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Stomatal evolution and plant adaptation to future climate

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Abstract

Global climate change is affecting plant photosynthesis and transpiration processes, as well as increasing weather extremes impacting socio-political and environmental events and decisions for decades to come. One major research challenge in plant biology and ecology is the interaction of photosynthesis with the environment. Stomata control plant gas exchange and their evolution was a crucial innovation that facilitated the earliest land plants to colonize terrestrial environments. Stomata couple homoiohydry, together with cuticles, intercellular gas space, with the endohydric water-conducting system, enabling plants to adapt and diversify across the planet. Plants control stomatal movement in response to environmental change through regulating guard cell turgor mediated by membrane transporters and signaling transduction. However, the origin, evolution, and active control of stomata remain controversial topics. We first review stomatal evolution and diversity, providing fossil and phylogenetic evidence of their origins. We summarize functional evolution of guard cell membrane transporters in the context of climate changes and environmental stresses. Our analyses show that the core signaling elements of stomatal movement are more ancient than stomata, while genes involved in stomatal development co-evolved *de novo* with the earliest stomata. These results suggest that novel stomatal development-specific genes were acquired during plant evolution, whereas genes regulating stomatal movement, especially cell signaling pathways, were inherited ancestrally and co-opted by dynamic functional differentiation. These two processes reflect the different adaptation strategies during land plant evolution.

KEYWORDS

abiotic stresses, climate change, membrane transporters, molecular evolution, photosynthesis, stomatal guard cell, sustainable plant production

1 | INTRODUCTION

Stomata are small pores surrounded by guard cells (guard cells and subsidiary cells for some species) on the epidermal surface in most plants. Stomata are primarily responsible for the exchange of gases, such as carbon dioxide (CO_2) and oxygen (O_2), and water (H_2O) through opening and closing, promoting the plant's continual adaptation to its environment and climate extremes (Lawson and Matthews, 2020; Li et al., 2022). During photosynthesis, stomata allow the absorption of CO_2, which is essential for the synthesis of

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sugars and other organic compounds through the process of photosynthesis (Inoue et al., 2022). Powered by sunlight, CO_2 combines with water to produce carbohydrates and oxygen (Ding et al., 2023). Transpiration, the loss of water vapor via stomata, regulates temperature, uptake of H_2O and essential nutrients, and cell turgidity (Xu et al., 2023). Therefore, stomata are crucial for plant growth and survival, particularly in response and adaptation to climate changes (Driever et al., 2023).

The acquisition of stomata is regarded as a key innovation that contributed to successful terrestrial colonization and radiation in the earliest land plants (Clark et al., 2022). So far, the fossil record shows the first appearance of stomata at 450 million years ago (Mya), but more solid evidence for stomatal diversification is found, in well-preserved plant fossils from 420 Mya (Wellman et al., 2003). Bryophytes (hornworts, mosses, and liverworts) are the earliest diverging extant land plants, but their fossil record is poor. Liverworts (Marchantiophyta), which evolved around 475 Mya, lack stomata and use air pores for gas exchange (Bowman et al., 2017). While mosses and hornworts, like most other land plants, possess sporophyte stomata usually formed by a pair of kidney-shaped guard cells (Chater et al., 2016; Clark et al., 2022). A far more recently diverging lineage, monocots, in particular the grasses, have an alternative stomata structure, often 'dumb-bell'-shaped, composed of a pair of guard cells and two subsidiary cells (Chen et al., 2017; Wang & LI, LI, et al., 2020). Why have land plants evolved stomata with increasing complexity and function?

The stomatal guard cell is one of the most studied cell types in plants. Its physiological, and ecological roles in balancing of the carbon and water cycles in our ecosystems are paramount for humans and animals. Much has been learnt about guard cell membrane transport and stomatal development since the 1980s, followed by the discovery of molecular identities of key transporters and regulatory proteins since the release of the Arabidopsis and rice genomes in early 2000s. Benefiting from the increased number of sequenced genomes for key plant species and gene editing tools in plant models including early divergent stomatous as mosses (e.g., *Physcomitrium patens*), ferns (e.g., *Ceratopteris richardii*), and model angiosperms (Arabidopsis, rice) including trees (*Populus spp.*), researchers can compare stomatal function and molecular evolution across plant species. In line with their primary role, the angiosperm stomata exhibit opening response to light and low atmospheric CO_2 levels, while they close in reaction to decreased air humidity. Conversely, our understanding of their counterparts in bryophytes and early diverging vascular plants (such as ferns and lycophytes) remains incomplete. For instance, some studies have shown that the drought hormone abscisic acid (ABA) induces stomatal closure in mosses, lycophytes, and ferns (Cai et al., 2017; Chater et al., 2013; Chater et al., 2016; Chater, 2024; Hõrak et al., 2017; Meigas et al., 2024; Ruszala et al., 2011), however, other experiments on lycophytes and ferns have indicated that the stomata of these species are insensitive to ABA (Brodribb and McAdam, 2011; Duckett and Pressel, 2018). Thus, the evolution of stomata may have contributed significantly to angiosperm diversification and speciation enabling them to become the most dominate clade in the plant kingdom, while stomata of earlier divergent lineages (e.g., mosses, lycophytes) may possess less complex responses which may have limited their spread to drier and hotter climatic regions.

Here, we summarize the fossil and molecular evidence to trace the most likely origin of stomata. We review the function of stomata in photosynthesis and transpiration in response to environmental stresses. We discuss the function and regulation of guard cell membrane transporters in the speed of stomatal opening and closure. Evolutionary bioinformatics demonstrates that the origin of genes related to plant stomata development is closely associated with the origin of stomata themselves. However, the genes governing stomatal movement in the plant genome pre-existed the origin stomata, and gave rise to a greater complexity in regulatory mechanisms with the emergence of stomata and changes in the environment. For excellent recent reviews, the readers are guided to topics including the origin and evolution of stomata (Clark et al., 2022), stomatal response to abiotic stress (Buckley, 2019), stomatal function (Lawson and Matthews, 2020), and stomatal modelling and genetic engineering (Nguyen et al., 2023).

2 | EVOLUTION AND DIVERSITY OF STOMATA IN PLANTS

2.1 | Fossil, morphological, and phylogenetic evidence of early evolution of stomata

The fossil record provides solid evidence for the origin and evolution of stomata. Although the earliest phase of land plant evolution is complex to interpret, stomata have been found neither in fossils of the earliest plants nor in living representatives of the common ancestor of extant land plants (McElwain and Steinthorsdottir, 2017). Acquisition of stomata occurred about 450 Mya, which is ~20 Mya later than the earliest fossil record of land plants in the Ordovician (Raven, 2002). An Ordovician plant macrofossil at Zbrza possesses a putative stomatal complex (Clark et al., 2022), but the first unequivocal evidence of stomata in abundant plant life on land is found in fossils dating from the end of the Silurian (~ 425 Mya) which exhibiting sporangial morphologies and stomatal distributions similar to extant mosses (Renzaglia et al., 2020). In the Devonian (419 ~ 358 Mya), a period of rapid land plant radiations, there is significant evidence in well-described plant species for morphologically distinct stomata composed of kidney-shaped guard cells (Edwards et al., 1998). However, fossil records provide neither the evidence of which plant lineages lacked stomatal movement, nor which lineages gradually acquired functional complexity. Moreover, as yet no fossils have been identified that display intermediate forms between pavement and guard cells, and thus it is hard to explain if or how stomata first developed from other epidermal cells (Clark et al., 2022).

Morphological evidence of stomata in extant plants such as bryophytes is much easier to obtain compared to fossil stomatal records. A major difference between liverworts and all other extant land plants, is their complete absence of stomata, which are widespread but not ubiquitous in hornworts and mosses (Chen et al., 2017; Morris et al., 2018; Renzaglia et al., 2017; Renzaglia et al., 2020). Howeverkey genes for stomatal development are retained in liverworts, which indicates that the ancestor to liverworts possessed stomata that were subsequently lost (Moriya et al., 2023; Raven, 2002). There are two well-documented losses of stomata in the hornwort clade (Renzaglia et al., 2017) and the two oldest moss clades (Takakiales and Andreaeopsida) have no stomata, suggesting that moss stomata may have been even more expendable during early diversification than they are today. Moss stomata are located on sporangia and appear to aid processes of sporogenesis and spore dispersal (Chater et al., 2016), divergent functions from Tracheophyta stomata (Cai et al., 2017; Renzaglia et al., 2020). These lines of evidence suggest that ancestral stomatal functions, like those of bryophyte stomata, may have differed from more recently diverged lineages. Nevertheless, the appearance of stomata and their subsequent innovations mark a major turning point for the success of the plants that have dominated our biosphere.

