frontiers | Frontiers in Plant Science

TYPE Mini Review
PUBLISHED 04 November 2024
DOI 10.3389/fpls.2024.1491428



OPEN ACCESS

EDITED BY
Bhumi Nath Tripathi,
Indira Gandhi National Tribal University, India

REVIEWED BY
Dinakar Challabathula,
Central University of Tamil Nadu, India
Kapuganti Jagadis Gupta,
National Institute of Plant Genome Research (NIPGR), India

*CORRESPONDENCE
Agepati S. Raghavendra
as_raghavendra@yahoo.com;
asrsl@uohyd.ernet.in

†PRESENT ADDRESS
Shashibhushan Gahir
Department of Botany,
Government Autonomous College,
Phulbani, Kandhamal, Odisha, India

‡These authors have contributed equally to this work

RECEIVED 04 September 2024
ACCEPTED 16 October 2024
PUBLISHED 04 November 2024

CITATION
Bharath P, Gahir S and Raghavendra AS (2024) Cytosolic alkalinization in guard cells: an intriguing but interesting event during stomatal closure that merits further validation of its importance. *Front. Plant Sci.* 15:1491428. doi: 10.3389/fpls.2024.1491428



# Cytosolic alkalinization in guard cells: an intriguing but interesting event during stomatal closure that merits further validation of its importance

Pulimamidi Bharath‡, Shashibhushan Gahir†‡ and Agepati S. Raghavendra*

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India

Stomatal closure is essential to conserve water and prevent microbial entry into leaves. Alkalinization of guard cells is common during closure by factors such as abscisic acid, methyl jasmonate, and even darkness. Despite reports pointing at the role of cytosolic pH, there have been doubts about whether the guard cell pH change is a cause for stomatal closure or an associated event, as changes in membrane potential or ion flux can modulate the pH. However, the importance of cytosolic alkalinization is strongly supported by the ability of externally added weak acids to restrict stomatal closure. Using genetically encoded pH sensors has confirmed the rise in pH to precede the elevation of $Ca^{2+}$ levels. Yet some reports claim that the rise in pH follows the increase in ROS or $Ca^{2+}$. We propose a feedback interaction among the rise in pH or ROS or $Ca^{2+}$ to explain the contrasting opinions on the positioning of pH rise. Stomatal closure and guard cell pH changes are compromised in mutants deficient in vacuolar $H^+$-ATPase (V-ATPase), indicating the importance of V-ATPase in promoting stomatal closure. Thus, cytosolic pH change in guard cells can be related to the rise in ROS and $Ca^{2+}$, leading to stomatal closure. We emphasize that cytosolic pH in stomatal guard cells deserves further attention and evaluation.

KEYWORDS

**alkalinization, ATPases, ion efflux, secondary messenger, signal transduction, V-ATPase, stomatal closure**

## Introduction

The cytosolic pH in plant cells is believed to be relatively stable. However, the available evidences suggest that transient changes in intracellular pH can exert short- and long-term effects. Alkalinization or acidification is often a pre-requisite for plant processes like root hair growth (Monshausen et al., 2007), gravitropism (Felle, 2001), defense responses (Mathieu

et al., 1996), phytohormone signaling (Hager, 2003; Li et al., 2022a), and pollen tube elongation (Behera et al., 2018) and stomatal movement (Li et al., 2021; Raghavendra et al., 2023). Changes in intracellular pH are crucial for regulating plant metabolism (Felle, 2001; Zhou et al., 2021; Trinh and Masuda, 2022). As a result, it is debated if cellular pH could be considered a secondary messenger or signaling component, either by itself or along with ROS and $Ca^{2+}$ (Gilroy and Trewavas, 1994; Felle, 2001; Roos et al., 2006).

Stomata regulate the transpirational water loss and restrict the entry of microbial pathogens into leaves. Stomatal opening is induced when guard cells swell due to turgor. Flaccid guard cells shrink and causes stomatal closure. Changes in guard cell turgidity are due to either the loss or accumulation of $K^+$, anions (chloride/malate), and organic solutes such as sucrose (Agurla et al., 2018; Yang et al., 2020; Zhang et al., 2024). Whether for opening or closure, guard cell signal transduction ensures ion channels and ion flux modulation, leading to turgor changes. A typical stress hormone, such as abscisic acid (ABA), is sensed and transduced through several signaling components, including receptors, reactive oxygen species (ROS), and cytosolic $Ca^{2+}$. Modulation of these signaling components: ROS, $Ca^{2+}$, and $Ca^{2+}$-dependent protein kinases (CDPK), converge to modulate ion channels and promote ion efflux from guard cells (Kim et al., 2010; Murata et al., 2015; Cotelle and Leonhardt, 2019; Chen et al., 2020; Bharath et al., 2021; Hsu et al., 2021; Liu et al., 2022).

Stomatal movements are associated with pH changes in guard cells (Gonugunta et al., 2009; Islam et al., 2010). However, there has been a debate over the primary importance of cytosolic pH change among the intracellular events leading to stomatal closure. The most intriguing aspect is the relative positioning of pH change with ROS or $Ca^{2+}$ production. Several authors have demonstrated that the pH change preceded ROS or $Ca^{2+}$ production (Irving et al., 1992; Suhita et al., 2004; Islam et al., 2010; Li et al., 2021; Pei et al., 2022; Huang et al., 2023). In contrast, a few reports suggest that cytosolic pH changes were due to elevated ROS/$Ca^{2+}$ (Zhang et al., 2001; Rhaman et al., 2020). In other words, the alkalinization may not always be an early event.

We advocate that the cytosolic pH change can be important in guard cells. While agreeing that cytosolic alkalinization may not be the primary event, we argue that the rise in cytosolic pH in guard cells can promote stomatal closure. We propose an interactive mechanism to explain the argument that pH changes occur either downstream or upstream of ROS or $Ca^{2+}$ rise. Changes in guard cell pH occur during stomatal opening, too, but this aspect has not been much considered in the present article. Similarly, the possible interrelationship of guard cell pH and NO is also not discussed due to the ambiguity of the essentiality of NO for stomatal closure (Ribeiro et al., 2009; Lozano-Juste and León, 2010; van Meeteren et al., 2020).

