termed halotropism (Galvan-Ampudia et al., 2013). Also, the emergence and outgrowth of lateral roots changes, which is predicted to alter the uptake of Na^+ from the soil (Julkowska et al., 2014). How sensing of salt induces responses and eventually salt tolerance, we do not fully comprehend (Deinlein et al., 2014; Shabala et al., 2015). Nevertheless,

hormonal changes during salt stress that occur in plants are well described, e.g. for auxin, jasmonates, ethylene and abscisic acid (Roychoudhury et al., 2013; Kazan, 2015; Zhang et al., 2016; Korver et al., 2018). One of the most important hormones altering root growth rate, root growth direction and root system architecture during salt stress is auxin. Auxin dynamics have been well described in different processes; perception (Salehin et al., 2015; Strader and Zhao, 2016), signalling (Peer, 2013), biosynthesis (Mano and Nemoto, 2012; Korasick et al., 2013; Tivendale et al., 2014), conjugation and oxidation (Zhang and Peer, 2017) and transport (Kleine-Vehn and Friml, 2008; Adamowski and Friml, 2015; Armengot et al., 2016). Although changes during salt stress have received less attention, reviewing recent knowledge on changes in all auxin related processes during stress has highlighted the importance of both auxin distribution and local auxin biosynthesis in root responses to salt and osmotic stress (Korver et al., 2018; Chapter 2, this thesis). This thesis furthermore addresses cellular responses in auxin carrier polarity and endocytosis during salt stress. The next sections function as background information to the different chapters in this thesis.

Which way to go, root growth direction during salt stress

Salt stress first and most of all affects the roots of the plant and studying plant root systems during salt stress is a fast growing field of research. Therefore, new helpful tools to study the root system under abiotic stress are being developed e.g. (Lobet et al., 2011; Clark et al., 2013; Jeudy et al., 2016). During stress, in addition to changes in the root system architecture (Sauter, 2013; Robbins and Dinneny, 2015; Khan et al., 2016; Koevoets et al., 2016; Julkowska et al., 2017), roots are capable to change their root growth direction temporarily when growth towards or away from specific soil conditions is required. The most common examples of alteration of root growth direction due to stress are hydrotropism (Eapen et al., 2005), halotropism (Galvan-Ampudia et al., 2013; Han et al., 2017), thigmotropism (Massa and Gilroy, 2003) and chemotropism (Ferrieri et al., 2017). During these tropisms the first step is sensing environmental stimuli. For gravitropism the sensing mechanism is known and for hydrotropism the sensing mechanisms are being unravelled (Dietrich et al., 2017), however the salt sensing mechanism remains elusive, although some theories have been proposed (Shabala et al., 2015). The signalling pathways downstream of the sensing mechanism then finally result in shifts in the auxin flow in the root. Auxin maxima and minima in the root cause local alterations in cell elongation rates, transition to cell differentiation (Di Mambro et al., 2017) and organ initiation. Changes in local

auxin concentrations are believed to be mainly the result of changes in polar auxin transport (PAT). On the other hand, for salt stress, recently more evidence has been found that suggests larger roles for short-distance auxin transport, auxin biosynthesis and conjugation (Korver et al., 2018). Changing PAT is a result of the internalization and re-localization of auxin carriers. However, the endocytosis pathways involved are a major gap in our knowledge on plant salt stress responses. In this thesis I fill parts of these gaps with novel knowledge on auxin carrier polarity and endocytosis in plant roots under stress.

Salt stress induced endocytosis of auxin carriers

The constitutive intracellular cycling of auxin carriers provides a rapidly adjustable process when local polarity needs to be changed to adjust PAT (Adamowski and Friml, 2015; Naramoto, 2017). Two protein families of auxin efflux carriers have been characterized; PINs (Friml et al., 2003; Wisniewska et al., 2006) and ABCBs (Geisler et al., 2017). Additionally the AUX/LAX family of auxin influx carriers transports auxin into the cell (Bennett et al., 1996; Peret et al., 2012; Rutschow et al., 2014). Auxin influx also occurs passively while efflux is only regulated by active processes. The auxin carriers often show a polar distribution in the different cell tissues (Wisniewska et al., 2006; Kleine-Vehn et al., 2008). Altering this polarity causes shifts in auxin flow which in the end affects plant developmental processes (Tanaka et al., 2013).