Phylogenetics is another effective method that can be used to infer the origin and evolution of stomata. We can interpret the evolution of stomata via the relationships among green plant lineages and the evolution of stomatal development and regulation related genes. Two major monophyletic land plant lineages are supported by phylogenomic analyses: Tracheophyta and Bryophyta (One Thousand Plant Transcriptomes, 2019). The relationship among bryophytes has been resolved, that hornworts are sister to a moss and liverwort clade, and liverworts and mosses are sister lineages forming the group setaphytes. Phylogenetic evidence has rejected the hypothesis that liverworts are sister to all other extant land plant lineages (Moriya et al., 2023; One Thousand Plant Transcriptomes, 2019; Raven, 2002). Additionally, the topology reconstructed by new genomes and transcriptomes supports bryophyte monophyly (Harris et al., 2020; One Thousand Plant Transcriptomes, 2019).

It is highly likely that stomata have their origin in the shared ancestor of extant bryophytes and tracheophytes, but the exact origin remains a fundamental question to be pursued by plant evolutionary biologists. In summary, further genomic, morphological and fossil evidence showing clear relationships among early land plants will be critical for understanding the evolution of stomata in land plants.

2.2 | Loss of stomata depending on the adaption of plants to their environments

Compelling evidence show that stomata losses occurred in species that have evolved to become aquatic, poikilohydric or holoparasitic, reflecting the reductive evolution of stomata from the phylogenomic viewpoint (Harris et al., 2020; Olsen et al., 2016; Raven, 2002). In many bryophytes, stomata may facilitate CO_2 uptake and spore ripening and drying for dispersal (Chater et al., 2016; Kubásek et al., 2021; Pressel et al., 2014), but there have been at least 63 independent stomatal losses in mosses (Renzaglia et al., 2020). Stomatal loss across moss and hornwort lineages highlights that it is an ongoing process. Their presence in mosses and hornworts implies that stomata evolved once in the common ancestor of all land plants, before the divergence (~495–515 Mya) of tracheophytes and bryophytes (Clark et al., 2022; Raven, 2002). Their absence is more likely to be due to secondary losses rather than ancestral absence. The marine angiosperm seagrass *Zostera marina* lost its entire repertoire of stomata-related genes and consequently stomata when adapting to the marine environment (Olsen et al., 2016). Another water-living angiosperm, the lotus (*Nelumbo nucifera*), has stomata only on the upper surface of its floating leaves; furthermore lotus stomata have gained the novel function to actively regulate convective airflow (Matthews and Seymour, 2014). Thus, diverse and novel functions of stomata in specific plant species may reflect their adaptative evolution to specific ecological niches and environmental stresses.

2.3 | Evolutionary insights of stomatal development

Numerous investigations on stomatal development provide invaluable insights into stomatal evolution particularly through studies of eudicot and monocot models. We summarize this complex process in Figures 1 and 2. In Arabidopsis, stomata initiate through the asymmetric cell division of a meristemoid mother cell (MMC), which results in the formation of a meristemoid and a larger stomatal lineage ground cell (SLGC) to generate a guard mother cell (GMC). Subsequently, the GMC undergoes symmetric division to become a pair of guard cells (Vaten and Bergmann, 2012). Key molecular regulators driving this process are the basic helix-loop-helix (bHLH) transcription factors: SPEECHLESS (SPCH) to initiate meristemoid production, MUTE for GMC development, and FAMA for symmetric division into guard cells (Mckown et al., 2023). Inducer Of CBF Expression1/Scream (ICE1/SCRM) and SCRM2, form heterodimers with SPCH, MUTE, and FAMA, thereby facilitating all three stomatal fate transitions (Kanaoka et al., 2008). SCARECROWs (SCRs) are expressed in the initial cells that control the asymmetric cell division (Wu et al., 2019). SHORTROOT (SHR) acts as a positional signal that moves from the vasculature to the surrounding cell layer where it is bound by SCR (Cui et al., 2007). The stomatal polarity protein Breaking of Asymmetry In The Stomatal Lineage (BASL) regulates cell size and fate asymmetries and is also an effector of peptide signaling to coordinate the division frequencies of sister cells produced by asymmetric cell divisions (Gong et al., 2021). The polar BREVIS RADIX (BRX)-like family and Polar Localization during Asymmetric Division and Redistribution (POLAR) proteins function in stomatal development and epidermal patterning (Chowdhury et al., 2021). A mitogen-activated protein kinase (MAPK) cascade, regulated by peptides known as epidermal patterning factors (EPFs), negatively regulates stomatal lineage fate (Wang et al., 2007). Upstream of

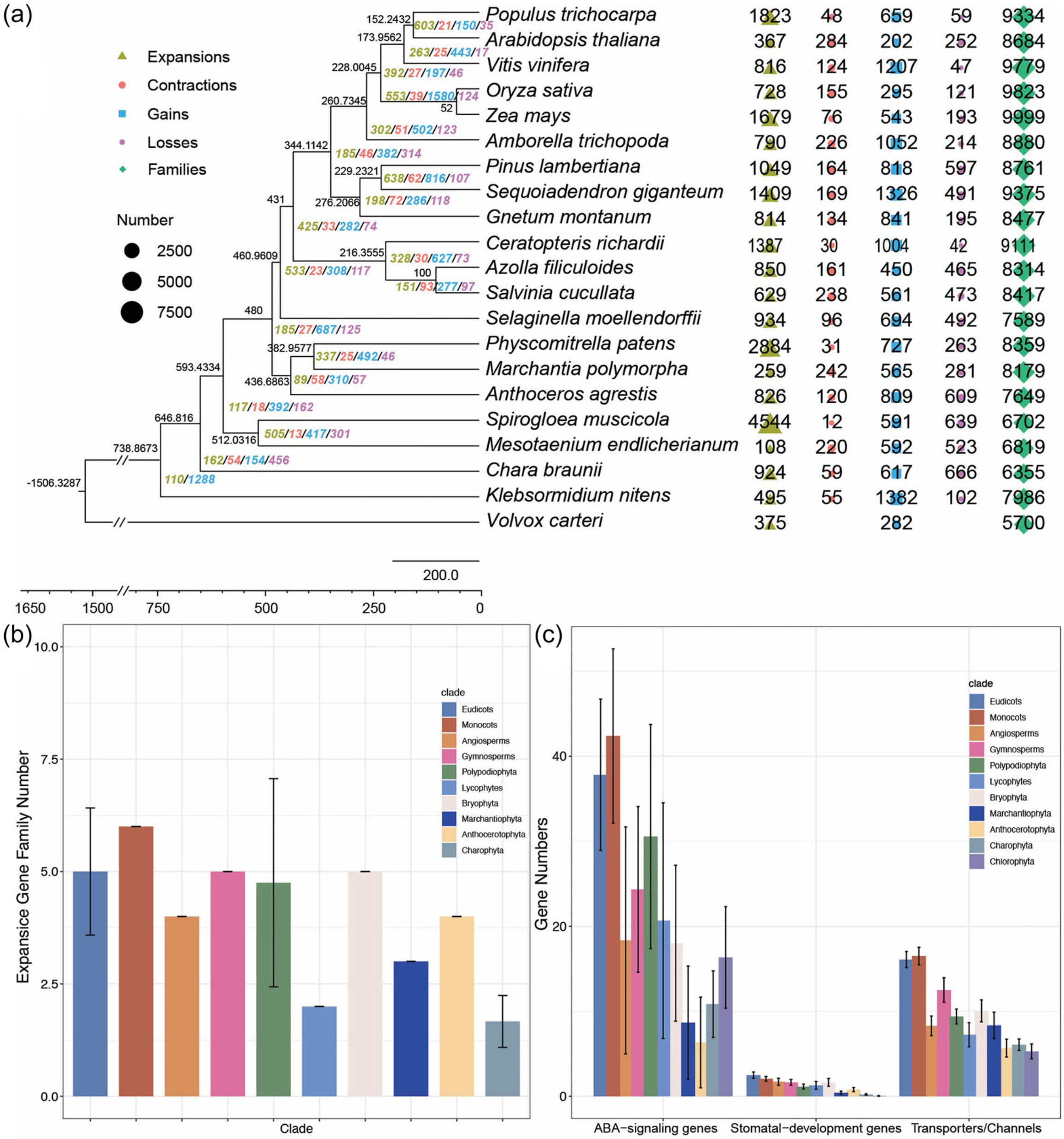

FIGURE 1 Ancestral reconstruction of gene family expansion/contraction and gain/loss in green plants. (a) general landscape of gene family expansions and contractions in land plants. (b) identified expanded gene family numbers out of 38 gene families analysed in this study in different plant clades. (c) statistics of average gene members in different plant clades. The gene families for analysis are the same as the 38 gene families analysed in this study. The phylogeny of selected 21 representative green plants was reconstructed based on the concatenated super-genes from the low copy orthologs clustered from published whole genomes. We selected *Volvox carteri* as the outgroup. [Color figure can be viewed at wileyonlinelibrary.com]

intracellular signaling cascades, stomatal spacing is modulated by secreted peptides belonging to the EPF-LIKE (EPFL) family, as well as three leucine-rich repeat receptor kinases (LRR-RLKs), namely ERECTA (ER), ERECTA-LIKE 1 (ERL1), and ERL2 and one LRR-receptor-like protein, too many mouths (TMM) (Hara et al., 2009). Peptides EPF1 and EPF2 and STOMAGEN (STOM, also referred to as EPFL9) act as competing ligands to the RLKs, to modulate stomatal development through MAP kinase pathway (Hunt et al., 2010; Sugano et al., 2010).