## Elevation of guard cell pH is typical during stomatal closure

Cytosolic alkalinization precedes the increase in ROS or $Ca^{2+}$ of guard cells during stomatal closure induced by several factors, including hormones, elicitors, and others. Examples are ABA, methyl jasmonate (MeJA), pyrabactin (an analog of ABA), ethylene, sphingosine-1-phosphate (S1P), chitosan, $H_2O_2$, UV-B, and even external $Ca^{2+}$ (Table 1). However, the mechanism of how alkalinization could raise ROS or $Ca^{2+}$ levels is not entirely understood. Also, the origin of such pH changes in guard cells too is under debate.

The changes in cytosolic pH may depend on the vacuolar and other intracellular components. There have been very few reports on the status and pH changes in the vacuole, chloroplast, or other internal membranes of guard cells. The acidic pH of apoplast facilitated stomatal opening, while apoplast alkalinization triggered stomatal closure (Blatt and Armstrong, 1993; Felle et al., 2004; Geilfus, 2017; Inoue and Kinoshita, 2017). The extent of pH change in the cytosol has also been substantial (Ye et al., 2021).

The occurrence of cytosolic pH changes is endorsed by at least three experimental approaches: Modulation of cellular pH by external agents, the use of optimized genetically encoded pH sensors and finally, overexpression/suppression of ATPases. Methylamine and benzylamine (alkalinizing agents) induce stomatal closure in a way similar to ABA or $H_2O_2$, by inducing cytosolic alkalinization followed by $H_2O_2$ production in guard cells (Zhang et al., 2001; Ma et al., 2013; Zhu et al., 2014). In contrast, butyrate or acetate (weak acidifiers), suppress stomatal closure (due to ABA, MeJA, UV-B, $H_2O_2$ or darkness) by reducing cytosolic pH and $H_2O_2$ production in guard cells (Suhita et al., 2004; Islam et al., 2010; Ma et al., 2013; Huang et al., 2014; Zhu et al., 2014).

Most of the pH measurements in plant cells, including guard cells, are made with the pH-sensitive fluorescent dye, 2′,7′-bis-(2-carboxyethyl)-5,(6)-carboxyfluorescein (BCECF) or its membrane-permeant acetoxymethyl ester (BCECF-AM). Recently developed genetically encoded sensors (such as ClopHensor and CapHensor) provide strong evidence that cytosolic pH changes occur along with those of $Cl^-$ and $Ca^{2+}$ in guard cells (Arosio et al., 2010; Demes et al., 2020; Li et al., 2021, 2024; Mirasole et al., 2023). Other genetically encoded green fluorescent proteins, including Pt-GFP, pHluorins and At-pHluorins, have demonstrated changes in the cytosolic pH of plant cells (Gao et al., 2004; Schulte et al., 2006; Martinière et al., 2018; Pecherina et al., 2021) but are yet to be tested on guard cells. These recent pH sensors can monitor cytosolic pH and ions such as $Ca^{2+}$ or chloride in real time, thus providing an advantage in measuring pH and ion dynamics.

External agents such as methylamine/benzylamine provide indirect evidence of pH changes. So far most of the pH changes in guard cells are monitored by using fluorescence dye, BCECF-AM. However, doubts are expressed about the preciseness of BCECF-AM. Recent studies with advanced pH sensors indicate that elevation of pH changes can occur as early as 2 mins followed by $Ca^{2+}$/ROS changes (Li et al., 2024). Arabidopsis mutants deficient in $H^+$-ATPases (PM-/V) also could be important for asserting their involvement during stomatal closure. Among these, advanced pH sensors and the use of ATPase mutants can provide convincing evidence of cytosolic pH changes during stomatal closure.

Changes in guard cell pH can occur when ATPases are modulated. This aspect is discussed in the next section.

10.3389/fpls.2024.1491428

TABLE 1 Elevation of cytosolic pH in guard cells and its consequences on the ROS and $Ca^{2+}$ levels during stomatal closure.

| Trigger | Consequence of cytosolic alkalization | Plant | References |
|---|---|---|---|
| **Hormones** | | | |
| Abscisic acid (ABA) | Increase in ROS<br>Increase in ROS followed by $Ca^{2+}$ | *Pisum sativum*<br>*Nicotiana tabacum, Arabidopsis thaliana* | Gonugunta et al., 2009;<br>Li et al., 2021;<br>Pei et al., 2022 |
| Methyl jasmonate | Elevated ROS | *A. thaliana* | Suhita et al., 2004;<br>Gonugunta et al., 2009 |
| Pyrabactin<br>(ABA analogue) | Increase in ROS | *P. sativum* | Puli and Raghavendra, 2012 |
| **Elicitors** | | | |
| Chitosan | Increased ROS | *P. sativum* | Gonugunta et al., 2009 |
| Yeast Elicitor (YEL) | ROS accumulation | *A. thaliana* | Salam et al., 2013 |
| **Others** | | | |
| Allyl isothiocynate | Elevated ROS, led to rise in cytosolic $Ca^{2+}$ | *A. thaliana* | Sobahan et al., 2015;<br>Afrin et al., 2020 |
| Phytosphingosine-1-Phosphate (PhytoS1P) | ROS production and ion channel modualtion | *Vicia faba* | Ma and Niu, 2017 |
| Sphingosine-1-phosphate (S1P) | $H_2O_2$ production | *Vicia faba* | Ma et al., 2012 |
| Darkness | Induced ROS production | *Vicia faba* | Ma et al., 2013 |
| UV-B | Rise in the levels of $H_2O_2$ | *A. thaliana* | Zhu et al., 2014 |
| High $SO_2$ | Increased $Ca^{2+}$ levels | *Tagetes erecta* | Wei et al., 2015 |
| Chloride | Transient alkalinization followed by elevation of cytosolic ABA | *V. faba* | Geilfus et al., 2015 |
| **pH modulators** | | | |
| Methylamine | Induction of $H_2O_2$ production | *A. thaliana* | Zhu et al., 2014 |
| Benzylamine | Mimicked $H_2O_2$ and promoted cytosolic alkalinizations | *V. faba* | Zhang et al., 2001 |

## The origin of pH-rise in guard cells: Involvement of vacuolar-ATPases

Stomatal opening is restricted when plasma membrane-ATPase (PM-ATPase) is inhibited (Takemiya and Shimazaki, 2010). Upregulation of PM $H^+$-ATPase activity appears to be necessary for stomatal opening. However, the role of PM-ATPase during stomatal closure is ambiguous. Two dominant mutations in the *open stomata 2* (*OST2*) gene result in constitutive activation of AHA1 (gene encoding PM-ATPase), abolishing ABA-induced closure and keeping stomata open (Merlot et al., 2007). Stomatal closure by ABA is compromised in loss-of-function mutants of *aha2-6* and *aha2-6 bak1-4* double mutants (Pei et al., 2022). Thus, the role of PM-ATPase during stomatal closure is confusing, and a question arises if the two forms of AHA1 and AHA2 act differently. Further work is needed to establish if PM-ATPase has a dual role during stomatal opening or closure.