Changes in auxin carrier polarity are caused by altered targeting of proteins during re-cycling (Zhang et al., 2010; Mei et al., 2012; Baster et al., 2013) or altered auxin carrier internalization (Chen et al., 2012; Galvan-Ampudia et al., 2013). Auxin carrier internalization and re-localization is essential for fast changes of auxin flow and thus auxin asymmetry in the root. Nonetheless, the endocytosis pathway responsible for stress induced internalization of auxin carriers remains unknown. While in animal systems multiple clathrin independent endocytosis pathways have been well characterized, in plants much less is known. Up to date, only clathrin dependent endocytosis (CME) has been well studied. For the auxin efflux carrier Pin-formed 2 (PIN2), CME involved in cycling and polarity has been established with both cell biological and genetic evidence (Wisniewska et al., 2006; Kleine-Vehn and Friml, 2008). However, whether the stress induced endocytosis of PIN2 is CME has become questionable. Up to date, tyrphostinA23 (TyrA23) has mostly been used as a CME inhibitor and TyrA23 has recently been shown to cause cytoplasm acidification (Dejonghe et al., 2016), raising serious questions about its specificity as a CME inhibitor. Thus,

as evidence supporting a clathrin independent, putatively sterol dependent, pathway has increased, the need to elucidate the salt induced endocytosis pathway internalizing auxin carriers is larger than ever. When looking at animal systems, other possible endocytosis pathways include; Caveolin-mediated endocytosis, flotillin mediated endocytosis and microdomain-mediated endocytosis. Caveolin-mediated endocytosis as found in animal cells is absent in plants due to the absence of caveolins. Flotillins, which have a similar structure as caveolins, are present in plants, where they form domains at the plasma membrane which facilitate endocytosis, resulting in endosomal structures approximately 3 times larger than CCV's (Li et al., 2012). No link between flotillins and salt stress has however been described. Membrane microdomains, which consist mainly of sterols and sphingolipids, are involved in microdomain-mediated endocytosis (MME) in plants (Grebe et al., 2003). During salt stress, it has been found that Respiratory burst oxidase homolog D (RbohD) is internalized via cooperative CME and MME (Hao et al., 2014). Likewise, it was shown that despite blocking recruitment of clathrin to the PM with a NAA pre-treatment, PIN2 accumulated inside the cell during salt stress (Baral et al., 2015). The aquaporin Plasma membrane Intrinsic Protein 2;1 (PIP2;1) that facilitates water transport across plasma membranes was shown to diffuse into and out of membrane microdomains. Nonetheless, upon salt stress its diffusion is restricted and internalization of PIP2;1 increased (Li et al., 2011). Loss of cyclopropylsterol isomerase1-1 (CPI1-1) results in defects in sterol-biosynthesis, the *cpi1-1* mutant has thus been shown to alter membrane sterol composition. Interestingly this mutant was also found to have alterations in PIN2 polarity and root gravitropism (Men et al., 2008). In the *cpi1-1* line the localization of PIN2 to newly formed membranes was abolished. Arabidopsis roots treated with fenpropimorph (FEN), an inhibitor of the sterol biosynthetic enzyme C14 sterol reductase FACKEL, have also been reported to reverse the inhibition of PIN2-GFP endocytosis by auxin (Pan et al., 2009). This suggests membrane sterols are required for inhibition of PIN2 endocytosis. All these results taken together suggest salt induced internalization of membrane proteins and provide a strong link between PIN2 and MME, making MME a candidate for the salt induced internalization of auxin carriers.