Phylogenetic analysis of gene families provides insights into the origin, evolution, duplication, divergence, and loss of genes. We analyzed the family homologs of stomatal development-related genes across with the evolution of green plant species (Figure 3). FAMA, SPCH, and MUTE are paralogues, and their orthologues are present in all tested land plant species, even in the stomata-free liverworts. Duplication of those gene families occurred in lycophytes, then further expanded in ferns and seed plants. EPFs, TMMs, SHRs, and SCRs appear in bryophytes and tracheophytes, but have been lost in

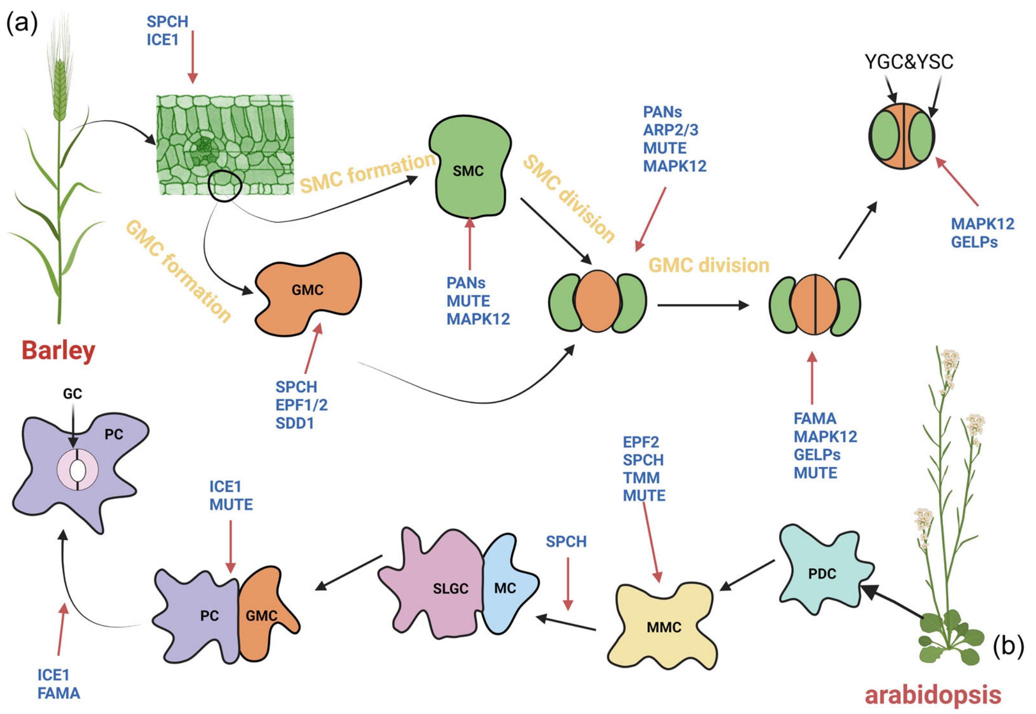

FIGURE 2 Model of stomatal lineage in stomatal development in *Arabidopsis thaliana* (eudicot) versus *Hordeum vulgare* (monocot). The development processes of different types of stomatal are shown. (a) barley and (b) Arabidopsis stomata are chosen as examples. [Color figure can be viewed at wileyonlinelibrary.com]

some lineages and species. The NAP and PIR peptides originated earlier than the appearance of stomata and are found in Charophyta. The BASL orthologues are found only in eudicots. These findings point to an origin of stomatal development-related genes in the common ancestor of all embryophytes. Combined with the species phylogeny and insights into stomatal function described above, this supports an origin of stomata in the common ancestor of all land plants. The increasing complexity of stomatal development from bryophytes to angiosperms bestowed later diverging lineages with the tools to exert greater control of this ongoing process of evolutionary adaptation to the environment. Similarly, genetic control of stomata-related traits such as gas exchange and guard cell size are important targets for selection and breeding for high photosynthetic performance and yield under different environments in many crop species.

In recent years, multi-omics studies (Wang et al., 2023), especially the application of single-cell omics technology, have further deepened our understanding of both the dynamic developmental processes of stomatal cell lineages and the dynamic cell physiology of opening and closing (Lopez-Anido et al., 2021; Sun et al., 2022; Zhang et al., 2021b). A model of cell fate determination via cell-cell communication to drive stomatal production and epidermal patterning in *Arabidopsis* has been proposed (Pillitteri and Torii, 2012). Single-cell RNA sequencing (scRNA-seq) in Arabidopsis demonstrated that WRKY33 and BASIC PENTACYSTEINE 1 (BPC1), BPC2, BPC4, BPC6 are potential factors that regulate stomatal lineage cell fate (Liu et al., 2020). In both monocot and eudicot plants, the stomatal density is usually higher on the lower leaf surface compared to that of the upper leaf surface. Compared to eudicot plants, each stomatal complex in grasses, such as wheat, rice, maize and barley, typically consists of two specialized dumbbell-shaped guard cells and their subsidiary cells (SCs) (Nunes et al., 2020). Six main developmental stages of grass stomata have been defined (Figure 2): (1) specification of the stomatal file; (2) GMC formation; (3) formation and polarization of the subsidiary mother cell (SMC); (4) asymmetric division of SMCs to generate SCs; (5) symmetric division of GMCs producing paired GCs; and (6) differentiation and morphogenesis of the four-celled stomatal complex. Single-nucleus RNA-seq applied on maize leaf epidermal peels has identified cell wall-related genes, as new candidates, which may play important roles in the morphogenesis of dumbbell-shaped of developing and mature stomata (Sun et al., 2022). In addition, an Arabidopsis proteome study of enriched GC protoplasts, identified approximately 3,000 proteins not previously found in the GC proteome with more than 600 proteins that may be specific to GCs, for example, a guard cell-specific kinase RAF15 (Wang et al., 2023). In the future, single-cell sequencing of the stomata of representative members in moss, lycophyte, and fern species (Marchant et al., 2022), such as *Lemna minuta* (Abramson et al., 2022) and *Physcomitrium patens* (Kubo et al., 2019), is expected to reveal changes in stomatal development and regulation during evolution, as well as their adaptive evolution to their environments.

