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# Regulation of PIN polarity in response to abiotic stress

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**Abstract**

Plants have evolved robust adaptive mechanisms to withstand the ever-changing environment. Tightly regulated distribution of the hormone auxin throughout the plant body controls an impressive variety of developmental processes that tailor plant growth and morphology to environmental conditions. The proper flow and directionality of auxin between cells is mainly governed by asymmetrically localized efflux carriers – PINs – ensuring proper coordination of developmental processes in plants. Discerning the molecular players and cellular dynamics involved in the establishment and maintenance of PINs in specific membrane domains, as well as their ability to readjust in response to abiotic stressors is essential for understanding how plants balance adaptability and stability. While much is known about how PINs get polarized, there is still limited knowledge about how abiotic stresses alter PIN polarity by acting on these systems. In this review, we focus on the current understanding of mechanisms involved in (re)establishing and maintaining PIN polarity under abiotic stresses.

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## Introduction

The challenges posed by climate change, including changes in temperature, precipitation patterns and atmospheric composition, have significant implications for plant survival [1]. Most studies to date have focused on how traits like biomass are impacted by plant responses to these changes. However, future research needs to focus on molecular and cellular responses in order to improve our mechanistic understanding of plant acclimation and adaptation to abiotic and biotic stress to mitigate the negative impact of such stresses [1].

Plants have an impressive capacity to acclimatize to various stressors by initiating an intricate pattern of signal transduction, involving several integrated pathways. In particular, the plant hormone auxin, which is a key factor mediating endogenous developmental signals to shape overall plant architecture, has been shown to play a role in the regulation of plant growth and development under both biotic and abiotic stresses [2–4]. Auxin undergoes tightly regulated directional transport from one cell to another and its precise distribution is crucial for the formation and maintenance of local auxin gradients as well as auxin minima and maxima. Its stable and/or fluctuating asymmetric distribution is primarily governed by the cell-type specific, plasma membrane (PM)-localized auxin efflux carriers PIN-FORMED proteins (PINs), with coordinated asymmetric (polar) subcellular localization [5–7]. Although research focused on the molecular mechanisms that regulate auxin transport at the cellular level is making significant progress, our understanding of the way that this process responds to abiotic stress is still fragmented. Understanding how plants can re-establish and maintain PIN polarity, enabling them to redirect auxin flow and adapt to stressful conditions, remains a significant research question. In this review, we focus on recent advances in understanding the re-establishment and maintenance of PIN polarity under abiotic stress, specifically considering temperature, drought, and salt stresses. The focus is primarily on the root meristem, as genetic and cell biological approaches have yielded remarkable insights about PIN polarity in this tissue compared to other plant organs.

## PIN polarity in Arabidopsis roots in a nutshell

PINs are integral components of the PM that function as auxin efflux carriers and, through their coordinated polar localization within plant tissues, establish the direction of auxin transport between neighbouring cells [7]. This polarized distribution of PINs plays a crucial role in connecting cellular and tissue polarity, forming the foundation for various developmental processes in plants [6,8]. In brief, most PINs in the Arabidopsis root exhibit basal (rootward) localization in the stele (PIN1, PIN3, PIN4, and PIN7) and cortex cells (PIN2). However, in the epidermis and lateral root cap, PIN2 is

apically localized (shootward), while PIN3 shows lateral localization in the pericycle cells and non-polar distribution in columella cells [8]. The coordinated activity of differentially expressed and localized PINs drives the so-called "reverse fountain" movement of auxin, which describes the downward transport of auxin through the vascular tissue towards the root tip, where it is redirected sideways and subsequently transported upward via the root's outer layers. Eventually, auxin is transported back into the meristem, reinforcing the establishment of an auxin maximum. This dynamic auxin flux is crucial for proper organ development and patterning [9,10].