Lipid signals during salt stress

Cellular signalling pathways are an essential part of the response to any environmental cue or interaction with harmful or beneficial organisms. An important aspect is lipid signalling, the formation of lipid second messengers

in the membrane. What sets lipid signalling molecules apart from structural lipids is their low abundance and rapid turnover upon the sensing of stress. The main lipid messengers in plants are phosphatidic acid (PA), polyphosphoinositides (PPIs), sphingolipids, lysophospholipids and oxylipins. For salt stress, PA (Testerink and Munnik, 2005; Hong et al., 2010) and PPIs (Hou et al., 2016) are known to be involved in signaling the stress response. The formation of PA in cellular membranes acts as a spatiotemporal signal reporting on the cell's environment during stress (McLoughlin and Testerink, 2013). Furthermore, PA acts as a binding site for PA-binding proteins during stress including clathrin adaptor proteins such as Epsin-like Clathrin Adaptor 1 (ECA1) and Epsin-like Clathrin Adaptor 4 (ECA4) (McLoughlin et al., 2013). Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) abundance increases during salt stress (Pical et al., 1999; DeWald et al., 2001; Darwish et al., 2009). Moreover, through co-localization studies between PI(4,5)P₂ and clathrin, involvement of PI(4,5)P₂ in salt-induced CCV formation has been proposed (Konig et al., 2008). Phosphatidylinositol 3-kinase (PI3K) mutants, which are inhibited in the formation of phosphatidylinositol 3-phosphate (PtdIns3P or PI3P), have been shown to have reduced salt-induced endocytosis and ROS production (Leshem et al., 2007). The role of sphingolipids in salt stress remains to be uncovered (Zhang et al., 2012; Wu et al., 2015).

Unravelling the cellular mechanisms behind halotropism - Thesis outline

In this thesis, the focus is on the underlying cellular mechanisms that control auxin flow in the root when it encounters salt stress. Internalization and re-localization of auxin carriers creates local auxin maxima and minima, which regulate root growth direction, root growth rate and root development. The work continues on the previously found internalization of PIN2 during halotropism and the involvement of Phospholipase D zeta during this process (Galvan-Ampudia et al., 2013). In **Chapter 2** first recent published findings on changes in Polar Auxin Transport (PAT) are discussed. Additionally, evidence is provided that the role of short distance auxin transport should not be overlooked in the establishment of local auxin concentrations during salt stress. Passive auxin transport into cells, regulated by apoplast pH, and cellular compartments control the auxin available for auxin perception. Moreover, the role of local auxin biosynthesis is stressed and the literature on auxin biosynthesis is reviewed. This includes a meta-analysis on previously published gene expression data, revealing relevant IAA biosynthesis and conjugation related gene expression patterns during salt stress.

The question whether changes in PIN2 polarity alone are sufficient for the difference in auxin concentration between the side of the root facing the higher salt concentration and the side facing the lower salt concentration, causing root bending, is answered in **Chapter 3**. Here, a combination of computational modelling and *in planta* experiments is used to determine the changes of the auxin influx carrier AUX1 and other putatively relevant auxin efflux carriers PIN1 and PIN3 and the effect of these changes on auxin flow. Interestingly, we find that the positive feedback of auxin on AUX1 abundance on the side of the root facing the lower salt concentration is required for a sufficient increase of auxin to reach levels that inhibit cell elongation. Nevertheless, with the addition of the auxin-induced AUX1 changes, the timing of the auxin asymmetry remains too slow. Therefore, we study PIN1 dynamics during halotropism for an additional input of auxin from the shoot to increase the speed of auxin asymmetry build up. Indeed, we find a transient increase of PIN1 after exposure to a salt gradient. In addition, a *pin1* mutant is observed to have a delayed halotropic response.