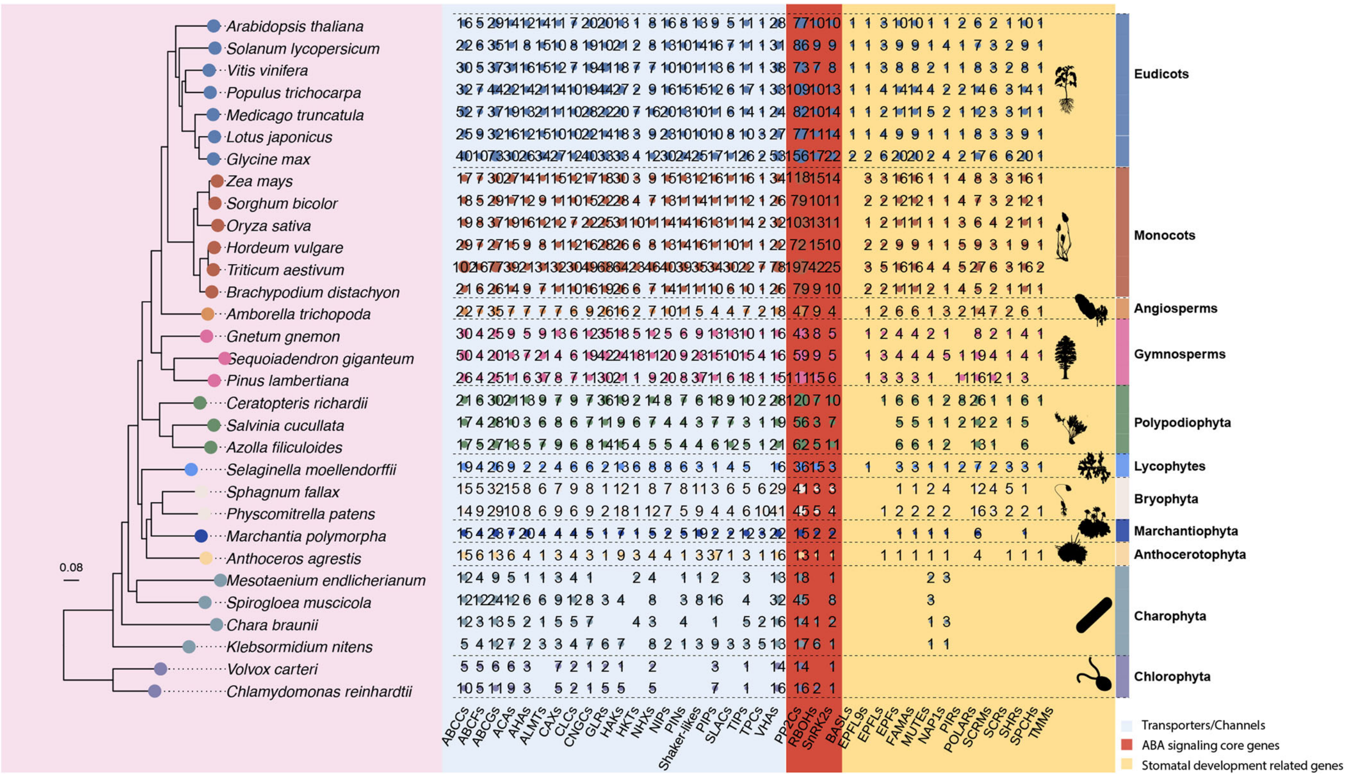

FIGURE 3 Diagram of gene family evolutionary of guard cell membrane transporters, ABA reception complex, and stomatal-development regulators in plants. Twenty-one ion channels/transporters (blue background), three ABA signalling core elements (brick background), and fourteen stomatal development related gene families (yellow background) were analysed. The gene family members were count and shown in texts and points with different size. The colors of points represent different plant clades. Abbreviations: ABCC, ATP-binding cassette C transporter; ACA, autoinhibited Ca^{2+}-ATPase; AHA, Arabidopsis plasma membrane H^+-ATPase; ALMT, aluminum-activated malate transporter; AVP, Arabidopsis vacuolar H + -pyrophosphatase; BASL, breaking of asymmetry in the stomatal lineage; CAX, cation proton exchanger; CLC, chloride channel; CNGC, cyclic nucleotide gated channel; EPFs, epidermal patterning factors; EPFLs, epidermal patterning factor-like; GLR, glutamate receptor-like Ca^{2+} channel; HAK, high-affinity K^+ transporter; HKT, high-affinity K^+/Na^+ transporter; KAT, guard cell inwardly-rectifying K^+ channel; NHX, Na^+/H^+ antiporter; OST1, open stomata 1; PIP, plasma membrane intrinsic protein; POLAR, localization during asymmetric division and redistribution; PP2C, protein phosphatase 2C; RCAR, regulatory component of ABA receptor; SCRs, scarecrows; SCRMs, inducer of CBF expression1/scream (ICE1/SCRM); SHRs, shortroot; SLAC, slow anion channel; SPCH, speechless; TIP, tonoplast intrinsic protein; TPC, two-pore Ca^{2+} channel; TPK, tonoplast K^+ channel; VHA, vacuolar H^+-ATPase. [Color figure can be viewed at wileyonlinelibrary.com]

3 | FUNCTIONAL EVOLUTION OF GUARD CELL MEMBRANE TRANSPORTERS

Achieving control of stomatal pore opening and closing to regulate gas exchange and transpiration is pivotal to temporal plant adaptation to various environmental stimuli. The regulation of ion and solute transport between guard cells and adjacent cells is achieved by multiple signaling transduction pathways (Chen et al., 2017). Elevated concentrations of osmotically active ions and solutes within the guard cell promote the uptake of water, facilitated by aquaporins, leading to an increase in turgor pressure (Eisenach and De Angeli, 2017). Potassium ions are the predominant inorganic osmotic regulators in guard cells and are balanced by chloride ions as well as organic anions, such as malate (Roux and Leonhardt, 2018). While the relationships between solute content, turgor pressure, guard cell volume, and stomatal aperture are evident, these associations are modulated by the complex coordination of ion, solute, and water fluxes across the plasma membrane (PM) and tonoplast. Precise guard cell control involves a network of transport proteins that facilitate the transport of ions and metabolites. This section focuses on the evolutionary role of genes encoding K^+ and anion transporters and aquaporins as key examples. The details of many other transporters (e.g., Ca^{2+} transporters, H^+ pumps) are not discussed here.

3.1 | Evolution of potassium transport for stomatal regulation

The most extensively studied ion channels in guard cells are the voltage-gated potassium channels (K^+ channels) (Nieves-Cordones et al., 2019). The Shaker like family potassium channels have been studied in many species. In Arabidopsis, KAT1, KAT2, and AKT1 are inward-rectifying ion channels activated by hyperpolarization, that facilitate K^+ entry and localize to the plasma membrane to promote stomatal opening (Lebaudy et al., 2010; Xicluna et al., 2007). Conversely, GORK is an outward-rectifying channel mediating the efflux of K^+ from the cell that can be activated by depolarization (Ache et al., 2000). AKT2, a weakly rectifying channel, mediates both the entry and exit of K^+ to and from the plant cell. KAT3 (KC1) is a unique member that exhibits no channel activity but is activated when heterooligomerized with either KAT1 or AKT1 (Sharma et al., 2013). Furthermore, these K_{in} channels can form heteromeric K^+ channels with each other to enhance the currents (Lebaudy et al., 2010; Xicluna et al., 2007). Interestingly, maize KAT1-like channel KZM2 and KZM3 form a heteromeric channel to regulate fast stomatal opening (Gao et al., 2017). Furthermore, the conserve functions of voltage-dependent K^+ channel are observed in angiosperms such as barley, rice, and foxtail millet (Adem et al., 2020; Feng et al., 2020; Zhang et al., 2022). However, whether Shaker-like potassium channels control stomatal movement in the early diverged plant species remain to be addressed. An electrophysiological study has demonstrated that guard cells from two fern species *Polypodium vulgare* and *Asplenium scolopendrium* possess voltage-dependent inward- and outward-rectifying K^+ channels, which exhibit properties like the Shaker channels found in angiosperms. However, the Ca^{2+}-dependent responses in fern stomata demonstrated guard cell interconnections by plasmodesmata, which appears to be lost in seed plants during evolution (Voss et al., 2018). Although phylogenetic evidence indicates that Shaker-like genes are present in the Chlorophyta, the lycophyte *Selaginella moellendorffii* appears to lack inward-rectifying Shaker K^+ channels, and the mosses *Physcomitrium patens* and *Sphagnum fallax* appear to lack outward-rectifying ones, even though they are present in the liverwort *Marchantia polymorpha* (Sussmilch et al., 2019). Previous studies observed that desiccation induced the increase of potassium levels in *Sphagnum* moss pseudostomata, in contrast to Arabidopsis guard cells which show decreased potassium content (Duckett et al., 2009; Hosy et al., 2003). This difference may be due to the function of GORK, which facilitates K^+ efflux during water stress-induced stomatal closure (Hosy et al., 2003). Taken together, this may suggest some loss or divergence of stomata closure control mediated by K^+ efflux in moss (Brodribb et al., 2020).

The high-affinity K^+ transporters (HKTs) and K^+ uptake permease (HAK/KUP/KTs) are critical for K^+ homeostasis in plant cells. The loss of *KUP6* and *KUP8* in Arabidopsis causes failure of ABA-induced stomatal closure (Osakabe et al., 2013). The Arabidopsis $Na^+(K^+)/H^+$ antiporter NHX family members NHX1 and NHX2 act redundantly in turgor pressure regulation and facilitate stomatal movement (Barragán et al., 2012). Orthologues of HKTs, HAKs, and NHXs are found in all land plants and algae, but their functions in stomatal movement regulation remain unknown in other clades beyond the angiosperms (Figure 3). Considering their importance in K^+ balance in plant cell turgor regulation, further study is crucial to decipher their functions in stomatal regulation in the changing environment.