However, PINs localisation is not static at the PM. Rather, PIN localization is highly dynamic, responding to developmental or environmental stimuli. The primary mechanisms in the establishment and maintenance of the polarity of PINs are subcellular trafficking, phosphorylation, clustering, and feedback loops. These mechanisms have been thoroughly described in recent reviews, providing comprehensive insights into the intricate processes governing the polarity of PINs (reviewed in Refs. [11–13]). However, it is important to note that all these processes are highly dependent on the composition and integrity of the PM and numerous studies have provided evidence that changes in PM lipid homeostasis can have a significant impact on the establishment and maintenance of PIN polarity. Modifying specific lipid components, such as sphingolipids [14,15], sterols [16,17] and phospholipids [18,19], has been shown to impact PIN polarity and/or abundance at the PM. For example, Marhava and colleagues established a model for PIN1-mediated auxin transport in protophloem cells, regulated by elements of a molecular rheostat that modulates auxin efflux in a dynamic fashion [20]. In-depth investigation of PM localization revealed that PIN1 is localized in a specific polar domain, resembling a donut shape that largely depends on a local increase in phospholipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) levels. Disruption of the level of $PI(4,5)P_2$ at the PM destabilizes the molecular rheostat module and leads to aberrant PIN1 localization, which results in altered auxin distribution [21–23]. These studies have demonstrated the crucial role of membrane lipids in PIN-mediated auxin transport, highlighting the significance of PM composition in (re) establishing and maintaining appropriate auxin levels during plant growth and development.

## The PM as a primary target of environmental stress

The PM is a crucial target for environmental stress, as it perceives and translates changes in environmental signals into intracellular responses. To fulfil this task, the PM has evolved into an extremely complex and well-organized structure comprising a multitude of proteins and lipids – a structure whose integrity and fluidity is a prerequisite for plant survival [24]. Environmental stress causes changes in PM physical properties and chemical composition, ultimately affecting signalling output, metabolism, and overall cellular homeostasis [24,25]. Thus, it is imperative for the biological membranes to be adaptive, yet stable, to ensure proper functioning of the cell for plant growth and survival. As stresses directly alter the fluidity of bio-membranes, the ability of plants to remodel membrane lipid and protein composition plays a crucial role in their acclimation and adaptation. Specifically, a sudden increase in ambient temperature makes the PM more fluid (fluidization) and permeable, while a reduction in temperature, water scarcity and salt stress reduce membrane fluidity (rigidification) (Figure 1) [24]. This can have substantial effects not only on the membrane composition, but also on specific interactions between lipids and proteins, and can even modify the conformation and function of membrane proteins and thus modulate signal transduction pathways [26,27].