Building on our previous work (Galvan-Ampudia et al., 2013), which described the involvement of Phospholipase D $\zeta 2$ in PIN2 internalization during halotropism, in **Chapter 4** we assess the role of Phospholipase D $\zeta 1$ in auxin carrier internalization and re-localization. Here, we establish that PLD $\zeta 1$ regulates PIN2 but not AUX1 polarity in control conditions. Moreover, PIN2 polarity shifts during salt stress are altered in the absence of PLD $\zeta 1$. This results in predicted differences in auxin distribution and observed halotropic and gravitropic responses as well as root architecture during salt stress in a *pld $\zeta 1$* mutant. Furthermore, we describe osmotic stress-induced membrane structures (OSIMS) that arise shortly after the application of a salt or osmotic treatment in a wildtype root and putatively influence auxin carrier endocytosis and cycling. Moreover OSIMS are observed for a prolonged time in salt-stressed *pld $\zeta 1$* mutant root cells.

In **Chapter 5**, to assess possible PLD $\zeta 1$ and PLD $\zeta 2$ redundancy, we study *pld $\zeta 1$* , *pld $\zeta 2$* and the double mutant during halotropism and salt stress on a physiological and a cell biological level. Interestingly, the *pld $\zeta 1$ /pld $\zeta 2$* double mutant is found to phenocopy the different single KO's for different traits. *pld $\zeta 1$* is phenocopied in PIN2 polarity and inhibition of root hair length by salt. Additionally, the double mutant is similar to the *pld $\zeta 2$* single mutant with respect to the number of PIN2 containing vesicles in response to salt stress and during halotropism. This indicates that PLD $\zeta 1$ and PLD $\zeta 2$ are involved in different cellular processes in the salt stress response.

In **Chapter 6**, to elucidate the endocytosis pathway involved in the internalization of auxin carriers during salt stress, we study the function of Epsin-like clathrin adaptor 1 (ECA1) and 4 (ECA4) during salt stress. We observe distinct phenotypes for loss of function ECA1 and 4 mutants in their halotropism response. Furthermore, ECA4 interactions with proteins involved in cell division, vacuolar sorting, cell polarity and energy flux are identified. In **Chapter 7** results from all previous chapters is put in perspective to the current knowledge on the cellular mechanisms involved in regulating auxin during salt stress.

References

- Adamowski M, Friml J** (2015) PIN-dependent auxin transport: action, regulation, and evolution. *Plant Cell* **27**: 20-32
- Armengot L, Marques-Bueno MM, Jaillais Y** (2016) Regulation of polar auxin transport by protein and lipid kinases. *J Exp Bot* **67**: 4015-4037
- Baral A, Irani NG, Fujimoto M, Nakano A, Mayor S, Mathew MK** (2015) Salt-Induced Remodeling of Spatially Restricted Clathrin-Independent Endocytic Pathways in Arabidopsis Root. *Plant Cell*: tpc-15
- Baster P, Robert S, Kleine-Vehn J, Vanneste S, Kania U, Grunewald W, De Rybel B, Beeckman T, Friml J** (2013) SCF(TIR1/AFB)-auxin signalling regulates PIN vacuolar trafficking and auxin fluxes during root gravitropism. *EMBO J* **32**: 260-274
- Bennett M, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA** (1996) Arabidopsis AUX1 Gene: A Permease-Like Regulator of Root Gravitropism. *Science* **273**: 948-950
- Britto DT, Kronzucker HJ** (2015) Sodium efflux in plant roots: what do we really know? *J Plant Physiol* **186-187**: 1-12
- Chen X, Naramoto S, Robert S, Tejos R, Lofke C, Lin D, Yang Z, Friml J** (2012) ABP1 and ROP6 GTPase signaling regulate clathrin-mediated endocytosis in Arabidopsis roots. *Curr Biol* **22**: 1326-1332
- Clark RT, Famoso AN, Zhao K, Shaff JE, Craft EJ, Bustamante CD, McCouch SR, Aneshansley DJ, Kochian LV** (2013) High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. *Plant Cell Environ* **36**: 454-466
- D'Odorico P, Bhattachan A, Davis KF, Ravi S, Runyan CW** (2013) Global desertification: Drivers and feedbacks. *Advances in Water Resources* **51**: 326-344
- Daliakopoulos IN, Tsanis IK, Koutroulis A, Kourgialas NN, Varouchakis AE, Karatzas GP, Ritsema CJ** (2016) The threat of soil salinity: A European scale review. *Sci Total Environ* **573**: 727-739
- Darwish E, Testerink C, Khalil M, El-Shihy O, Munnik T** (2009) Phospholipid signaling responses in salt-stressed rice leaves. *Plant Cell Physiol* **50**: 986-997