3.2 | Evolution of anion transport in stomatal movements

Three anion channel gene families including slow anion channel (SLACs), aluminum activated malate transporter (ALMTs), and chloride channel (CLCs) members are important and extensively studied in plant stomatal regulation. In angiosperms such as Arabidopsis, SLAC1-type anion channels can be distinctly categorized into three groups: the SLAH1/4-group, the SLAH2/$_{3}^{-}$group, and the SLAC1-group (Dreyer et al., 2012). The SLAH1/4-group diverged from the other groups in an early event predating the emergence of bryophytes. The functional and structural diversity within the SLAC/SLAH class further expanded with the separation of the SLAH2/3 group from the SLAC1 group, occurring after the appearance of bryophytes but before the advent of lycophytes (Figure 4) (Dreyer et al., 2012). Following the transition to land, SLAC1-type anion channels were present in green plant lineages that predate seed plants, including green algae (represented by *Klebsormidium nitens* in Figure 4), liverworts (*Marchantia polymorpha*), mosses (*Physcomitrium* patens), lycophytes (*Selaginella moellendorffii*), and ferns (*Ceratopteris richardii*), but lost in streptophyte algae (*Chara hraunii*, *Spirogloea muscicola* and *Mesotaenium endlicherianu*) (Figure 1 and Figure 4). The absence of SLAC1 in Arabidopsis results in plants with open stomata under ozone, CO_2, and a decrease in air humidity conditions (Laanemets et al., 2013). The SLAC1 homolog SLAH3, also highly expressed in guard cells mediated nitrate-induced anion currents to control stomatal closure (Zhang et al., 2016). SLAC1 and SLAH3 share similar characteristics, although SLAH3 presents higher permeability to NO_3^- than SLAC1 (Geiger et al., 2011). The selectivity of SLAC1 varies in different plant species. Eudicot SLAC1 channels, such as those from Arabidopsis and tomato, are permeable to chloride and nitrate-insensitive (Schäfer et al., 2018), but the nitrate-to-chloride permeability of monocot SLAC1 channels, such as those from date palm *Phoenix dactylifera*, barley and rice, is higher compared to *At*SLAC1 (Müller et al., 2017; Schäfer et al., 2018). However, recent studies on the eudicot pear *Pyrus bretschneideri* observed that *Pbr*SLAC1 is a nitrate-selective anion channel in guard cells (Chen et al., 2022). Furthermore, *Physcomitrium patens Pp*SLAC1, *Selaginella moellendorffii Sm*SLAC1, and fern *Ceratopteris richardii Cr*SLAC1 all demonstrate nitrate permeable anion channel activity, but whether they have chloride selectivity is unknown (Mcadam et al., 2016). This indicates that the selectivity of SLAC1/SLAHs is species-specific. New evidence showed that, except for nitrate, SLAC1 activity can be directly activated by extracellular malate and stimulated Ca^{2+} signaling in Arabidopsis guard cells (Mimata et al., 2022). With the increasingly diverse and complex regulatory mechanisms underpinning SLAC1 activity in guard cells, as well as its central role in stomatal movement, active investigations on SLAC1 are ongoing.

The ALMTs are also known as QUAC (quick-activating channels), reflecting their function in facilitating rapid-type currents. There are 14 ALMT members identified in Arabidopsis (Figure 3). To date, four of the vacuolar *At*ALMTs have been identified as being expressed in

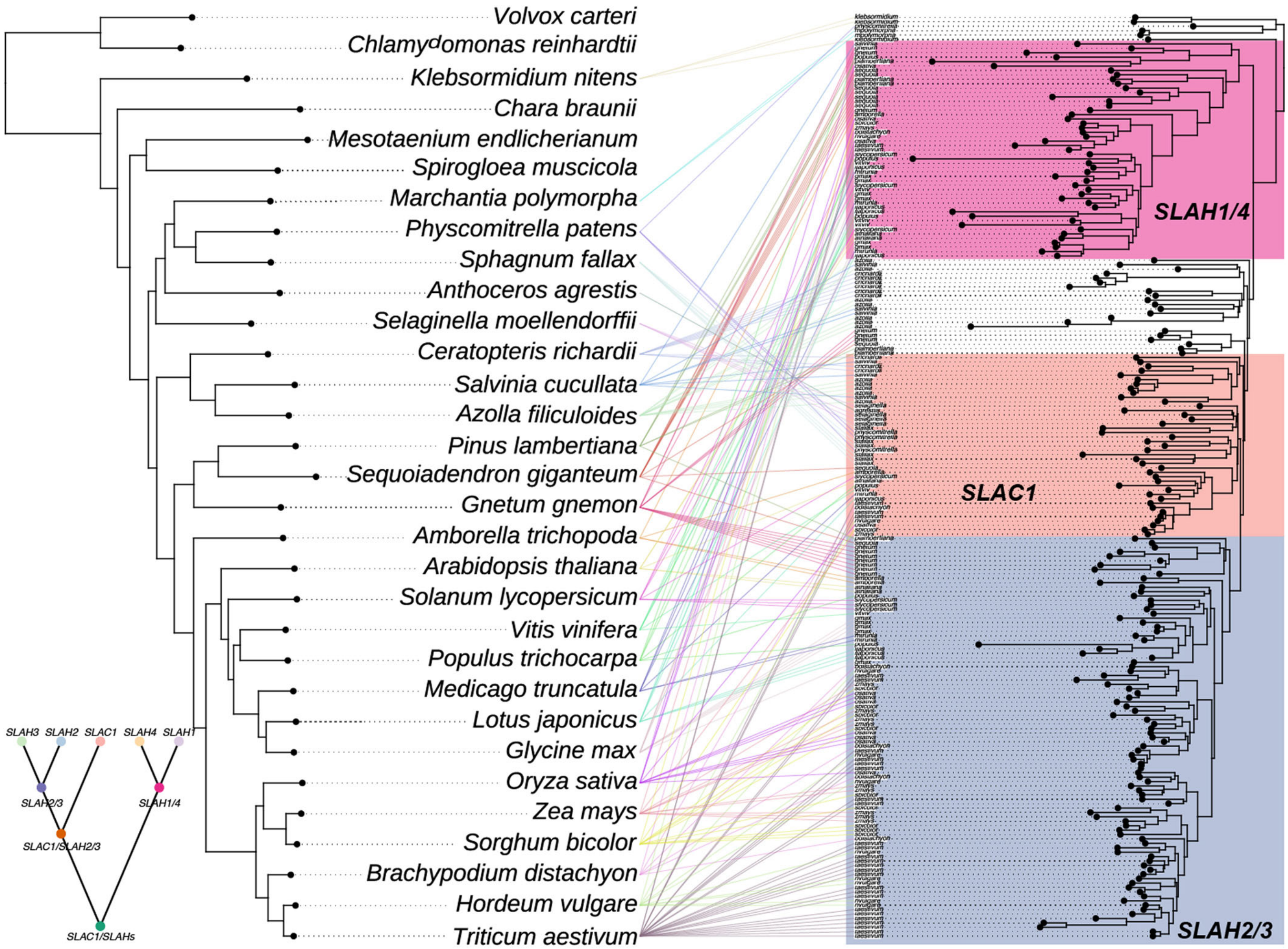

FIGURE 4 Evolutionary processes of SLAC1s in green plants. The phylogeny in the left panel represents the species tree, which is built based on genomic data. The phylogeny in the right panel is the gene tree constructed by *SLAC1* gene family members. In total 104 duplication events were identified in the *SLAC1* gene family during evolution in selected species. The comparison of species and gene trees are linked with lines in different colors. Background in the gene tree presents different clades of *SLAC1* and *SLAH*s. The panel on the lower left corner is the evolutionary model of SLAC1 in plants. [Color figure can be viewed at wileyonlinelibrary.com]