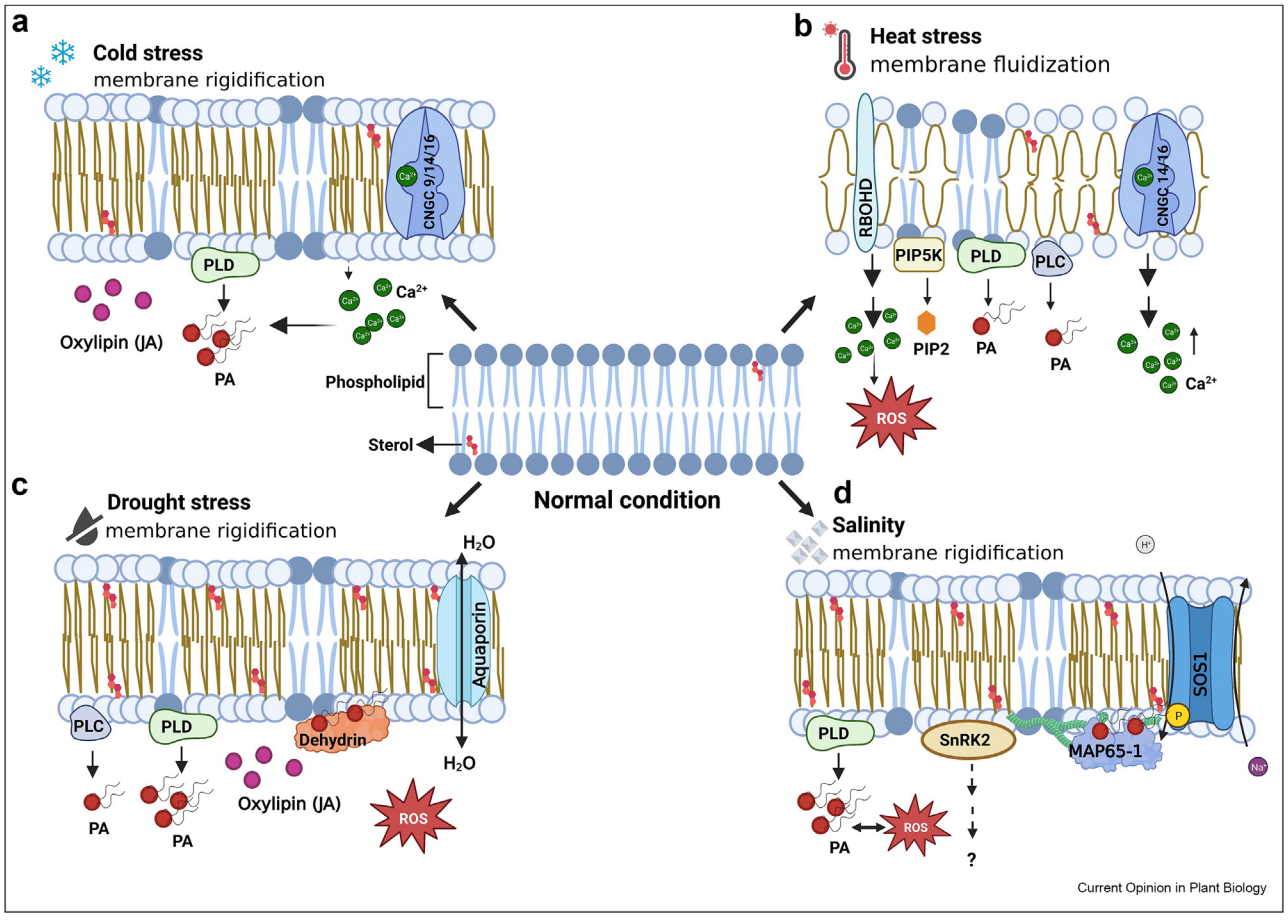
Membrane fluidity relies on two key aspects of lipid composition: the degree of unsaturation and length of the fatty acid chains, as well as changes in the relative proportions of various lipid classes [24,26]. For example, elevated temperatures trigger an increase in the levels of saturated and monounsaturated fatty acids [26], as well as changes in abundance of sterols, glycosides, fatty acids-containing phospholipids and triacylglycerols. Conversely, exposure to cold and salinity stress causes an increase in fatty acid saturation of membrane lipids, leading to membrane rigidification. However, halophytes like *Thellungiella halophila* demonstrate an intriguing adaptation by increasing the unsaturation of fatty acid chains, thus promoting membrane fluidity to facilitate proper cell functioning [26].

## Temperature stress

It has been established that both low and high temperatures have an impact on PIN-mediated auxin transport (28, 34). In this context, it is important to understand how temperature influences the functioning of the PM together with the activity and localization of auxin carriers within it, as this directly affects auxin transport.

In Arabidopsis roots, short term exposure (up to 4 h) to moderate–high temperature stress (29 °C) leads to changes in the intracellular auxin response by stimulating the auxin level within the cells. However, this increase is counterbalanced by enhanced shootward auxin efflux in a PIN2-dependent manner that is regulated, in part, by a component of endosomal sorting machinery SORTING NEXIN 1 (SNX1) that retrieves PIN2 from late endosomes and directs them to the PM to maintain an optimal balance of auxin within the root