- de Boer AH, Volkov V** (2003) Logistics of water and salt transport through the plant: structure and functioning of the xylem. *Plant Cell Environ* **26**: 87-101
- Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI** (2014) Plant salt-tolerance mechanisms. *Trends Plant Sci* **19**: 371-379
- Dejonghe W, Kuenen S, Mylle E, Vasileva M, Keech O, Viotti C, Swerts J, Fendrych M, Ortiz-Morea FA, Mishev K, Delang S, Scholl S, Zarza X, Heilmann M, Kourelis J, Kasproicz J, Nguyen le SL, Drozdzecki A, Van Houtte I, Szatmari AM, Majda M, Baisa G, Bednarek SY, Robert S, Audenaert D, Testerink C, Munnik T, Van Damme D, Heilmann I, Schumacher K, Winne J, Friml J, Verstreken P, Russinova E** (2016) Mitochondrial uncouplers inhibit clathrin-mediated endocytosis largely through cytoplasmic acidification. *Nat Commun* **7**: 11710
- DeWald DB, Torabinejad J, Jones CA, Shope JC, Cangelosi AR, Thompson JE, Prestwich GD, Hama H** (2001) Rapid Accumulation of Phosphatidylinositol 4,5-Bisphosphate and Inositol 1,4,5-Trisphosphate Correlates with Calcium Mobilization in Salt-Stressed Arabidopsis. *Plant Physiol* **126**: 759-769
- Di Mambro R, De Ruvo M, Pacifici E, Salvi E, Sozzani R, Benfey PN, Busch W, Novak O, Ljung K, Di Paola L, Maree AFM, Costantino P, Grieneisen VA, Sabatini S** (2017) Auxin minimum triggers the developmental switch from cell division to cell differentiation in the Arabidopsis root. *Proc Natl Acad Sci U S A* **114**: E7641-E7649
- Dietrich D, Pang L, Kobayashi A, Fozard JA, Boudolf V, Bhosale R, Antoni R, Nguyen T, Hiratsuka S, Fujii N, Miyazawa Y, Bae T-W, Wells DM, Owen MR, Band LR, Dyson RJ, Jensen OE, King JR, Tracy SR, Sturrock CJ, Mooney SJ, Roberts JA, Bhalerao RP, Dinneny JR, Rodriguez PL, Nagatani A, Hosokawa Y, Baskin TI, Pridmore TP, De Veylder L, Takahashi H, Bennett MJ** (2017) Root hydrotropism is controlled via a cortex-specific growth mechanism. *Nature Plants* **3**: 17057
- Duan L, Dietrich D, Ng CH, Chan PM, Bhalerao R, Bennett MJ, Dinneny JR** (2013) Endodermal ABA signaling promotes lateral root quiescence during salt stress in Arabidopsis seedlings. *Plant Cell* **25**: 324-341
- Eapen D, Barroso ML, Ponce G, Campos ME, Cassab GI** (2005) Hydrotropism: root growth responses to water. *Trends Plant Sci* **10**: 44-50
- Ferrieri AP, Machado RAR, Arce CCM, Kessler D, Baldwin IT, Erb M** (2017) Localized micronutrient patches induce lateral root foraging and chemotropism in *Nicotiana attenuata*. *J Integr Plant Biol* **59**: 759-771
- Flowers TJ, Colmer TD** (2015) Plant salt tolerance: adaptations in halophytes. *Ann Bot* **115**: 327-331
- Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jurgens G** (2003) Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature* **426**: 147-153
- Galvan-Ampudia CS, Julkowska MM, Darwish E, Gandullo J, Korver RA, Brunoud G, Haring MA, Munnik T, Vernoux T, Testerink C** (2013) Halotropism is a response of plant roots to avoid a saline environment. *Curr Biol* **23**: 2044-2050
- Geisler M, Aryal B, di Donato M, Hao P** (2017) A Critical View on ABC Transporters and Their Interacting Partners in Auxin Transport. *Plant Cell Physiol* **58**: 1601-1614