On the other hand, there is strong evidence for the role of vacuolar $H^+$-ATPase (V-ATPase) mediated vacuolar acidification and cytosolic alkalinization during stomatal closure. Alkalinization of guard cells by $H_2O_2$ or phosphatidylinositol 3,5 bisphophate [PI(3,5)P2] is due to $H^+$-efflux from the cytosol into the vacuole involving V-ATPase (Zhang et al., 2001; Bak et al., 2013). Suppression of V-ATPase (as in *vha-a* mutant) results in enhanced stomatal aperture (Zhang et al., 2013). Arabidopsis V-ATPase double mutant (*vha-a2 vha-a3*) has no vacuolar $H^+$-pumping activity and exhibits delayed vacuolar acidification and annulled stomatal closure in response to ABA (Bak et al., 2013). Down-regulation of phosphatidylinositol3-kinase (*pi3k*), a protein kinase that activates V-ATPase, results in low vacuolar acidification and limited stomatal closure in response to MeJA (Liu et al., 2016). A deficiency of V-ATPase (as in *de-etiolated-3*/*det3* mutant) or RNAi interference, results in enhanced opening (Allen et al., 2000; Zhang et al., 2013; Seidel, 2022). Thus, vacuolar acidification was closely associated with cytosolic alkalinization.

5-aminolevulinic acid, a potential plant growth regulator, promotes stomatal opening and reverses ABA-induced closure

by downregulating V-ATPase and restricting guard cell pH and $H_2O_2$ levels in apple leaves (Hu et al., 2019). Cytosolic pH and ROS levels are low in several of these instances. An active V-ATPase can cause cytosolic alkalinization and raise $H_2O_2$ levels in guard cells during stomatal closure. Besides V-ATPase, vacuolar-PPase (V-PPase) can cause rapid acidification of vacuoles during stomatal closure induced by ABA (Eisenach and De Angeli, 2017). But, the specific role of V-PPase needs to be examined in detail.

# Discussion

## Cytosolic alkalinization in relation to the scheme of signaling events during stomatal closure

Stomata close when guard cells lose their $K^+/Cl^-$ triggered by an increase in intracellular $Ca^{2+}$ of guard cells. Whenever the stomata are exposed to biotic/abiotic stress signals, the levels of two major secondary messengers, ROS and $Ca^{2+}$, increase in guard cells. The perception of a signal such as ABA (a plant hormone), or flagellin (microbial elicitor) activates OST1 kinase and NADPH oxidase to promote $H_2O_2$ production. The elevated ROS initiates the efflux $Ca^{2+}$ from endo-cytomembranes, an influx of external $Ca^{2+}$, or both. This scheme of signaling events during stomatal closure is well accepted (Bharath et al., 2021; Hsu et al., 2021; Meddya et al., 2023; Zhang et al., 2024).

The temporal studies indicate that the increase in guard cell pH is the earliest, followed by ROS or $Ca^{2+}$ (Suhita et al., 2004; Ma et al., 2012; 2013; lozanoZhu et al., 2014). Using genetically encoded pH/$Ca^{2+}$ sensor, Li et al. (2021) have observed that ABA elevated cytosolic pH by ~2 min, followed by $Ca^{2+}$ in >5 min. However, the mechanism of pH-induced ROS production in guard cells has yet to be elucidated. One of the possibilities is that alkalinization and subsequent release of $Ca^{2+}$ (from endo-cytomembranes) could facilitate the activation of NADPH oxidase through the $Ca^{2+}$-dependent phosphorylation of SnRK-type OST kinase (Han et al., 2019; Kimura et al., 2022; Liu et al., 2022).

The secondary messengers, ROS and $Ca^{2+}$, may act either upstream or downstream of cytosolic alkalinization in an interactive manner to promote ion efflux and stomatal closure (Figure 1). In addition to ROS or $Ca^{2+}$, other signaling molecules that can induce cytosolic pH changes include ethylene, S-1-P/phyto S-1-P (Table 1) and PI(3,5)P2 (Bak et al., 2013). However, their action seems to converge at ROS or $Ca^{2+}$ or both. Further studies are needed to identify the exact conditions when alkalinization precedes or co-occurs with ROS generation. Parallelly, the cytosolic pH can directly modulate the outward $K^+$-channels and promote $K^+$ efflux from guard cells (Blatt, 1990; Blatt and Armstrong, 1993; Grabov and Blatt, 1997). In addition, the modulation of OST1 kinase/NADPH oxidase/ROS/ion channels and increased ion flux leading to stomatal closure can also occur independent of cytosolic pH change.

