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Chloride transport and homeostasis in plants

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Abstract

The micronutrient chloride (Cl^-) plays key roles in plant physiology, from photosystem II and vacuolar ATPase activity to osmoregulation, turgor maintenance and drought resilience, while also posing toxicity risks at high concentrations. This review examines Cl^- uptake, transport and homeostasis, focussing on adaptations balancing its dual roles as a nutrient and toxicant. Key transporters, including NPF, SLAH, ALMT, CLC and CCC families, mediate Cl^- fluxes to maintain ionic balance and prevent toxicity. Plants employ strategies such as selective uptake and vacuolar compartmentalization to cope with high salinity. Cl^- also influences nitrogen-use efficiency and plant productivity. Advances in transporter biology reveal the role of Cl^- in water-use efficiency, drought resilience and stress adaptation.

1. Essential functions and beneficial roles of chloride

Chlorine is a micronutrient for higher plants, which is required in trace amounts. The chloride ion (Cl^-), a monovalent anion, is delivered from the soil to the root primarily via mass flow. Thus, its uptake is closely associated with the movement of water through the soil. The critical deficiency concentration of Cl^- varies across non-halophytic plants: rice (*Oryza sativa*) requires approximately 3 mg g^{-1} shoot dry weight (DW), while barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) need 4 mg g^{-1} DW. Lettuce (*Lactuca sativa*) and maize (*Zea mays*), on the other hand, have much lower demands, showing deficiency symptoms at concentrations around 0.14 mg g^{-1} DW and 0.1 mg g^{-1} DW, respectively (Geilfus, 2018b). Kiwifruit (*Actinidia deliciosa*) stands out for its relatively high Cl^- requirement, with leaf concentrations of 225–550 µmol g^{-1} DW needed for optimal growth (Smith et al., 1987). Species with lower Cl^- demands, such as lettuce and maize, often receive sufficient amounts from rainfall, which typically supplies 4–8 kg ha^{-1} annually in non-coastal regions (Geilfus, 2018b). When Cl^- concentrations fall below these thresholds, plants tend to exhibit a reduction in photosynthesis (Terry, 1977) and leaf area, the latter being often associated with decreased cell division rates (Franco-Navarro et al., 2016).

Foliar Cl^- concentrations vary between species depending on Cl^- fertilization levels. For example, *Arabidopsis thaliana* can accumulate around 25 mg Cl^- g^{-1} DW (Cubero-Font, 2017), while the Solanaceae species tomato (*Solanum lycopersicum*) (Rosales et al., 2012), and tobacco (*Nicotiana tabacum*) can accumulate up to 50 mg Cl^- g^{-1} DW (Franco-Navarro et al., 2016). Cl^- is essential for chloroplast function and is the preferred substrate of VCCN1, a Cl^- channel in plastids that finetunes the electrical potential across the thylakoid membrane (Herdean et al., 2016). Cl^- stabilizes the oxygen-evolving complex of photosystem II (PSII-OEC), initiating the electron flux from the stroma to the thylakoid lumen. When Cl^- is deficient, electron transfer is impeded by the formation of a salt bridge between *lys317* of *D2* and *asp61* of *D1* (Kawakami et al., 2009; Raven, 2017). For its catalytic role in PSII-OEC, Cl^- is enriched by the extrinsic proteins PsbP and PsbQ. These extrinsic proteins possess high-affinity Cl^- binding sites that enhance the Cl^- binding affinity of the Mn–Ca–Cl complex (Kakiuchi et al., 2012; Nishimura et al., 2014; Seidler, 1996) and thus aid in saturating the Cl^- PSII-OEC requirement at chloroplastic Cl^- concentrations that are, for example, 40 mM in non-halophytes such as *Triticum* and *Hordeum vulgare* (Bose et al., 2017). At higher chloroplastic Cl^- levels, this Cl^- enrichment is dispensable for maintaining PSII activity (Seidler, 1996). This is consistent with some halophytes, which typically have higher chloroplastic Cl¯ levels, for example, 100 mM in *Beta vulgaris* and *Sueda australis*, where PsbQ is often absent and PsbP levels are reduced, while PSII operation remains functional (Bose et al., 2017). In addition to the catalytic role of Cl¯ in the fully assembled PSII-OEC, Cl¯ is required for OEC synthesis and resynthesis after photodamage (Vinyard et al., 2019). This Cl¯ requirement is significantly higher than that required to operate already assembled OEC, as demonstrated by a doubling of the oxygen evolution rate after a 5-min assembly with 100 mM Cl¯ instead of 20 mM in an *in vitro* experiment in the non-halophyte spinach (Vinyard et al., 2019). The higher estimated Cl¯ requirement to saturate the (re-)assembly of PSII-OEC compared to PSII-OEC operation with 40 mM and 2 mM Cl¯, respectively, could provide a rationale for the positive effects of supplying Cl¯ to crops beyond micronutrient levels (as described below), as it might enhance the rate of OEC assembly and the repair of photodamaged thylakoids, thereby supporting a more efficient photosynthesis (Raven, 2020).