guard cells. It was revealed that *At*ALMT6 facilitates inward currents of Mal^{2-} (malate) and Fum^{2-} (fumarate) and is activated by micromolar concentrations of cytosolic Ca^{2+}, and acidic vacuolar pH (Dreyer et al., 2012). However, no discernible stomatal aperture phenotype has been detected in almt6 knockout plants, perhaps due to redundancy of malate transporters in guard cells (Meyer et al., 2011). *At*ALMT9 is localized at the vacuolar membrane functions in light-induced stomatal opening; *almt9* knockout mutants exhibit reduced stomatal opening. *At*ALMT9 is a voltage-gated inward-rectifying chloride channel, activated by negative membrane potentials and cytosolic malate (De Angeli et al., 2013). *At*ALMT12 and *At*ALMT4 mediate vacuolar Mal^{2-} efflux leading to stomatal closure (Eisenach & Baetz, Huck, et al., 2017; Meyer et al., 2010). The malate-selective R-type anion channel *AtALMT12* is highly expressed in guard cells, playing key roles in the dark- and CO_2-induced stomatal closure (Meyer et al., 2010). The Arabidopsis loss-of-function mutants of AtALMT4 have normal stomatal opening, but present delayed and incomplete stomatal closure in response to ABA (Eisenach & Baetz, Huck, et al., 2017). Functional comparison of tomato *Sl*ALMT11 and *At*ALMT12 indicate that they are both activated by malate and mediate malate efflux that cause stomatal closure (Sasaki et al., 2022). The biological functions of ALMTs are conserved in monocots and eudicots. The overexpression of *HvALMT1*, a barley guard cell malate efflux channel, does not totally impair stomatal function but instead slows stomatal closure responses to low light (Gruber et al., 2011). However, *Hv*ALMT1's electrophysiological characters are unclear. The anion current characters of *Brachypodium distachyon Bd*ALMT12 is similar to *At*ALMT12, where both malate and Ca^{2+}/CaM coregulate channel activation n (Luu et al., 2019). Although ALMT homologues are identified in the algal sister groups to land plants, and therefore arose much earlier than the origin of stomata, the molecular functions of ALMTs in nonflowering plants are still unknown (Figure 3). Understanding ALMT function in early divergent plants like ferns, lycophytes, and mosses will enhance our understanding of the origins of precise stomatal control and could provide guidance for evolutionary adaptation to future climates. The storage of monovalent anions in the guard cell vacuole is also facilitated by chloride channel CLC transporters. CLC family members located in intracellular organelles promote nitrate homeostasis and pH adjustments. In Arabidopsis, *At*CLCc is a tonoplast channel strongly expressed in guard cells that limits stomatal opening by controlling Cl^- accumulation when response to salt stress, light and ABA (Jossier et al., 2010). It is probable that CLCc coordinates the transport of Cl^- into vacuoles with an H^+ flux into the cytosol (Jossier et al., 2010). *AtCLCa*, another highly guard cell$^-$expressed NO_3^-/H^+ antiporter, allows inward NO^{3-}currents into guard cell vacuoles, to activate stomatal opening in response to light (Demes et al., 2020). Surprisingly, another study observed that *At*CLCa also mediate anions efflux resulting in stomatal closure induced by ABA (Wege et al., 2014). These diverse and flexible anion channel regulatory mechanisms allow plants to achieve more precise control over stomata using limited components to swiftly respond to environmental changes.

3.3 | Aquaporins in stomatal movements

In guard cells, aquaporins, such as Plasma Membrane Intrinsic Proteins (PIPs), gamma Tonoplast Intrinsic Proteins (TIPs), and NOD26-like Intrinsic Proteins (NIPs), enable rapid water and solutes movement across different membranes during stomatal opening and closure (Vaziriyeganeh et al., 2023). There are 18 members of PIPs in *Arabidopsis* (Figure 3). *AtPIP1;2* show decreased expression during drought stress, leading to reduced water permeability of guard cells and promoting stomatal closure (Heckwolf et al., 2011). This adaptive response helps higher plants conserve water during periods of water scarcity. However, unlike *Arabidopsis*, *PpPIP1;1* is downregulated under high humidity in the moss *P. patens* (Yooyongwech et al., 2009), potentially reducing water permeability of guard cells and preventing excessive water uptake. This adaptive response helps maintain proper stomatal function and prevents water-related damage. In Arabidopsis, elevated CO_2 levels lead to decreased expression of *AtPIP1;1* and increased expression of *AtPIP2;1*, resulting in reduced water permeability and smaller stomatal apertures (Yang et al., 2022b). This adaptation helps plants optimize water-use efficiency under elevated CO_2 conditions through the newly discovered CO_2 sensing mechanisms in guard cells. It was found that Arabidopsis mitogen activated protein kinase 4 (MPK4) and MPK12, together with high leaf temperature 1 (HT1), constitute the primary stomatal CO_2/bicarbonate sensor upstream of the CONVERGENCE OF BLUE LIGHT AND CO_2 1 (CBC1) kinase (Takahashi et al., 2022). Later, CO_2-dependent stomatal responses were confirmed rely on MPK12's ability to bind to HT1, but not its kinase activity (Yeh et al., 2023). All these studies demonstrate how aquaporins in guard cells can adapt their expression and activity in response to various environmental cues. Such adaptations allow plants to better regulate stomata for optimizing water use efficiency, thereby ensuring their survival and productivity under changing environmental conditions.

Overall, our bioinformatics analysis showed that the evolutionary origins of anion channels (SLACs, ALMTs), potassium transporters (HKTs), aquaporins (NIPs, PIPs), as well as auxin transporters (PINs) arose from the common ancestor of Chlorophytes and land plants. In contrast, the ABC transporters (ABCCs, ABCFs and ABCGs), calcium transporters (ACAs, CAXs, CLCs, CNGCs), glutamate receptor-like channels (GLRs), potassium transporters (HAKs), Shaker-like transporters, cation/proton antiporters (NHXs), aquaporins (TIPs), and H^+ pumps (AHAs, VHAs) may have originated from the common ancestor of all green plants (Figure 3). The gene numbers in each gene family are also representative of the increasing trend in complexity across the evolution of diverse plant clades (Figure 1a). Surprisingly, further exploration of gene family expansions and constructions in representative plants (Figure 3) shows that 18 out of 21 channels/transporters analyzed can be classified as rapidly evolved (Table S1). In addition, PP2Cs, RBOHs, SnRK2s, and four stomatal-development related gene families the bHLHs (SPCHs, FAMAs, and MUTEs) as well as EPFLs are shown to be evolving rapidly (Table S1). These genes have undergone significant changes over a relatively short period and are assumed to rapidly evolve in response to environmental stresses like drought, high temperatures, or other ecological pressures, ensuring the plant's survival and reproduction (Steensels et al., 2021; Wang & LI, LI, et al., 2020). Gene family expansions are found in all plant clades with varying gene family numbers (Figure 1b, Table S1). These results support our hypothesis that ion channels and the ABA signaling pathway elements underwent strong functional differentiation during plant evolution, contributing to increasingly refined stomatal regulation. The gene structure and functional diversity and differentiation caused by gene family expansions have aided plant survival and niche expansion under various environmental stresses (Figure 1c).

Land plants possess the toolkit to regulate stomatal aperture, environmental regulation of stomatal conductance may have evolved early in land plant history and subsequently underg1 secondary losses along with stomatal losses in liverworts, some mosses, and many aquatic tracheophytes (Matthews and Seymour, 2014; Olsen et al., 2016; Pressel et al., 2014; Renzaglia et al., 2020). There are still many gaps in our understanding of how early diverged plants like lycophytes and mosses regulate stomatal movement to adapt to external environmental changes. Investigations of ion channels/transporter functionality in these lineages will expand our understanding of the processes by which these genes underwent functional differentiation throughout the evolution of plants.

4 | EVOLUTION OF THE REGULATORY COMPONENTS OF GUARD CELL MEMBRANE TRANSPORT

The regulation of guard cell membrane transport is a complex and tightly controlled process that plays a central role in stomatal movement and plant responses to environmental stimuli. Diverse regulatory mechanisms including protein phosphorylation, cytosolic signaling molecules, and hormonal signaling, fine-tune guard cell membrane transport (Lawson and Matthews, 2020). The knowledge of the origin of stomatal control in plants is evolving. In sporophytes of some hornworts and mosses, once the stomata are mature, they are open, thus benefiting in spores drying and release (Chater et al., 2016). Gas exchange studies fail to detect moss stomatal responses to light or CO_2 (Kubásek et al., 2021), perhaps due to conditional effects. Similarly, several fern and lycophyte studies showed that no decline in gas exchange with ABA and CO_2, suggesting an ancestrally passive stomatal regulation closely tied to hydraulic status (Sussmilch et al., 2019). However, distinct stomatal responses to elevated CO_2, light, and ABA in ferns have also been observed in other studies (Cai et al., 2017; Cai et al., 2021, Meigas et al., 2024), suggesting that fern aperture responses may be both environment- and species-dependent (Cardoso et al., 2020). What is the molecular evidence for these diverse and sometimes apparently conflicting responses of stomata in the early divergent plant lineages?