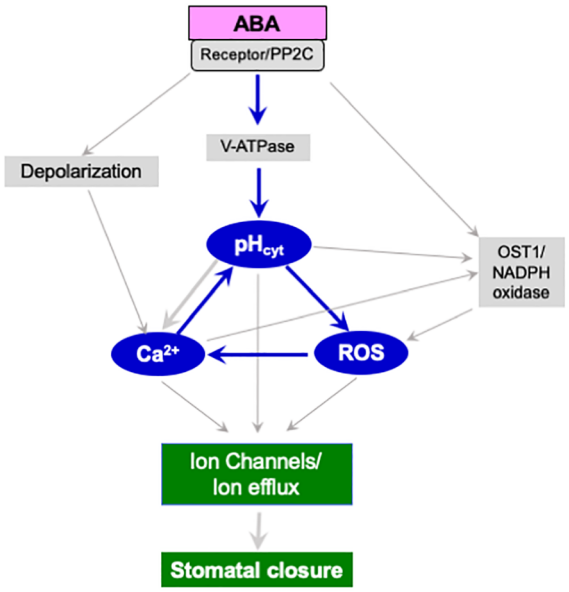


FIGURE 1
A hypothetical scheme of signaling components participating in stomatal closure induced by ABA. Changes in the cytosolic pH ($pH_{cyt}$) of guard cells can modulate ROS and cytosolic $Ca^{2+}$. The cytosolic alkalinization caused by ABA seems to be due to the activation of vacuolar $H^+$-ATPase (V-ATPase). The stomatal closure by ABA involves the binding of ABA to its receptor (PYR/PYL/RCAR), inhibition of protein phosphatase (PP2C), and activation of protein kinase (OST1). In turn, OST1 kinase activates NADPH oxidase to produce ROS. Elevated ROS can increase cytosolic $Ca^{2+}$ levels and directly affect ion channels like SLAC1 (Gilroy et al., 2016; Liu et al., 2022). Parallelly, cytosolic alkalinization promotes membrane depolarization, increased $Ca^{2+}$ and a rise in pH. At the same time, elevated pH, ROS, and $Ca^{2+}$ upregulate outward channels of $K^+$, $Cl^-$, and $NO_3^-$, causing a net efflux of ions, loss of guard cell turgor, and stomatal closure. These components may all interact. As per our feedback activation model (indicated in blue), adding $H_2O_2$, or $Ca^{2+}$, can promote the rise in pH and *vice-versa*. The published evidence endorses the increase in ROS by guard cell alkalinization (Islam et al., 2010), the elevation of $Ca^{2+}$ by ROS (Liu et al., 2022), the rise in pH by $Ca^{2+}$ (Li et al., 2021; Huang et al., 2023). The upregulation of pH by $Ca^{2+}$ is also known (Islam et al., 2010).

# Ambiguities to be resolved

Changes in guard cell pH can be mediated by the membrane potential and ion fluxes and *vice versa*. During stomatal closure, the cytosolic pH increases, followed by membrane depolarization and increased $K^+/Cl^-$ efflux from guard cells (Blatt and Armstrong, 1993; Miedema and Assmann, 1996; Pandey et al., 2007; Chen et al., 2020). Membrane depolarization and $Ca^{2+}$ influx can modulate pH in plant cells, including guard cells (Brault et al., 2004; Meimoun et al., 2009; Pottosin et al., 2014). As per Roelfsema et al. (2004), ABA can cause membrane depolarization and activation of outward ion channels. Such a situation still does not decrease the importance of cytosolic alkalinization during stomatal closure.

ATPases, particularly PM-ATPase and V-ATPase are among the most important proteins that can modulate intracellular pH (Roelfsema and Hedrich, 2005; Kim et al., 2010). Ion-transporters (particularly $Ca^{2+}$, $Cl^-$ or $NO_3$) and CONSTITUTIVE

PHOTOMORPHO-GENIC 1 (COP1, a light-sensitive, negative regulator of stomatal opening) can also drive pH-changes in guard cells to modulate stomatal movement (Eisenach and De Angeli, 2017; Demes et al., 2020; Dreyer et al., 2022; Li et al., 2024; Cha et al., 2024). A few other proteins, such as cation/$H^+$ transporters and transcription factors (PacC, a dominant transcription factor), are known to modify the cellular pH, but their role in guard cells is uncertain (Pittman and Hirschi, 2016; Li et al., 2022b). However, ion-transporters' role in modulating guard cell pH is unclear and needs further study.

Another criticism is that the rise in pH may not all be cytoplasmic. The dye BCECF-AM, with a pKa value of 6.98, is expected to stay within the cytosol (Boyer and Hedley, 1994; Paradiso et al., 1984). Further, BCECF-AM has been widely used to detect the pH changes in root hairs and pollen tissues besides guard cells (Gehring et al., 1990; Kosegarten et al., 1997; Han and Burgess, 2010; Wilkins et al., 2015; Yemelyanov et al., 2020). We, therefore, believe that the elevated fluorescence by BCECF-AM upon ABA or MeJa treatment originates mainly from the cytosol of guard cells. An additional confusion arises when the buffer strength of cellular components is considered. The buffer strength of cytosol is expected to be several times that of apoplast. However, Oja et al. (1999) have suggested that fast cytoplasmic pH changes can occur due to the pumping of protons into the vacuole. Using genetically encoded sensors that are much more robust than the fluorescent dyes also validates the cytosolic alkalinization in guard cells during closure (see Section 2).

## Future perspectives

The importance of guard cell pH in mediating stomatal closure cannot be ignored. We emphasize that the guard cell pH can be an important event that modulates stomatal movements, even if the pH change in guard cells is not necessarily the primary cause. Other aspects that need critical re-evaluation in guard cells are pH changes in different intracellular compartments, the exact values of cytosolic pH and time-dependent dynamics of pH change. The relationship between changes in pH, ROS and $Ca^{2+}$ can vary depending on the trigger, for e.g. ABA or flagellin (Li et al., 2021). The availability of genetically encoded dual pH, $Ca^{2+}$ or K+ sensors would be extremely useful in resolving some of these issues.

It is quite fascinating to consider the possible mechanism of "pH-sensing" in guard cells. The occurrence of pH sensors in plant cells is often discussed, but the mechanism of pH sensing is still unclear (Li and Yang, 2023). Among the possible molecules that could be relevant to guard cells are phosphatidic acid (Li et al., 2019), PP2C D-clade proteins (Wong et al., 2019) and vacuolar transporters (Demes et al., 2020). We expect our article on guard cell pH will trigger further research into this intriguing but fascinating topic of cytosolic pH as a key event. Stomatal guard cells are promising model systems for examining pH's role in plant tissues.

## Author contributions

PB: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. SG: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. AR: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. Our work on guard cell signaling was supported by a grant from the Department of Biotechnology (BT/PR9227/PBD/16/748/2007) to ASR.

## Acknowledgments

Our work on guard cell signaling was supported by a grant from the Department of Biotechnology (BT/PR9227/PBD/16/748/2007) and an INSA Senior Scientist Research Grant to ASR. SG held a Senior Research Fellowship from University Grants Commission, New Delhi. We thank DST-FIST, UGC-SAP-CAS, and DBT-BUILDER (all from New Delhi, India) for the facilities in the Department of Plant Sciences and School of Life Sciences.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Publisher's note

# References


Afrin, S., Okuma, E., Tahjib-Ul-Arif, M., Jahan, M. S., Nakamura, T., Nakamura, Y., et al. (2020). Stomatal response to isothiocyanates in *Arabidopsis thaliana*. *J. Exp. Bot.* 71, 6921–6931. doi: 10.1093/jxb/eraa420

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., and Raghavendra, A. S. (2018). Mechanism of stomatal closure in plants exposed to drought and cold stress. *Adv. Exp. Med. Biol.* 1081, 215–232. doi: 10.1007/978-981-13-1244-1_12