Another potential essential function is related to the activity of the vacuolar proton-pumping ATPase (V-H^+-ATPase) that depends on Cl¯ for maximal activity, as described by Churchill and Sze (1984). Beyond these essential roles, Cl¯ can fulfil additional, less specific functions that can also be fulfilled by other anions. Among them are processes like the regulation of enzyme activities such as the asparagine synthetase (Rognes, 1980) and α-amylases (*α-1,4-glucan-4-glucanohydrolase*) (Metzler, 1979; D'amico et al., 2000). Cl¯ also contributes to turgor maintenance and osmoregulation, which are additional non-essential but beneficial functions (Nieves-Cordones et al., 2019; Shelke et al., 2019). Here, Cl¯ might have an advantage over nitrate, as Cl¯ has a tighter bound hydration shell compared to nitrate, which might prevent further water loss (Wege et al., 2017). However, once Cl¯ reaches higher levels (toxicity), molecular crowding might lead to the disruption of many cellular processes, particularly in chloroplasts. In addition, influx of Cl¯ into guard cells is relevant for the opening of leaf pores (Humble & Hsiao, 1969). For these beneficial functions, Cl¯ is required in much greater quantities than for its essential role in chloroplasts, which necessitates only 0.1–0.2 mg g^{-1} (~2.8–5.5 μmol) based on shoot DW (Geilfus, 2018a; Marschner, 2011). To support these additional functions, Cl¯ concentrations in the millimolar (mM) range are often necessary. When Cl¯ is given in concentrations typical for a macronutrient, Franco-Navarro et al. (2019) demonstrated that Cl¯ treatments ranging from 0.075 to 5.075 mM in the growth medium increased biomass production and improved water relations in tobacco plants. Their studies suggest that Cl¯ regulates leaf osmotic potential and turgor, enhances cell growth and increases drought tolerance in tobacco. These effects were also observed in other species tested, including lettuce, spinach and tomato. Koch et al. (2021) investigated the effects of Cl¯ on potato (*Solanum tuberosum L.*) using four different Cl¯ doses: 0, 200, 400 and 800 mg Cl¯ kg^{-1} soil. They found that Cl¯ application significantly altered soil–plant water relations, with the highest dose (800 mg Cl¯ kg^{-1} soil) improving plant hydration status without reducing tuber yield or dry matter. Cl¯ was suggested to play a crucial role in improving water-use efficiency (WUE) by reducing transpiration through lowered stomatal conductance. Overall, these authors discuss that Cl¯ doses above those of a micronutrient but below toxicity thresholds enhance biomass production and improve WUE. This review focusses on Cl¯; for sodium homeostasis, see the article by Munns, Tyerman and Bose in this special issue.