4.1 | Evolution of phytohormone signaling pathways in land plants

Lines of evidence are yielding new insights into the evolutionary mechanisms of stomatal regulation. The drought hormone ABA, the core regulator of stomatal closure induced by abiotic stresses, is used here as a key example of the evolution of phytohormone signaling pathways in land plants. In Arabidopsis, ABA is first perceived by a receptor complex composed of the PYR/PYL/RCAR family of ABA receptors and the PP2C family of negative regulators (Park et al., 2009). When ABA binds to the receptor complex, it inhibits the activity of PP2Cs, leading to the activation of SnRK2 kinase SnRK2.6/OST1. Activated SnRK2 kinases phosphorylate specific target proteins, including SLAC1(Acharya et al., 2013; Lind et al., 2015; Yang et al., 2022a). SnRK2s are highly conserved in protein sequences and function among plants, while SLAC1 presents phylogenic and functional diversity across land plant evolution (Figure 3, Figure S1) (Chen et al., 2017). In experiments conducted using the *Xenopus* oocyte expression system, it was observed that *Pp*SLAC2 could be weakly activated by *Pp*OST1.2 from the moss *P. patens*. However, when various SLAC1 homologs from algae, liverworts, lycophytes, and ferns were tested, none of them were activated by native OST1 kinases (Lind et al., 2015; Mcadam et al., 2016). Replacement of the *Pp*SLAC1 N terminal with that of *At*SLAC1, resulted in *At*Nt-*Pp*SLAC1 which was activated by all OSTs tested, not just by *At*OST1 and *Pp*OST1.2 (Lind et al., 2015). Recent studies on *P. patens* and Arabidopsis revealed that Raf-like kinase (ARK) is a primary activator of OST1, which is negatively regulated by PP2C-A in response to ABA and osmotic stress, thus regulating the activation of SLAC1 (Islam et al., 2021; Katsuta et al., 2020; Saruhashi et al., 2015). This suggests that the mechanism of regulation of SLAC1 is complex but is functionally conserved during the evolution. Moreover, Ca^{2+}-dependent protein kinases (CPKs) function as $[Ca^{2+}]_{cyt}$ sensors that mediate the Ca^{2+}-dependent regulation of S-type anion channels. SLAC1 is activated by CPK3, CPK6 CPK21, and CPK23 in a calcium-dependent way, and this activation is inhibited by PP2Cs (Scherzer et al., 2012). *In vitro* and in vivo studies have identified a mechanism by which the Ca^{2+}-CPK-dependent pathway integrates with PYR/PYL/RCAR-PP2C-SnRK2 in guard cells (Huang et al., 2023). The CBL-interacting protein kinase CIPK23 is critical in regulating stomatal opening in plants by direct phosphorylation of potassium channel AKT1 to enhance the K^+ uptake in guard cells (Inoue et al., 2020) and inhibiting SLAC1-type channels to prevent anion efflux from guard cells, thereby maintaining stomata open (Huang et al., 2023).

These studies all suggest diverse molecular regulation of ABA signaling pathways across land plants, particularly between angiosperms and early divergent plant lineages. Our gene family orthologue analysis reveals that the key ABA-signaling elements, PP2Cs, SnRK2s, and downstream transporters, exist in all clades of test species, indicating their evolution pre-dated stomatal acquisition in plants (Figure 1). These results imply that the upstream ABA signaling pathway is highly conserved, while the functional divergence of transporters and channels across evolution is more closely related to plant adaptation to different environments. Understanding how plants adapt to changing climates has always been a crucial aspect of plant adaptation and evolution. However, current knowledge, especially research on nonvascular and early vascular plant lineages, remains notably limited. There is, therefore, a pressing need to bolster research efforts in molecular biology of stomatal regulation in nonvascular and early diverging vascular plants.

4.2 | Evolution of key components of cytoskeleton regulation in stomata

Guard cells rapidly and routinely undergo massive changes of up to 70% of their cell volume, caused by the flux of water and solutes during stomatal opening and closure (Hills et al., 2012). The cytoskeleton including actin filaments (AFs) and microtubules (MTs), is pivotal for this stomatal volume change, enabling the regulation of guard cell turgor pressure (Khanna et al., 2014). AFs and MTs also function in cell division and cell wall synthesis, directly affecting guard cell shape, structure, and mechanics (Muroyama et al., 2020).

AF dynamics power stomatal opening and closing by regulating ion channels and NADPH oxidase. Cytochalasin D, an inhibitor of actin polymerization, induced the opening of Ca^{2+}-permeable channel at the single-channel level and enhanced the current magnitude at the whole-cell level. However, pretreatment with a filamentous actin (F-actin) stabilizer, phalloidin, limited channel activation and current enhancements in *Vicia faba* guard cells (Zhang and Fan, 2009). Latrunculin B depolymerizes AFs and enhances ABA-induced RbohD and ROS accumulation (Li et al., 2014). Actin-binding proteins (ABPs) contribute to the dynamic adjustments of AFs in response to environmental fluctuations (Li et al., 2015). Recent research has unveiled the involvement of several ABPs in the regulation of stomata. Notably, mutations affecting subunits of the ARP2/3 complex, such as *arpc4*, *arpc5*, *arpc2* (referred to as *hsr3*), *arp2* (referred to as *wrm*), or *arp3* (referred to as dis1), produce a consistent phenotype characterized by diminished or completely impaired stomatal closure in darkness in response to ABA and H_2O_2, and delayed light-induced stomatal opening (Isner et al., 2017; Li et al., 2014). The ARP2/3 complex in conjunction with its upstream regulator, the SCAR/WAVE complex, plays an essential role in stomatal movement by influencing the disorganization and remodeling of AFs. A mutation in the *PIR1* gene, which encodes a subunit of the SCAR/WAVE complex, leads to a reduction in the dark-induced stomatal closure (Isner et al., 2017). However, the response to ABA or $CaCl_2$ remains unaffected, and the insensitivity to darkness can be reversed through treatment with latrunculin B or cytochalasin D (Isner et al., 2017). Actin-depolymerizing Factor (ADF), a conserved class of APBs, participates in stomatal movement regulation through the ABA signaling pathway. The key link between stomatal regulation and AF is the formation of the ABA-PYR/PYLs/PP2Cs complex in guard cells. It was shown the activation of Casein Kinase 1-Like Protein 2 (CKL2) and subsequently the CKL2 phosphorylation of ADF4 can induce stomatal closure under drought or ABA treatment through regulating actin reorganization (Shi et al., 2022).

Microtubules (MTs) serve as guides for cellulose synthesis complexes and determine the orientation of cellulose microfibrils, potentially contributing to the high tensile strength exhibited by guard cells during stomatal movement (Rui and Anderson, 2016). In Arabidopsis, MTs guide the process of cellulose synthesis complexes (CSCs) for cellulose production at the cell surface (Gutierrez et al., 2009). The co-localization between CSCs and MTs diminishes, and the motility speed of CSCs increases during the closure of stomata induced by darkness (Rui and Anderson, 2016). Moreover, MTs determine the mode of alignment for callose deposition, and the disruption of MTs, as induced by oryzalin, perturbs the pattern of callose deposition in guard cells of the fern *Asplenium nidus* L. (Apostolakos et al., 2009). Microtubule-associated proteins (MAPs) participate in MT organization and stomatal movement regulation with their upstream regulators. WAVE-DAMPENED2 (WVD2)/WVD2-LIKE 7 (WDL7) acts as an MT stabilizer via binding to MTs directly, preventing ABA-induced stomatal closure, and MICROTUBULE RELATED E3 LIGASE 57 (MERL57) can target and ubiquitinate WDL7 directly for degradation (Dou et al., 2021). The ubiquitin JAV1-ASSOCIATED UBIQUITIN LIGASE1 (JUL) functions downstream of H_2O_2 and Ca^{2+} in ABA-induced microtubule disorganization and stomatal closure (Yu et al., 2020). Loss of SUN-INTERACTING NUCLEAR ENVELOPE PROTEIN 1 (SINE1) and SINE2 results in MT disorder in guard cells during stomatal movement (Biel et al., 2020). Consequently, the organization of MTs is likely to influence the configuration of the cell wall during stomatal movement.

The majority of current research into the stomatal functions and molecular mechanisms of AFs and MTs focus on the model plant Arabidopsis and therefore few evolutionary perspectives can be drawn. To address this, future investigations on AFs and MTs should be expanded to the early divergent land plant species, and their interactions with hormone signaling pathways and ion channels in the regulation of stomata.