Allen, G. J., Chu, S. P., Schumacher, K., Shimazaki, C. T., Vafeados, D., Kemper, A., et al. (2000). Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis det3* mutant. *Science* 289, 2338–2342. doi: 10.1126/science.289.5488.2338

Arosio, D., Ricci, F., Marchetti, L., Gualdani, R., Albertazzi, L., and Beltram, F. (2010). Simultaneous intracellular chloride and pH measurements using a GFP-based sensor. *Nat. Methods* 7, 516–518. doi: 10.1038/nmeth.1471

Bak, G., Lee, E. J., Lee, Y., Kato, M., Segami, S., Sze, H., et al. (2013). Rapid structural changes and acidification of guard cell vacuoles during stomatal closure require phosphatidylinositol 3,5-bisphosphate. *Plant Cell* 25, 2202–2216. doi: 10.1105/tpc.113.110411

Behera, S., Zhaolong, X., Luoni, L., Bonza, M. C., Doccula, F. G., De Michelis, M. I., et al. (2018). Cellular $Ca^{2+}$ signals generate defined pH signatures in plants. *Plant Cell* 30, 2704–2719. doi: 10.1105/tpc.18.00655

Bharath, P., Gahir, S., and Raghavendra, A. S. (2021). Abscisic acid-induced stomatal closure: An important component of plant defense against abiotic and biotic stress. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.615114

Blatt, M. R. (1990). Potassium channel currents in intact stomatal guard cells: rapid enhancement by abscisic acid. *Planta* 180, 445–455. doi: 10.1007/BF00198799

Blatt, M. R., and Armstrong, F. (1993). $K^+$ channels of stomatal guard cells: abscisic-acid-evoked control of the outward rectifier mediated by cytoplasmic pH. *Planta* 191, 330–341. doi: 10.1007/BF00195690

Boyer, M. J., and Hedley, D. W. (1994). Measurement of intracellular pH. *Methods Cell Biol.* 41, 135–148. doi: 10.1016/S0091-679X(08)61714-8

Brault, M., Amiar, Z., Pennarun, A. M., Monestiez, M., Zhang, Z., Cornel, D., et al. (2004). Plasma membrane depolarization induced by abscisic acid in Arabidopsis suspension cells involves reduction of proton pumping in addition to anion channel activation, which are both $Ca^{2+}$ dependent. *Plant Physiol.* 135, 231–243. doi: 10.1104/pp.104.039255

Cha, S., Min, W. K., and Seo, H. S. (2024). Arabidopsis COP1 guides stomatal response in guard cells through pH regulation. *Commun. Biol.* 7, 150. doi: 10.1038/s42003-024-05847-w

Chen, K., Li, G. J., Bressan, R. A., Song, C. P., Zhu, J. K., and Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. *J. Integr. Plant Biol.* 62, 25–54. doi: 10.1111/jipb.12899

Cotelle, V., and Leonhardt, N. (2019). ABA signaling in guard cells. *Adv. Bot. Res.* 92, 115–170. doi: 10.1016/bs.abr.2019.10.001

Demes, E., Besse, L., Cubero-Font, P., Satiat-Jeunemaitre, B., Thomine, S., and De Angeli, A. (2020). Dynamic measurement of cytosolic pH and $[NO_3^-]$ uncovers the role of the vacuolar transporter *AtCLCa* in cytosolic pH homeostasis. *Proc. Nat. Acad. Sci. U.S.A.* 117, 15343–15353. doi: 10.1073/pnas.2007580117

Dreyer, I., Li, K., Riedelsberger, J., Hedrich, R., Konrad, K. R., and Michard, E. (2022). Transporter networks can serve plant cells as nutrient sensors and mimic transceptor-like behavior. *iScience* 25, 104078. doi: 10.1016/j.isci.2022.104078

Eisenach, C., and De Angeli, A. (2017). Ion transport at the vacuole during stomatal movements. *Plant Physiol.* 174, 520–530. doi: 10.1104/pp.17.00130

Felle, H. H. (2001). pH: signal and messenger in plant cells. *Plant Biol.* 3, 577–591. doi: 10.1055/s-2001-19372

Felle, H. H., Herrmann, A., Hanstein, S., Hückelhoven, R., and Kogel, K. H. (2004). Apoplastic pH signaling in barley leaves attacked by the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. *Mol. Plant Microbe Interact.* 17, 118–123. doi: 10.1094/MPMI.2004.17.1.118

Gao, D., Knight, M. R., Trewavas, A. J., Sattelmacher, B., and Plieth, C. (2004). Self-reporting Arabidopsis expressing pH and $Ca^{2+}$ indicators unveil ion dynamics in the cytoplasm and in the apoplast under abiotic stress. *Plant Physiol.* 134, 898–908. doi: 10.1104/pp.103.032508

Gehring, C. A., Irving, H. R., and Parish, R. W. (1990). Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. *Proc. Natl. Acad. Sci. U.S.A.* 87, 9645–9649. doi: 10.1073/pnas.87.24.9645

Geilfus, C. M. (2017). The pH of the apoplast: dynamic factor with functional impact under stress. *Mol. Plant* 10, 1371–1386. doi: 10.1016/j.molp.2017.09.018

Geilfus, C. M., Mithöfer, A., Ludwig-Müller, J., Zörb, C., and Muehling, K. H. (2015). Chloride-inducible transient apoplastic alkalinizations induce stomata closure by controlling abscisic acid distribution between leaf apoplast and guard cells in salt-stressed *Vicia faba*. *New Phytol.* 208, 803–816. doi: 10.1111/nph.13507

Gilroy, S., Białasek, M., Suzuki, N., Górecka, M., Devireddy, A. R., Karpiński, S., et al. (2016). ROS, calcium, and electric signals: Key mediators of rapid systemic signaling in plants. *Plant Physiol.* 171, 1606–1615. doi: 10.1104/pp.16.00434

Gilroy, S., and Trewavas, A. (1994). A decade of plant signals. *BioEssays* 16, 677–682. doi: 10.1002/bies.950160914

Gonugunta, V. K., Srivastava, N., and Raghavendra, A. S. (2009). Cytosolic alkalinization is a common and early messenger preceding the production of ROS and NO during stomatal closure by variable signals, including abscisic acid, methyl jasmonate and chitosan. *Plant Signal. Behav.* 4, 561–564. doi: 10.4161/psb.4.6.8847