**Figure 1**




Schematic representation of changes in membrane fluidity followed by acclimation process in response to various abiotic stressors. (**a**) Low or freezing temperature causes frame shift in the plasma membrane from liquid crystalline phase to gel-like solid state that triggers membrane-associated enzymes to form PA and oxylipin. Membrane bound $Ca^{2+}$ channel CNGC 9/14/16 contributes to cold-induced $Ca^{2+}$ spikes that trigger the release of PA from PLDs. (**b**) High temperature increases membrane fluidization that triggers membrane-associated enzymes like PIP5K, PLC, PLD to form PIP2 and PA respectively. HSR activates membrane bound RBOHD to release $Ca^{2+}$ in the cytosol that leads to a rapid increase in $H_2O_2$ and initiates the ROS/redox signalling pathway. CNGC 14/16 plays a significant role in sensing heat-induced alterations in membrane lipid fluidity by generating heat-induced $Ca^{2+}$ spikes leading to changes at gene expression level. (**c**) Drought generates ROS that causes lipid peroxidation leading to the formation of more saturated fatty acids that decrease membrane fluidity. To counteract this, membrane bound phospholipases activate PA, oxylipin and also increase the plasma membrane sterol levels. PA also interacts with membrane bound dehydrins proteins to prevent denaturation of macromolecules. Plants also activate water channels like aquaporin to regulate the movement of water in and out of the cell membrane. (**d**) Salinity stress generates ROS that causes lipid peroxidation leading to the formation of more saturated fatty acids that decrease membrane fluidity. Salinity-induced PA also interacts and activates protein kinase SnRK2 that causes downstream changes in gene expression through an unknown mechanism. $NA^+/H^+$ antiporter SOS1 ion channel that pumps $Na^+$ ions out of the cell is also activated. PA also binds to MAPK65-1 and stabilizes microtubule organization. The downstream components of lipid signalling are still unknown but changes in gene expression cause various physiological effects resulting in adaptation to abiotic stress. Solid arrows indicate positive regulation. Dashed arrows indicate unknown mechanism. Bidirectional arrow indicates two-way regulatory events. PLC, Phospholipase C; PLD, Phospholipase D; PA, Phosphatidic Acid; ROS, Reactive Oxygen Species; PIP5K, Phosphatidylinositol-4-phosphate 5-kinase; PIP2, Phosphatidylinositol-4,5-bisphosphate; RBOHD, Respiratory Burst oxidase Homolog D: CNGC, Cyclic Nucleotide-Gated Calcium; SOS, Salt Overly Sensitive 1; SnRK2, Sucrose Non-Fermenting 1-Related Protein Kinase 2; MAPK65-1, Microtubule Associated Protein 65-1; HSR, Heat Shock Response. Reviewed in Refs. [24,26].

in response to elevated ambient temperature [28]. However, recent work by Haiyue et al. (2023) described the opposite effect of short-term temperature stress (28 °C) on PIN localization at the PM [29]. When seedlings were exposed to an elevated temperature for 4 h, the fluorescent signal produced by PIN1-GFP and PIN2-GFP in the root meristem virtually disappeared, although this disappearance seemed to be transient. In addition, PIN2 has been shown to undergo a shift in the lateral-to-basal PM of cortical cells [28,29] as well as in epidermal cells, where PIN2 undergoes a shift in the lateral-to-apical PM to enhance shootward auxin efflux [28,29]. Further research is needed to draw firm conclusions about how PIN localization is (re)stablished

and/or maintained in response to short term exposure to moderate—high temperature stress. On the other hand, prolonged exposure (72 h) to moderate—high temperature stress (29 °C) has been shown to reduce the abundance of both PIN1 and PIN2 at the PM without affecting their transcription, resulting in the altered auxin distribution in the Arabidopsis root meristem [30]. Interestingly, a similar phenomenon has been observed in the *ctl1/cher1* mutant, which has perturbed membrane lipid composition, due to defects in a (putative) choline transporter. While pleiotropic, this mutant also displays reduced abundance of PIN1 and PIN3 proteins at the PM due to aberrant endomembrane trafficking, while other PINs remain unaffected — defects, which do not correlate with PIN transcription levels (Wang et al., 2007). These findings, whether influenced by temperature or not, highlight the significance of membrane lipid composition (e.g. sphingolipid abundance and length of fatty acids) in regulating PIN abundance at the PM and, consequently, maintaining proper auxin distribution. Furthermore, temperature-induced alteration in PIN abundance is dependent on a cytoskeletal component — actin, specifically the subclass II actin isovariant ACTIN 7 (ACT7), albeit with a stronger effect on PIN2 [30]. Previous literature further supports the notion that PIN abundance and/or polarity is regulated by actin dynamics in response to various abiotic stresses. For instance, under low phosphate conditions, increased actin bundling was found to decrease MAX2-dependent PIN2 trafficking and polarization in the PM [31]. Interestingly, in both studies, the role of actin cytoskeleton has a different effect on PIN localization compared to AUX1 [30,31], which is an auxin influx carrier localized on the apical side of the cell [32]. However, cellular trafficking of PINs and AUX1 are regulated by two distinct regulatory pathways [33].