- Geng Y, Wu R, Wee CW, Xie F, Wei X, Chan PM, Tham C, Duan L, Dinneny JR** (2013) A spatio-temporal understanding of growth regulation during the salt stress response in Arabidopsis. *Plant Cell* **25**: 2132-2154
- Grebe M, Xu J, Möbius W, Ueda T, Nakano A, Geuze HJ, Rook MB, Scheres B** (2003) Arabidopsis Sterol Endocytosis Involves Actin-Mediated Trafficking via ARA6-Positive Early Endosomes. *Current Biol* **13**: 1378-1387
- Han EH, Petrella DP, Blakeslee JJ** (2017) 'Bending' models of halotropism: incorporating protein phosphatase 2A, ABCB transporters, and auxin metabolism. *J Exp Bot*. **68**: 3071-3089
- Hao H, Fan L, Chen T, Li R, Li X, He Q, Botella MA, Lin J** (2014) Clathrin and Membrane Microdomains Cooperatively Regulate RbohD Dynamics and Activity in Arabidopsis. *Plant Cell* **26**: 1729-1745
- Hong Y, Zhang W, Wang X** (2010) Phospholipase D and phosphatidic acid signalling in plant response to drought and salinity. *Plant Cell Environ* **33**: 627-635
- Hou Q, Ufer G, Bartels D** (2016) Lipid signalling in plant responses to abiotic stress. *Plant, cell & environment* **39**: 1029-1048
- Jeudy C, Adrian M, Baussard C, Bernard C, Bernaud E, Bourion V, Busset H, Cabrera-Bosquet L, Cointault F, Han S, Lamboeuf M, Moreau D, Pivato B, Prudent M, Trouvelot S, Truong HN, Vernoud V, Voisin AS, Wipf D, Salon C** (2016) RhizoTubes as a new tool for high throughput imaging of plant root development and architecture: test, comparison with pot grown plants and validation. *Plant Methods* **12**: 31
- Julkowska MM, Hoefsloot HC, Mol S, Feron R, de Boer GJ, Haring MA, Testerink C** (2014) Capturing Arabidopsis root architecture dynamics with ROOT-FIT reveals diversity in responses to salinity. *Plant Physiol* **166**: 1387-1402
- Julkowska MM, Koevoets IT, Mol S, Hoefsloot H, Feron R, Tester MA, Keurentjes JJB, Korte A, Haring MA, de Boer GJ, Testerink C** (2017) Genetic Components of Root Architecture Remodeling in Response to Salt Stress. *Plant Cell* **29**: 3198-3213
- Julkowska MM, Testerink C** (2015) Tuning plant signaling and growth to survive salt. *Trends Plant Sci* **20**: 586-594
- Kazan K** (2015) Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends Plant Sci* **20**: 219-229
- Keisham M, Mukherjee S, Bhatla SC** (2018) Mechanisms of Sodium Transport in Plants-Progresses and Challenges. *Int J Mol Sci* **19**: 647
- Khan MA, Gemenet DC, Villordon A** (2016) Root System Architecture and Abiotic Stress Tolerance: Current Knowledge in Root and Tuber Crops. *Front Plant Sci* **7**: 1584
- Kleine-Vehn J, Friml J** (2008) Polar targeting and endocytic recycling in auxin-dependent plant development. *Annu Rev Cell Dev Biol* **24**: 447-473
- Kleine-Vehn J, Langowski L, Wisniewska J, Dhonukshe P, Brewer PB, Friml J** (2008) Cellular and molecular requirements for polar PIN targeting and transcytosis in plants. *Mol Plant* **1**: 1056-1066
- Koevoets IT, Venema JH, Elzenga JT, Testerink C** (2016) Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. *Front Plant Sci* **7**: 1335
- Konig S, Ischebeck T, Lerche J, Stenzel I, Heilmann I** (2008) Salt-stress-induced association of phosphatidylinositol 4,5-bisphosphate with clathrin-coated vesicles in plants. *Biochem J* **415**: 387-399