Grabov, A., and Blatt, M. R. (1997). Parallel control of the inward-rectifier $K^+$ channel by cytosolic free $Ca^{2+}$ and pH in *Vicia* guard cells. *Planta* 201, 84–95. doi: 10.1007/BF01258684

Hager, A. (2003). Role of the plasma membrane $H^+$-ATPase in auxin-induced elongation growth: historical and new aspects. *J. Plant Res.* 116, 483–505. doi: 10.1007/s10265-003-0110-x

Han, J., and Burgess, K. (2010). Fluorescent indicators for intracellular pH. *Chem. Rev.* 110, 2709–2728. doi: 10.1021/cr900249z

Han, J. P., Köster, P., Drerup, M. M., Scholz, M., Li, S., Edel, K. H., et al. (2019). Fine-tuning of RBOHF activity is achieved by differential phosphorylation and $Ca^{2+}$ binding. *New Phytol.* 221, 1935–1949. doi: 10.1111/nph.15543

Hsu, P. K., Dubeaux, G., Takahashi, Y., and Schroeder, J. I. (2021). Signaling mechanisms in abscisic acid-mediated stomatal closure. *Plant J.* 105, 307–321. doi: 10.1111/tpj.15067

Hu, J., An, Y. Y., Cai, C. Y., He, S. S., and Wang, L. (2019). Cytoplasmic pH is involved in 5-aminolevulinic acid (ALA)-induced stomatal opening in apple leaves. *Acta Hortic. Sinic.* 46, 1869–1881. doi: 10.16420/j.issn.0513-353x.2018-0880

Huang, A. X., She, X. P., Zhao, J. L., and Zhang, Y. Y. (2014). Inhibition of ABA-induced stomatal closure by fusicoccin is associated with cytosolic acidification-mediated hydrogen peroxide removal. *Bot. Stud.* 55, 33. doi: 10.1186/1999-3110-55-33

Huang, S., Shen, L., Roelfsema, M. R. G., Becker, D., and Hedrich, R. (2023). Light-gated channelrhodopsin sparks proton-induced calcium release in guard cells. *Science* 382, 1314–1318. doi: 10.1126/science.adj9696

Inoue, S. I., and Kinoshita, T. (2017). Blue light regulation of stomatal opening and the plasma membrane $H^+$-ATPase. *Plant Physiol.* 174, 531–538. doi: 10.1104/pp.17.00166

Irving, H. R., Gehring, C. A., and Parish, R. W. (1992). Changes in cytosolic pH and calcium of guard cells precede stomatal movements. *Proc. Natl. Acad. Sci. U.S.A.* 89, 1790–1794. doi: 10.1073/pnas.89.5.1790

Islam, M. M., Hossain, M. A., Jannat, R., Munemasa, S., Nakamura, Y., Mori, I. C., et al. (2010). Cytosolic alkalization and cytosolic calcium oscillation in Arabidopsis guard cells response to ABA and MeJA. *Plant Cell Physiol.* 51, 1721–1730. doi: 10.1093/pcp/pcq131

Kim, T. H., Böhmer, M., Hu, H., Nishimura, N., and Schroeder, J. I. (2010). Guard cell signal transduction network: advances in understanding abscisic acid, $CO_2$, and $Ca^{2+}$ signaling. *Annu. Rev. Plant Biol.* 61, 561–591. doi: 10.1146/annurev-arplant-042809-112226

Kimura, S., Kaya, H., Hashimoto, K., Wrzaczek, M., and Kuchitsu, K. (2022). Quantitative analysis for ROS-producing activity and regulation of plant NADPH oxidases in HEK293T cells. *Methods Mol. Biol.* 2526, 107–122. doi: 10.1007/978-1-0716-2469-2_8

Kosegarten, H., Grolig, F., Wieneke, J., Wilson, G., and Hoffmann, B. (1997). Differential ammonia-elicited changes of cytosolic pH in root hair cells of rice and maize as monitored by 2',7'-bis-(2-carboxyethyl)-5 (and -6)-carboxyfluorescein-fluorescence ratio. *Plant Physiol.* 113, 451–461. doi: 10.1104/pp.113.2.451

Li, B., Chen, Y., and Tian, S. (2022b). Function of pH-dependent transcription factor PacC in regulating development, pathogenicity, and mycotoxin biosynthesis of phytopathogenic fungi. *FEBS J.* 289, 1723–1730. doi: 10.1111/febs.15808

Li, K., Grauschopf, C., Hedrich, R., Dreyer, I., and Konrad, K. R. (2024). $K^+$ and pH homeostasis in plant cells is controlled by a synchronized $K^+/H^+$ antiport at the plasma and vacuolar membrane. *New Phytol.* 241, 1525–1542. doi: 10.1111/nph.19436

Li, K., Prada, J., Damineli, D. S. C., Liese, A., Romeis, T., Dandekar, T., et al. (2021). An optimized genetically encoded dual reporter for simultaneous ratio imaging of $Ca^{2+}$ and $H^+$ reveals new insights into ion signaling in plants. *New Phytol.* 230, 2292–2310. doi: 10.1111/nph.17202

Li, W., Song, T., Wallrad, L., Kudla, J., Wang, X., and Zhang, W. (2019). Tissue-specific accumulation of pH-sensing phosphatidic acid determines plant stress tolerance. *Nat. Plants.* 5, 1012–1021. doi: 10.1038/s41477-019-0497-6

Li, J., and Yang, Y. (2023). How do plants maintain pH and ion homeostasis under saline-alkali stress? *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1217193

Li, Y., Zeng, H., Xu, F., Yan, F., and Xu, W. (2022a). $H^+$-ATPases in plant growth and stress responses. *Ann. Rev. Plant Biol.* 73, 495–521. doi: 10.1146/annurev-arplant-102820-114551

Liu, J., Ji, Y., Zhou, J., and Xing, D. (2016). Phosphatidylinositol 3-kinase promotes activation and vacuolar acidification and delays methyl jasmonate-induced leaf senescence. *Plant Physiol.* 170, 1714–1731. doi: 10.1104/pp.15.00744

Liu, H., Song, S., Zhang, H., Li, Y., Niu, L., Zhang, J., et al. (2022). Signaling transduction of ABA, ROS, and $Ca^{2+}$ in plant stomatal closure in response to drought. *Int. J. Mol. Sci.* 23, 14824. doi: 10.3390/ijms232314824

Lozano-Juste, J., and León, J. (2010). Enhanced abscisic acid-mediated responses in *nia1nia2noa1-2* triple mutant impaired in NIA/NR- and AtNOA1-dependent nitric oxide biosynthesis in Arabidopsis. *Plant Physiol.* 152, 891–903. doi: 10.1104/pp.109.148023