2. Chloride toxicity and responses

Regions particularly affected by Cl¯ salinity include coastal areas, arid and semi-arid zones, and irrigated agricultural lands in countries such as Australia, the United States and parts of the Middle East (Munns & Tester, 2008). Cl¯ concentrations in soil solutions can vary widely: from 1 to 250 mg L^{-1} in freshwater streams and lakes, 500 to 5,000 mg^{-1} in brackish water and up to 19,400 mg^{-1} in seawater (Flowers, 2004). Cl¯ salinity is a common issue driven by both natural and human activities. Natural sources include the weathering of salt-rich materials and wind-borne salt depositions from oceans and lakes (Rengasamy, 2006), while human activities mainly involve irrigation with Cl¯-rich water and the use of Cl¯-containing fertilizers (Flowers & Yeo, 2012). In detail, fertilization with Cl¯-containing fertilizers can lead to high Cl¯ concentrations in the soil, particularly under conditions of limited water percolation that reduce leaching. Common fertilizers contain varying levels of Cl¯ by weight, with magnesium Cl¯ ($MgCl_2$) containing 74% Cl¯ by weight, ammonium Cl¯ (NH_4Cl) containing 66% Cl¯ by weight and potassium Cl¯ (KCl) containing 47% Cl¯ by weight (Turhan, 2021). Organic fertilizers like pig slurries also add substantial amounts of Cl¯. For example, applying 210 kg N ha^{-1} $year^{-1}$ via pig slurry can add 282–458 kg Cl¯ ha^{-1} $year^{-1}$ (Moral et al., 2008), while chicken and pigeon manure contribute 7.1 and 6.1 g Cl¯ per kg DW, respectively (Li-Xian et al., 2007). If there is insufficient rainfall to leach Cl¯ deeper into the soil, these high Cl¯ loads can accumulate in the root zone, leading to osmotic stress, ion toxicity and nutrient imbalances in plants, negatively impacting crop yield and quality (Teakle & Tyerman, 2010).

However, Cl¯ can be toxic to plants at excessive concentrations, with critical toxicity estimated to be 4–7 mg g^{-1} for Cl¯-sensitive species and 15–35 mg g^{-1} DW for Cl¯-tolerant species (Colmenero-Flores et al., 2019; Xu et al., 2000). Differences in tolerance to Cl¯ salinity are often observed not only between species but also within a single species. Under salt stress conditions, woody perennial plants exhibit toxicity symptoms due to Cl¯, rather than sodium accumulation in their leaves. This association exists not because Cl¯ is metabolically more harmful than sodium to these species but because these plants are able to secrete a greater amount of sodium into their woody organs (Gong et al., 2011; Shelke et al., 2019). This limits sodium transport to the leaves to reduce its impact upon cellular metabolism within photosynthetic organs (Li, Tester, & Gilliham, 2017b). The high level of Cl¯ accumulation leads to decreased transpiration, photosynthesis, crop yield and quality, and ultimately to plant death (Teakle & Tyerman, 2010). This phenomenon has been documented in fruit tree species grown on rootstocks, such as citrus (*Citrus*) (Storey & Walker, 1999; Tadeo et al., 2008), grapevine (*Vitis*) (Sykes, 1992) and avocado (*Persea Americana*) (Bar et al., 1997). In citrus, the ability to exclude Cl¯ is a critical factor in determining salt tolerance. Tolerant citrus rootstocks, such as *C. macrophylla* and *Poncirus trifoliata*, exclude Cl¯ primarily by reducing xylem loading and retaining Cl¯ in distal root tissues, resulting in leaf concentrations of 5–13 mg g^{-1} DW. In contrast, sensitive rootstocks like *C. aurantium* accumulate up to 30 mg g^{-1} DW, due to higher xylem Cl¯ loading and thus limited retention ability. This difference is partly regulated by genes like *CcICln*, which modulate Cl¯ transport and homeostasis (Brumós et al., 2010). Similarly, Sykes (1992) reported that grapevine rootstocks exhibit varying efficiencies in Cl¯ exclusion. Rootstocks such as *Ramsey* and *1103 Paulsen* are particularly effective, maintaining leaf Cl¯ concentrations below harmful thresholds (less than 10 mg g^{-1} DW) in saline conditions. In contrast, rootstocks such as

Salt Creek may accumulate more Cl^-, which cause symptoms of toxicity (Sykes, 1992). Genetic variation in Cl^- accumulation was also found for rice, with sensitive cultivars like *IR29* accumulating above 30 mg Cl^- g^{-1} DW, while the tolerant cultivar *Pokkali* maintained leaf Cl^- concentrations below 15 mg g^{-1} DW (Shannon et al., 1998).