- Korasick DA, Enders TA, Strader LC** (2013) Auxin biosynthesis and storage forms. *J Exp Bot* **64**: 2541-2555
- Korver RA, Koevoets IT, Testerink C** (2018) Out of Shape During Stress: A Key Role for Auxin. *Trends Plant Sci* **23**: 783-793
- Leshem Y, Seri L, Levine A** (2007) Induction of phosphatidylinositol 3-kinase-mediated endocytosis by salt stress leads to intracellular production of reactive oxygen species and salt tolerance. *Plant J* **51**: 185-197
- Li R, Liu P, Wan Y, Chen T, Wang Q, Mettbach U, Baluska F, Samaj J, Fang X, Lucas WJ, Lin J** (2012) A membrane microdomain-associated protein, Arabidopsis Flot1, is involved in a clathrin-independent endocytic pathway and is required for seedling development. *Plant Cell* **24**: 2105-2122
- Li X, Wang X, Yang Y, Li R, He Q, Fang X, Luu DT, Maurel C, Lin J** (2011) Single-molecule analysis of PIP2;1 dynamics and partitioning reveals multiple modes of Arabidopsis plasma membrane aquaporin regulation. *Plant Cell* **23**: 3780-3797
- Lobet G, Pages L, Draye X** (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiol* **157**: 29-39
- Mano Y, Nemoto K** (2012) The pathway of auxin biosynthesis in plants. *J Exp Bot* **63**: 2853-2872
- Massa GD, Gilroy S** (2003) Touch modulates gravity sensing to regulate the growth of primary roots of Arabidopsis thaliana. *The Plant J* **33**: 435-445
- McLoughlin F, Arisz SA, Dekker HL, Kramer G, de Koster CG, Haring MA, Munnik T, Testerink C** (2013) Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of Arabidopsis thaliana roots. *Biochem J* **450**: 573-581
- McLoughlin F, Testerink C** (2013) Phosphatidic acid, a versatile water-stress signal in roots. *Front Plant Sci* **4**: 525
- Mei Y, Jia WJ, Chu YJ, Xue HW** (2012) Arabidopsis phosphatidylinositol monophosphate 5-kinase 2 is involved in root gravitropism through regulation of polar auxin transport by affecting the cycling of PIN proteins. *Cell Res* **22**: 581-597
- Men S, Boutte Y, Ikeda Y, Li X, Palme K, Stierhof YD, Hartmann MA, Moritz T, Grebe M** (2008) Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity. *Nat Cell Biol* **10**: 237-244
- Naramoto S** (2017) Polar transport in plants mediated by membrane transporters: focus on mechanisms of polar auxin transport. *Curr Opin Plant Biol* **40**: 8-14
- Pan J, Fujioka S, Peng J, Chen J, Li G, Chen R** (2009) The E3 ubiquitin ligase SCFTIR1/AFB and membrane sterols play key roles in auxin regulation of endocytosis, recycling, and plasma membrane accumulation of the auxin efflux transporter PIN2 in Arabidopsis thaliana. *Plant Cell* **21**: 568-580
- Peer WA** (2013) From perception to attenuation: auxin signalling and responses. *Curr Opin Plant Biol* **16**: 561-568
- Peret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, James N, Casimiro I, Perry P, Syed A, Yang H, Reemmer J, Venison E, Howells C, Perez-Amador MA, Yun J, Alonso J, Beemster GT, Laplaze L, Murphy A, Bennett MJ, Nielsen E, Swarup R** (2012) AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during Arabidopsis development. *Plant Cell* **24**: 2874-2885