Recently, Hong and colleagues (2017) elegantly used experimental and computational approaches to reveal a unique sacrifice-for-survival mechanism in which chilling injury (4 °C/24 h) to root tips induced selective cell death of newly generated columella daughter stem cells (CSCD) [34]. In addition, chilling stress affected the abundance of PINs at the PM (increased level of PIN2 and decreased level of PIN1/3/4/7), which was associated with reduced auxin throughout the root meristem. The authors demonstrated that the death of newly generated CSCD was essential to cause an anatomical block in PIN-mediated auxin transport and hence higher auxin levels at the quiescent centre to maintain its activity. This one-of-a-kind survival strategy ensures the maintenance of a functional stem cell pool for recovery and survival [34]. Shibasaki and colleagues also demonstrated that chilling stress (4 °C for 12 h) induced altered formation of an asymmetric auxin gradient, and hence reduced root growth and gravity response. Live cell imaging of PIN2 and intracellular trafficking in experiments using the fungal toxin Brefeldin A have further revealed that the block in shootward flow of auxin is caused by inhibition of PIN2 cycling between endosomes and the PM rather than altering its polarity [3], a process that depends greatly on membrane lipid composition [35]. Moreover, studying the function of GNOM, a GDP/GTP exchange factor (GEF) for ADP ribosylation factors (ARF) provides additional evidence that the preservation of PIN2 trafficking activity enhances cold tolerance in Arabidopsis seedlings [36]. Nevertheless, treatment of Arabidopsis seedlings with membrane rigidifier DMSO was not found to affect PIN2 cycling or PIN3 dependent graviresponses, thus suggesting that membrane rigidification plays a limited role in cold stress-mediated inhibition of PIN trafficking [3]. However, it is noteworthy that the impact of low temperatures on the inhibition of root growth during cold stress can vary depending on the specific temperature conditions. Studies have shown that different low temperatures — 4 °C vs. 16 °C, for example — can have varying effects on the involvement of PIN proteins. Specifically, at a low temperature of 4 °C, PIN2 primarily contributes to enhancing cold tolerance in Arabidopsis seedlings. However, at 16 °C, PIN1, PIN3, and PIN7 participate in low temperature-mediated inhibition of root growth, with PIN1 playing a major role in this process [37].

Overall, our understanding of the mechanisms governing the (re)establishment and maintenance of PIN protein polar localization in response to temperature stress is still fragmented. However, it is clear that subcellular trafficking of PIN proteins, which is influenced by the organization and integrity of the PM [28—30,34], plays a significant role in the re-establishment of auxin transport. Further research is needed to uncover the specific regulatory pathways and molecular mechanisms involved in this process.

## Drought

The role of auxin machinery in the drought stress response is obscure. A previous report suggested that higher accumulation of ABA in Arabidopsis roots increased the transcript abundance of AUX1 and PIN2. The *aux1*-7 and *eir1-4* mutants exhibited shorter root length, lower root hair density and decreased PM $H^+$ ATPase activity and proton efflux under the control, PEG, and ABA treatments. All these results suggest that ABA-dependent auxin transport enhances proton secretion making Arabidopsis and rice roots more adaptable to moderate water stress [38]. In rice, a putative auxin efflux carrier similar to Arabidopsis PIN3 was isolated and characterized in response to auxin and drought stress. Knockdown lines of *OsPIN3t* showed crown root abnormalities and altered localization in the presence of polar auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA), indicating its role in auxin transport. In addition, overexpression lines of *OsPIN3t* exhibited higher expression of drought

responsive marker genes *DEHYDRATION RESPONSIVE ELEMNT BINDING FACTOR 2A* (*OsDREB2A)* and *OsAP37* and increased drought stress tolerance in terms of better root and shoot growth and more adventitious roots [39]. Recently, Mehra et al. (2022) revealed the dynamic regulatory events during xerobranching, which involves changes in the redistribution of hormones like ABA and auxin. When the root tip experiences transient drought stress, ABA-signalling triggers the closure of plasmodesmata and blocks radial auxin flow to the pericycle cells, a process required for initiating lateral roots. Surprisingly, no spatial changes were observed in auxin influx (AUX1) and efflux (PIN2) carriers, suggesting that during the xerobranching response, auxin moves through the plasmodesmata rather than through its well-characterized transporters [40]. Although over recent years several studies have investigated auxin movement via plasmodesmata [41], this topic remains largely understudied, especially in the context of abiotic stress. Furthermore, additional research is needed to gain a deeper understanding of how the localization of PINs is regulated in response to drought stress, particularly when the PM undergoes rigidification. Unravelling these mechanisms could provide valuable insights into the adaptive responses of plants to water scarcity and potentially lead to novel strategies for enhancing drought tolerance.