5 | RESPONSE AND EVOLUTION OF GUARD CELL MEMBRANE TRANSPORT TO ENVIRONMENTAL STRESSES

Guard cell machinery has evolved to be buffeted by extremes. Light plays a crucial role in stomatal regulation through a complex interplay of various signaling pathways and photoreceptors (Li et al., 2023; Wang et al., 2008) (Figure 5), yet excessive light induces stress in plants, leading to photoinhibition and damage to photosynthetic machinery (Wall et al., 2022). In response to high light, guard cells often close, arguably to reduce water loss through transpiration (Wang et al., 2008). Excessive light can also alter the movement of ions, particularly K^+, Ca^{2+}, and H^+, across the guard cell plasma membrane (Lamport, 2023). Photoinhibition can lead to the production of reactive oxygen species (ROS) within guard cells (Watkins et al., 2017) which trigger oxidative stress and damage cellular components, including the plasma membrane and its associated transporters. Excess light is also often accompanied by excess thermal energy, and the guard cell has also evolved to be highly responsive to heat. Stomata tend to open wider at higher temperatures and close at lower temperatures. Higher temperatures accelerate water loss from plant tissues through increased evapo-

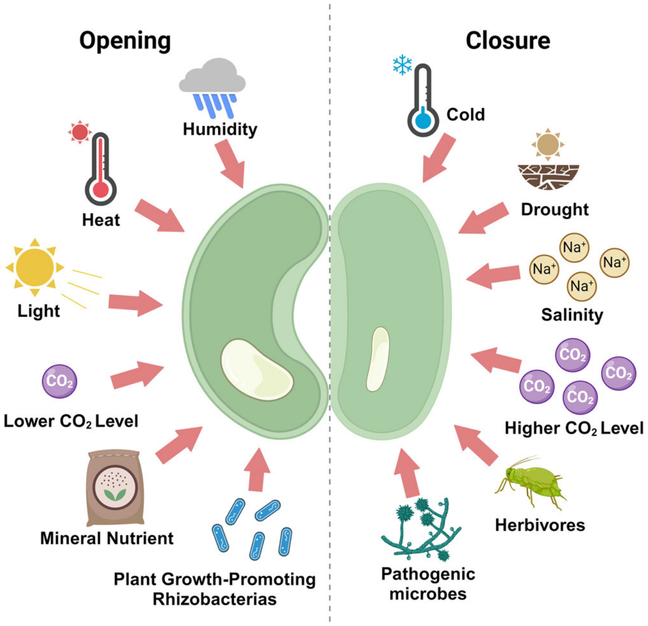

FIGURE 5 Schematic diagram of potential environmental factors to stimulating stomata movement. Different environmental stimuli are shown as illustrations, and the dicot guard cell was used as an example. The templates were obtained from Biorender (https://www.biorender.com). [Color figure can be viewed at wileyonlinelibrary.com]

transpiration (Abro et al., 2022) to help plants cool and to balance the trade-off between water loss and the need for CO_2 uptake for photosynthesis (Khanthavong et al., 2022). However, when heat stress is coupled with limited soil water availability this can exacerbate drought severity and other abiotic stresses. Extreme temperatures can also induce stomatal closure to prevent excessive water loss and reduce the risk of dehydration. Elevated temperatures can enhance ion transport, which promotes guard cell swelling and stomatal opening (Müller et al., 2017). Conversely, cooler temperatures can impede ion fluxes and result in stomatal closure (Ge et al., 2022). Temperature stress can also influence stomatal development process in different plant species. Some plants may respond to high temperature by reducing stomatal density, resulting in fewer stomata per unit area to minimize the risk of dehydration (Witoń et al., 2021). Excess light and heat can also be coupled with water deficit (drought) stress which almost invariably induces ABA biosynthesis and ABA-activated stomatal closure (Malcheska et al., 2017). Despite these combined and interconnected environmental stressors, guard cells have evolved parallel sensors of water availability and can respond independently to drought signals (QI et al., 2020). The suite of ABA receptors and signaling proteins that perceive ABA changes and the increasing regulatory complexity in ion channels and transporters are testament to this evolutionary feat (see Section 4.1 for more details).

As an essential nutrient, potassium (K^+), is also fundamental for control of stomatal aperture in many plants (Cochrane and Cochrane, 2009). Similarly, optimum levels of Nitrogen (N), Phosphorus (P), and Magnesium (Mg), as macronutrients, the proper levels of these nutrients are important for maintaining the physiological processes associated with stomatal regulation. Nutrient deficiencies or imbalances can disrupt stomatal regulation and compromise plant water relations, photosynthesis, and overall growth (Patel et al., 2020). Apart from essential mineral nutrients, salt and heavy metals negatively affect stomatal behaviour. Salinity stress, involving the accumulation of sodium ions (Na^+) and chloride ions (Cl^-), disrupts guard cell ion homeostasis and osmotic regulation (Niu et al., 2022). High salt concentrations also interfere with K^+ uptake, and triggers accumulation of guard cell ABA, contributing to stomatal closure (Zhang et al., 2021a). Little is known about the evolutionary origins or divergences of guard cell responses to ion stress and nutrient imbalance.

Guard cell signaling evolved not only as a dynamic interface with the abiotic environment, but also as the reactive and defensive gatekeeping sentries to a portal accessible to endosymbiotc and pathogenic microbiota. These organisms have themselves evolved ways to hijack the gates. Certain beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR), can produce compounds that promote stomatal opening (Tsai et al., 2020) (Figure 5). These compounds can include signaling molecules like cytokinins or volatile organic compounds (VOCs) that directly or indirectly stimulate guard cells, enhancing stomatal aperture (Liu et al., 2022). This enhanced stomatal opening facilitates improved gas exchange, increases photosynthetic efficiency, and promotes plant growth. Pathogenic microbes, such as bacteria, fungi, or viruses, can invade leaf tissue through open stomata (Zeng et al., 2010). Some pathogens have evolved mechanisms to manipulate stomatal behavior for their own benefit, by producing effector molecules that trigger stomatal closure, limiting the entry of other microorganisms or preventing the plant's defense responses (Meddya et al., 2023). This response involves the regulation of ion channels, such as anion channels, potassium (K^+) channels, and proton (H^+) pumps (Potkay and Feng, 2023).

Despite the importance of plant-environment interactions for the evolution of stomata, there are still limited studies on the response and evolution of guard cell functional proteins to environmental stresses in early diverging plant species. This situation is about to change given the successful sequencing of genomes and genetic transformation of early diverging plant species (e.g. *M. polymorpha*, *P. patens*, *C. richardii*), which can be compared to the extensive functional information of genes in model angiosperms (e.g. *A. thaliana*, *O. sativa*). Many comparative studies on multiple species can be conducted to unravel the evolutionary history and functional diversity of the key genes that regulate stomata.

6 | CONCLUSION AND FUTURE PERSPECTIVES

By summarizing the evolution and diversity of stomata in plants in terms of fossil, morphology, and phylogeny, it is thus proposed that stomata have the likely origin from the ancestors of extant bryophytes and the loss or divergence of stomata in certain species that is strongly influenced by natural selection and evolutionary adaptation. We also conducted analysis of the functional evolution of gene families for stomatal development, membrane transport and regulatory components. We conclude that the membrane transporters, channels and regulatory components are dynamic, evolved rapidly and functionally diverged to regulate stomatal movement, enhancing plant abiotic stress adaptation during evolution. Compared to stomatal movement, stomatal development-related genes present more conservation during evolution. Moreover, the evolution of membrane transporters in the context of abiotic and biotic stresses was also extensively review. We suggest that plants regulate stomatal movement and development through different strategies to adapt to diverse environments during the process of evolution.

There are many outstanding questions on stomatal evolution and the roles of stomata in plant adaptation to future climates, which need to be investigated by researchers in the future. When did stomata appear in green plants and evolve to regulate photosynthesis and transpiration? Discovery of new fossils is essential to solve the mystery of the origin of stomata and answer the key evo-devo (evolutionary developmental biology) questions on stomatal development. It is also crucial to identify whether there are any extant bryophytes that possess transitional intermediate form of cells between guard cells and epidermal cells. What are the dominant factors (membrane transport or stomatal structure and development, both, or indeed other factors) regulating the speed of stomatal opening and closure? For the second question, we propose that more research efforts should focus on early divergent land plants such as liverworts (as negative controls), mosses, lycophytes, and ferns, particularly the use of the model species of these lineages. What are the "ideal stomata" for improving crop yield, water use efficiency and resistance to abiotic and biotic stresses? What is 'ideal' under different ecophysiological and agricultural conditions? Is selective breeding sufficient or is genetic engineering required for the "ideal stomata" for better adaptation to future climate? To address those questions, a consortium should be formed by plant evolutionary biologists, molecular biologists, ecologists, plant physiologists, agronomists, and plant breeders, to push forward international collaborative research. These enormous joint research efforts will not only identify evolutionary insights into the molecular, developmental ecological, and structural advantages of the "ideal stomata" but also utilize genome editing, genetic engineering, multi-omics, and field trials to test the yield potential of crops with "ideal stomata" that are better adapted to future climates.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study. The data that support the findings of this study are available from the corresponding author upon reasonable request. The author responsible for the distribution of materials integral to the findings presented in this article is Zhong-Hua Chen.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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