- Pical C, Westergren T, Dove SK, Larsson C, Sommarin M** (1999) Salinity and hyperosmotic stress induce rapid increases in phosphatidylinositol 4,5-bisphosphate, diacylglycerol pyrophosphate, and phosphatidylcholine in *Arabidopsis thaliana* cells. *J Biol Chem* **274**: 38232-38240
- Plett DC, Moller IS** (2010) Na(+) transport in glycophytic plants: what we know and would like to know. *Plant Cell Environ* **33**: 612-626
- Robbins NE, 2nd, Dinneny JR** (2015) The divining root: moisture-driven responses of roots at the micro- and macro-scale. *J Exp Bot* **66**: 2145-2154
- Roychoudhury A, Paul S, Basu S** (2013) Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. *Plant Cell Rep* **32**: 985-1006
- Rutschow HL, Baskin TI, Kramer EM** (2014) The carrier AUXIN RESISTANT (AUX1) dominates auxin flux into *Arabidopsis* protoplasts. *New Phytol* **204**: 536-544
- Salehin M, Bagchi R, Estelle M** (2015) SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *Plant Cell* **27**: 9-19
- Sauter M** (2013) Root responses to flooding. *Curr Opin Plant Biol* **16**: 282-286
- Shabala S, Wu H, Bose J** (2015) Salt stress sensing and early signalling events in plant roots: Current knowledge and hypothesis. *Plant Sci* **241**: 109-119
- Smedema LA, Shiati K** (2002) Irrigation and salinity: a perspective review of the salinity hazards of irrigation development in the arid zone. *Irrig. Drain. Syst.* **16**: 161-174
- Strader LC, Zhao Y** (2016) Auxin perception and downstream events. *Curr Opin Plant Biol* **33**: 8-14
- Tanaka H, Kitakura S, Rakusova H, Uemura T, Feraru MI, De Rycke R, Robert S, Kakimoto T, Friml J** (2013) Cell polarity and patterning by PIN trafficking through early endosomal compartments in *Arabidopsis thaliana*. *PLoS Genet* **9**: e1003540
- Testerink C, Munnik T** (2005) Phosphatidic acid: a multifunctional stress signaling lipid in plants. *Trends Plant Sci* **10**: 368-375
- Tivendale ND, Ross JJ, Cohen JD** (2014) The shifting paradigms of auxin biosynthesis. *Trends Plant Sci* **19**: 44-51
- Ventura Y, Eshel A, Pasternak D, Sagi M** (2015) The development of halophyte-based agriculture: past and present. *Ann Bot* **115**: 529-540
- Wisniewska J, Xu J, Seifertova D, Brewer PB, Ruzicka IB, Rouquie D, Benkova E, Scheres B, Friml J** (2006) Polar PIN Localization Directs Auxin Flow in Plants. *Science* **312**: 883-883
- Wu JX, Li J, Liu Z, Yin J, Chang ZY, Rong C, Wu JL, Bi FC, Yao N** (2015) The *Arabidopsis* ceramidase AtACER functions in disease resistance and salt tolerance. *Plant J* **81**: 767-780
- Zhang H, Zhai J, Mo J, Li D, Song F** (2012) Overexpression of rice sphingosine-1-phosphate lyase gene OsSPL1 in transgenic tobacco reduces salt and oxidative stress tolerance. *J Integr Plant Biol* **54**: 652-662
- Zhang J, Nodzynski T, Pencik A, Rolcik J, Friml J** (2010) PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. *Proc Natl Acad Sci U S A* **107**: 918-922
- Zhang J, Peer WA** (2017) Auxin homeostasis: the DAO of catabolism. *J Exp Bot* **68**: 3145-3154

Zhang M, Smith JA, Harberd NP, Jiang C (2016) The regulatory roles of ethylene and reactive oxygen species (ROS) in plant salt stress responses. *Plant Mol Biol* **91**: 651-659