## Salinity

Survival in highly saline environment requires several growth adaptations in roots and reduced auxin response is a hallmark trait of salt-induced root growth inhibition. Halotropism, an adaptive growth response in which roots change gravitropic behaviour to avoid a highly saline area requires auxin redistribution through differential regulation of PIN2 proteins and their re-localization [42]. The activation of phospholipase D by salt induces an increase in clathrin-mediated endocytosis, a process regulated by $PI(4,5)P_2$, which leads to the internalization of PIN2 at the side of the root exposed to the high salt concentration [42]. As a result, the transport of auxin towards the shoot is reduced, leading to an accumulation of auxin on the non-saline side of the root. This imbalance in auxin levels results in reduced cell elongation and gravitropic bending away from the area of high salinity. Based on these observations it has been suggested that the phosphatidic acid (PA) plays a role in regulating the polar localization of PINs during halotropism [42]. The roots of the *pldζ1* mutant have been found to display an exaggerated gravitropic and reduced halotropic response induced by salt [43]. In wild type roots subjected to salt stress, there was an increase in the intracellular accumulation of PIN2, resulting in a decrease in its abundance at the apical side. With prolonged exposure to salt stress, PIN2 underwent relocation to the lateral membranes. However, the *pldζ1* mutant failed to properly redistribute PIN2 to the lateral membranes, although it initially responded similarly to wild type roots after 5 min of salt stress. This indicates a disrupted ability of the *pldζ1* mutant to properly redistribute PIN2 in response to salt-induced stress [43]. However, using both computational modelling and *in planta* experiments, it has been suggested that changes solely in PIN2 re-localization would be insufficient to generate an effective auxin asymmetry [44]. Instead, auxin-dependent regulation and distribution of AUX1, such as clustering in the PM and abundance at the apical-lateral polar domain, as well as a transient increase in PIN1 in the stele, are crucial for the swift establishment of auxin asymmetry during halotropism [2,44]. In addition, PA and PI4P interact with protein kinases PINOID (PID) and D6 PROTEIN KINASE (D6PK), which through phosphorylation, regulate the activity and polarity of PINs (reviewed in Ref. [13]). The authors proposed that salt-induced PA enhances PID activity, which alters PIN2 activity and abundance at the PM, resulting in altered auxin accumulation and increased root growth when subjected to salt stress [45]. However, to enhance the model of halotropic growth, the incorporation of additional factors such as apolarly localized ATP-binding cassette B (ABCB)-type auxin exporters [46], protein phosphatase 2 A (PP2A) activity (dephosphorylation of auxin transporters) and auxin metabolism has been proposed. These factors are believed to play important roles in shaping the complex processes involved in halotropism and should be considered when developing a more comprehensive understanding of plant growth under salt stress conditions (reviewed in Ref. [47]). Taken together, all the research over the past decade demonstrates how abiotic stress, such as salt stress, impacts the composition of the PM, which, in turn, leads to modified interactions between lipids and proteins, ultimately resulting in the reorganization of biological processes when plants encounter unfavourable environmental conditions.

## Conclusion

The hormone auxin acts as a key mediator in a plant's response to abiotic stress. One of the critical aspects of auxin regulation is its transport, which is tightly regulated by the polar localization of PINs. Under abiotic stress conditions, such as temperature, drought, and salt stresses, the polar localization and/or activity of PINs is dynamically altered, allowing plants to fine-tune auxin transport to optimize their response to the stressor. Although a few studies have made significant progress in this research field, the precise nature of these changes and the underlying molecular mechanisms are still not fully understood. Hence, understanding how plants adjust auxin transport in response to climate change factors requires an integration of multiple levels of biological research, including molecular studies of plant adaptation at the tissue and cell-type-specific levels in

connection with PM functioning. Furthermore, recent advances in technology (e.g. imaging techniques, single-cell analysis techniques) have brought about a revolutionary shift in the field of cell biology, allowing us to delve into the intricacies of biological mechanisms at even deeper molecular level [21]. This should shed more light on the control of PIN polarity, particularly in plants experiencing stress conditions, during which they must undergo adaptive reorganization to ensure survival. Overall, studies of abiotic stress and its impact on auxin transport provide valuable insights into how plants perceive and respond to their environment.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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