Ma, Y., and Niu, J. (2017). The role of phytosphingosine-1-phosphate (Phyto-S1P) and its relationships with cytosolic pH and hydrogen peroxide ($H_2O_2$) during stomatal closure by darkness in broad bean. *S. Afr. J. Bot.* 108, 237–242. doi: 10.1016/j.sajb.2016.11.002

Ma, Y. L., She, X. P., and Yang, S. S. (2012). Sphingosine-1-phosphate (S1P) mediates darkness-induced stomatal closure through raising cytosol pH and hydrogen peroxide ($H_2O_2$) levels in guard cells in *Vicia faba*. *Sci. China Life Sci.* 55, 974–983. doi: 10.1007/s11427-012-4386-8

Ma, Y. L., She, X. P., and Yang, S. S. (2013). Cytosolic alkalization-mediated $H_2O_2$ and NO production are involved in darkness-induced stomatal closure in *Vicia faba*. *Can. J. Plant Sci.* 93, 119–130. doi: 10.4141/cjps2012-040

Martinière, A., Gibrat, R., Sentenac, H., Dumont, X., Gaillard, I., and Paris, N. (2018). Uncovering pH at both sides of the root plasma membrane interface using noninvasive imaging. *Proc. Nat. Acad. Sci. U.S.A.* 115, 6488–6493. doi: 10.1073/pnas.1721769115

Mathieu, Y., Lapous, D., Thomine, S., Laurière, C., and Guern, J. (1996). Cytoplasmic acidification as an early phosphorylation- dependent response of tobacco cells to elicitors. *Planta* 199, 416–424. doi: 10.1007/BF00195734

Meddya, S., Meshram, S., Sarkar, D., R., S., Datta, R., Singh, S., et al. (2023). Plant Stomata: An unrealized possibility in plant defense against invading pathogens and stress tolerance. *Plants (Basel Switzerland)* 12, 3380. doi: 10.3390/plants12193380

Meimoun, P., Vidal, G., Bohrer, A. S., Lehner, A., Tran, D., Briand, J., et al. (2009). Intracellular $Ca^{2+}$ stores could participate to abscisic acid-induced depolarization and stomatal closure in *Arabidopsis thaliana*. *Plant Signal. Behav.* 4, 830–835. doi: 10.4161/psb.4.9.9396

Merlot, S., Leonhardt, N., Fenzi, F., Valon, C., Costa, M., Piette, L., et al. (2007). Constitutive activation of a plasma membrane $H^+$-ATPase prevents abscisic acid-mediated stomatal closure. *EMBO J.* 26, 3216–3226. doi: 10.1038/sj.emboj.7601750

Miedema, H., and Assmann, S. M. (1996). A membrane-delimited effect of internal pH on the $K^+$ outward rectifier of *Vicia faba* guard cells. *J. Memb. Biol.* 154, 227–237. doi: 10.1007/s002329900147

Mirasole, F. M., Nastasi, S. P., Cubero-Font, P., and De Angeli, A. (2023). Vacuolar control of stomatal opening revealed by 3D imaging of the guard cells. *Sci. Rep.* 13, 7647. doi: 10.1038/s41598-023-34273-x

Monshausen, G. B., Bibikova, T. N., Messerli, M. A., Shi, C., and Gilroy, S. (2007). Oscillations in extracellular pH and reactive oxygen species modulate tip growth of *Arabidopsis* root hairs. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20996–21001. doi: 10.1073/pnas.0708586104

Murata, Y., Mori, I. C., and Munemasa, S. (2015). Diverse stomatal signaling and the signal integration mechanism. *Ann. Rev. Plant Biol.* 66, 369–392. doi: 10.1146/annurev-arplant-043014-114707

Oja, V., Savchenko, G., Jakob, B., and Heber, U. (1999). pH and buffer capacities of apoplastic and cytoplasmic cell compartments in leaves. *Planta* 209, 239–249. doi: 10.1007/s004250050628

Pandey, S., Zhang, W., and Assmann, S. M. (2007). Roles of ion channels and transporters in guard cell signal transduction. *FEBS Lett.* 581, 2325–2336. doi: 10.1016/j.febslet.2007.04.008

Paradiso, A. M., Tsien, R. Y., and Machen, T. E. (1984). $Na^+$-$H^+$ exchange in gastric glands as measured with a cytoplasmic-trapped, fluorescent pH indicator. *Proc. Natl. Acad. Sci. U.S.A.* 81, 7436–7440. doi: 10.1073/pnas.81.23.7436

Pecherina, A., Grinberg, M., Ageyeva, M., Zdobnova, T., Ladeynova, M., Yudintsev, A., et al. (2021). Whole-plant measure of temperature-induced changes in the cytosolic pH of potato plants using genetically encoded fluorescent sensor Pt-GFP. *Agriculture* 11, 1131. doi: 10.3390/agriculture11111131

Pei, D., Hua, D., Deng, J., Wang, Z., Song, C., Wang, Y., et al. (2022). Phosphorylation of the plasma membrane $H^+$-ATPase AHA2 by BAK1 is required for ABA-induced stomatal closure in Arabidopsis. *Plant Cell* 34, 2708–2729. doi: 10.1093/plcell/koac106

Pittman, J. K., and Hirschi, K. D. (2016). CAX-ing a wide net: Cation/$H^+$ transporters in metal remediation and abiotic stress signalling. *Plant Biol.* 18, 741–749. doi: 10.1111/plb.12460

Pottosin, I., Velarde-Buendía, A. M., Bose, J., Fuglsang, A. T., and Shabala, S. (2014). Polyamines cause plasma membrane depolarization, activate $Ca^{2+}$-, and modulate $H^+$-ATPase pump activity in pea roots. *J. Exp. Bot.* 65, 2463–2472. doi: 10.1093/jxb/eru133

Puli, M. R., and Raghavendra, A. S. (2012). Pyrabactin, an ABA agonist, induced stomatal closure and changes in signalling components of guard cells in abaxial epidermis of *Pisum sativum*. *J. Exp. Bot.* 63, 1349–1356. doi: 10.1093/jxb/err364

Raghavendra, A. S., Ye, W., and Kinoshita, T. (2023). pH as a signal and secondary messenger in plant cells. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1148689