Control of Cl^- transport and Cl^- 'exclusion' from shoots is correlated with salt tolerance in many species, particularly for legumes (Franzisky et al., 2019). Luo et al. (2005) evaluated seedlings of two *Glycine max* cultivars (salt-tolerant *Nannong 1138-2* and salt-sensitive *Zhongzihuangdou-yi*) and two *Glycine soja* populations (*BB52* and *N23232*) under iso-osmotic conditions of 150 mM sodium, Cl^- and sodium chloride. They showed that *Glycine max* cultivars suffered more damage from Cl^- than from sodium, with leaf sensitivity linked to their ability to restrict Cl^- accumulation in roots and stems. The ability of soybean plants to exclude Cl^-, as well as sodium, from leaves is related to the activity of the putative cation proton exchanger GmSALT3 through a yet-unknown mechanism; plants without a functional *GmSALT3* gene show an increase in foliar Cl^- before sodium increases (Qu et al., 2020). A functional GmSALT3 protein is also present in the *G. max* relative, *Glycine soja*. Different *G. soja* populations are more susceptible to sodium toxicity than others, indicating that their salt tolerance relies on preventing leaf sodium accumulation. This may also be because wild soybean has a very high Cl^- exclusion capacity, making sodium toxicity more apparent compared to domesticated soybean.

In addition to soy, Teakle et al. (2007) studied another leguminous species under salinity and waterlogging conditions, *Lotus corniculatus*. They demonstrated that salinity and waterlogging conditions hinder growth in sensitive species by increasing sodium and Cl^- transfer to shoots. *L. corniculatus* had 50% higher Cl^- levels in xylem and shoots than *L. tenuis* in 200 mM aerated sodium Cl^-, but sodium and potassium levels were comparable. Under conditions of waterlogging and salt stress, *L. corniculatus* had double the xylem Cl^- and sodium concentrations as *L. tenuis*. *L. tenuis* roots had increased inherent porosity due to the production of aerenchyma, which is important for oxygen supply and ion 'exclusion'. This aerenchyma not only supports processes like compartmentalization and ion 'exclusion', but also limits the accumulation of Cl^- in roots. While the precise mechanisms remain to be elucidated, Cl^- exclusion is pivotal for salinity tolerance (White & Broadley, 2001). Franzisky et al. (2019) tested the toxicity of Cl^- on thirteen non-salt-tolerant *Vicia faba* genotypes. Plants were exposed to 100 mM sodium Cl^- hydroponically until necrotic leaf patches formed. At this time, Cl^- concentrations in the affected leaves were similar among genotypes, but sodium levels varied, suggesting that Cl^- toxicity predominantly caused the lesions (Slabu et al., 2009; Tavakkoli et al., 2010). Higher Cl^- accumulation in photosynthetic tissues negatively influenced growth and photosynthesis in sensitive genotypes. Tolerant cultivars, such as *Scoop* and *Nebraska*, likely limited Cl^- transfer to leaves, reducing damage and extending photosynthetic activity.

Grass species are also affected by Cl^- toxicity. Tavakkoli et al. (2010) evaluated four barley genotypes (Barque73, Clipper, Sahara and Tadmor) under different Cl^- levels in culture solution (control, 1.9 mM Cl^-; and high-Cl^- treatment, 125 mM Cl^-). They reported that the high-Cl^- treatment reduced photosynthetic capacity due to non-stomatal effects, causing chlorophyll degradation and a decrease in the actual quantum yield of electron transport in PSII. These effects were associated with both photochemical quenching and excitation energy capture efficiency. In addition, Khare et al. (2015) evaluated two rice genotypes, Panvel-3 (salt-tolerant) and Sahyadri-3 (salt-sensitive), under varying Cl^- levels in a hydroponic solution (15 mM $CaCl_2$, 15 mM $MgCl_2$ and 40 mM KCl). They found a significant decrease in DW in both cultivars, which was linked to higher levels of reactive oxygen species (ROS), such as H_2O_2 and O_2^-, and enhanced cell death. An accumulation of ROS causes oxidative damage, which in turn disrupts metabolic functions and hinders growth. Furthermore, as a defence mechanism, high ROS contents cause programmed cell death, which further reduces biomass.