Rhaman, M. S., Imran, S., Rauf, F., Khatun, M., Baskin, C. C., Murata, Y., et al. (2020). Seed priming with phytohormones: an effective approach for the mitigation of abiotic stress. *Plants (Basel)* 10, 37. doi: 10.3390/plants10010037

Ribeiro, D. M., Desikan, R., Bright, J., Confraria, A., Harrison, J., Hancock, J. T., et al. (2009). Differential requirement for NO during ABA-induced stomatal closure in turgid and wilted leaves. *Plant Cell Environ.* 32, 46–57. doi: 10.1111/j.1365-3040.2008.01906.x

Roelfsema, M. R. G., and Hedrich, R. (2005). In the light of stomatal opening: new insights into 'the Watergate'. *New Phytol.* 167, 665–691. doi: 10.1111/j.1469-8137.2005.01460.x

Roelfsema, M. R. G., Levchenko, V., and Hedrich, R. (2004). ABA depolarizes guard cells in intact plants, through a transient activation of R- and S-type anion channels. *Plant J.* 37, 578–588. doi: 10.1111/j.1365-313x.2003.01985.x

Roos, W., Viehweger, K., Dordschbal, B., Schumann, B., Evers, S., Steighardt, J., et al. (2006). Intracellular pH signals in the induction of secondary pathways–the case of Eschscholzia californica. *J. Plant Physiol.* 163, 369–381. doi: 10.1016/j.jplph.2005.11.012

Salam, M. A., Jammes, F., Hossain, M. A., Ye, W., Nakamura, Y., Mori, I. C., et al. (2013). Two guard cell-preferential MAPKs, MPK9 and MPK12, regulate YEL signalling in *Arabidopsis* guard cells. *Plant Biol.* 15, 436–442. doi: 10.1111/j.1438-8677.2012.00671.x

Schulte, A., Lorenzen, I., Böttcher, M., and Plieth, C. (2006). A novel fluorescent pH probe for expression in plants. *Plant Methods* 2, 7. doi: 10.1186/1746-4811-2-7

Seidel, T. (2022). The plant V-ATPase. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.931777

Sobahan, M. A., Akter, N., Okuma, E., Uraji, M., Ye, W., Mori, I. C., et al. (2015). Allyl isothiocyanate induces stomatal closure in *Vicia faba*. *Biosci. Biotechnol. Biochem.* 79, 1737–1742. doi: 10.1080/09168451.2015.1045827

Suhita, D., Raghavendra, A. S., Kwak, J. M., and Vavasseur, A. (2004). Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure. *Plant Physiol.* 134, 1536–1545. doi: 10.1104/pp.103.032250

Takemiya, A., and Shimazaki, K. (2010). Phosphatidic acid inhibits blue light-induced stomatal opening via inhibition of protein phosphatase 1. *Plant Physiol.* 153, 1555–1562. doi: 10.1104/pp.110.155689

Trinh, M. D. L., and Masuda, S. (2022). Chloroplast pH homeostasis for the regulation of photosynthesis. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.919896

van Meeteren, U., Kaiser, E., Malcolm Matamoros, P., Verdonk, J. C., and Aliniaeifard, S. (2020). Is nitric oxide a critical key factor in ABA-induced stomatal closure? *J. Exp. Bot.* 71, 399–410. doi: 10.1093/jxb/erz437

Wei, A., Fu, B., Wang, Y., Zhai, X., Xin, X., Zhang, C., et al. (2015). Involvement of NO and ROS in sulfur dioxide induced guard cells apoptosis in *Tagetes erecta*. *Ecotoxicol. Environ. Saf.* 114, 198–203. doi: 10.1016/j.ecoenv.2015.01.024

Wilkins, K. A., Bosch, M., Haque, T., Teng, N., Poulter, N. S., and Franklin-Tong, V. E. (2015). Self-incompatibility-induced programmed cell death in field poppy pollen involves dramatic acidification of the incompatible pollen tube cytosol. *Plant Physiol.* 167, 766–779. doi: 10.1104/pp.114.252742

Wong, J. H., Spartz, A. K., Park, M. Y., Du, M., and Gray, W. M. (2019). Mutation of a conserved motif of PP2C.D phosphatases confers SAUR immunity and constitutive activity. *Plant Physiol.* 181, 353–366. doi: 10.1104/pp.19.00496

Yang, J., Li, C., Kong, D., Guo, F., and Wei, H. (2020). Light-mediated signaling and metabolic changes coordinate stomatal opening and closure. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.601478

Ye, W., Koya, S., Hayashi, Y., Jiang, H., Oishi, T., Kato, K., et al. (2021). Identification of genes preferentially expressed in stomatal guard cells of *Arabidopsis thaliana* and involvement of the aluminum-activated malate transporter 6 vacuolar malate channel in stomatal opening. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.744991

Yemelyanov, V. V., Chirkova, T. V., Shishova, M. F., and Lindberg, S. M. (2020). Potassium efflux and cytosol acidification as primary anoxia-induced events in Wheat and Rice seedlings. *Plants (Basel).* 9, 1216. doi: 10.3390/plants9091216

Zhang, J., Chen, X., Song, Y., and Gong, Z. (2024). Integrative regulatory mechanisms of stomatal movements under changing climate. *J. Integr. Plant Biol.* 66, 368–393. doi: 10.1111/jipb.13611

Zhang, X., Dong, F. C., Gao, J. F., and Song, C. P. (2001). Hydrogen peroxide-induced changes in intracellular pH of guard cells precede stomatal closure. *Cell Res.* 11, 37–43. doi: 10.1038/sj.cr.7290064

Zhang, H., Niu, X., Liu, J., Xiao, F., Cao, S., and Liu, Y. (2013). RNAi-directed downregulation of vacuolar $H^+$-ATPase subunit a results in enhanced stomatal aperture and density in rice. *PLoS One* 8, e69046. doi: 10.1371/journal.pone.0069046

Zhou, J. Y., Hao, D. L., and Yang, G. Z. (2021). Regulation of cytosolic pH: The contributions of plant plasma membrane $H^+$-ATPases and multiple transporters. *Int. J. Mol. Sci.* 22, 12998. doi: 10.3390/ijms222312998

Zhu, Y., Ge, X. M., Wu, M. M., Li, X., and He, J. M. (2014). The role and interactions of cytosolic alkalization and hydrogen peroxide in ultraviolet B-induced stomatal closure in Arabidopsis. *Plant Sci.* 215-216, 84–90. doi: 10.1016/j.plantsci.2013.11.010