3. Chloride uptake at low chloride conditions

Cl^- has minimal affinity for soil components because most soil particles, such as clay and organic matter, are negatively charged. This negative charge tends to repel negatively charged ions like Cl^-. Consequently, Cl^- remains in the aqueous phase, where its movement is largely governed by water flow. This enables Cl^- to follow the path of water, making its transport within the soil highly dependent on moisture dynamics and mass flow (Geilfus, 2019; Moya et al., 2003). Cl^- uptake in plant roots can occur via an apoplastic, a symplastic or a transcellular pathway, with Casparian bands playing a crucial role in selectively regulating ion transport by limiting passive flow through the apoplast (Abbaspour et al., 2014; Barberon, 2017; Karahara et al., 2004). Casparian bands are key structural components of plant roots, situated at the innermost cortex cell layer, the endodermis. Casparian strips develop here in the radial and tangential walls of endodermal cells and prevent the unselective flux of ions from the soil to the vasculature (Abbaspour et al., 2014; Barberon, 2017; Karahara et al., 2004). Casparian bands therefore prevent ions, most likely including Cl^-, from passively reaching the xylem via simple diffusion.

Cl^- concentrations in non-saline soil solutions typically range from 1 to 4 mM (Tavakkoli et al., 2010; Wada et al., 2006), while cytosolic Cl^- concentrations in the root cells of *A. thaliana* are around 10 mM (Lorenzen et al., 2004). Similarly, in barley root cells, cytosolic Cl^- levels range from 8 to 10 mM under external Cl^- concentrations of 0.1 mM (Britto et al., 2004). Teakle and Tyerman (2010) found that cytosolic Cl^- concentrations between 5 and 20 mM are typical for many glycophytic plants not under excessive Cl^- stress. Additionally, Bazihizina et al. (2019) reported cytosolic Cl^- concentrations ranging from 2 to 50 mM under non-saline conditions in three halophytic plant species: *Suaeda maritima* (Hajibagheri & Flowers, 1989), *Atriplex amnicola* (Jeschke et al., 1986) and *Atriplex spongiosa* (Storey et al., 1983). Due to the negative potential across the plasma membrane (PM) of root cells, the uptake of Cl^- via anion channels is energetically unfavourable (Maathuis & Sanders, 1993; Wang et al., 2015). Thus, under low or moderate external Cl^- concentrations when $[Cl^-]_{out} < [Cl^-]_{cyt}$, energy is required to move Cl^- into the cytoplasm; i.e. it is an active transport (Felle, 1994; White & Broadley, 2001). Here, adenosine triphosphate (ATP) is consumed by H^+ pumps, and the term to be referred to is secondary active transport (Saleh & Plieth, 2013). With stoichiometry, the overall transport has a surplus of one positive elementary charge and thus is supported by the pH gradient, as well as the membrane potential (negative on the cytosolic side).

The transport proteins that enable the uptake of Cl^- are likely to be members of the nitrate transporter 1/peptide transporter family (NPF) (Léran et al., 2014). The first identified member of this group of transporters was NPF6.3 (NRT1.1), which facilitates the uptake of NO_3^- across the PM (Tsay et al., 1993). However, the homologous maize *Zm*NPF6.4 protein acts as a high-affinity

Cl^--selective pH-dependent transporter, whereas *Zm*NPF6.6 has a lower Cl^- affinity (Wen et al., 2017). Single-point mutations in these transporters had a strong impact on NO_3^-/ Cl^- selectivity, which suggests that genes in the NPF6 group encode H^+-coupled transporters with a variable selectivity of Cl^- versus NO_3^-. The ability of these proteins to transport both anions would also explain why high NO_3^- concentrations compete with Cl^- uptake from the soil (Bar et al., 1997).

Pitman (1982) estimated that the diffusion coefficient of monovalent ions, such as Cl^-, in the apoplast is approximately 10^{-10} m^2 s^{-1}. Cl^- transport by the symplastic pathway provides a more selective and regulated mechanism for ion movement. Once Cl^- enters the root symplast, it moves from cell to cell through plasmodesmata towards the cortex. According to Zhang et al. (2016), this intracellular movement distributes Cl^- throughout the root and may involve short-term storage in vacuoles, mediated by transporters of the chloride channel (CLC) family of plant nitrate and putative Cl^- proton antiporters (not channels as the name might suggest), which helps in maintaining osmotic pressure balance under stress. Additionally, during abiotic stress, signals like ATP, abscisic acid (ABA) and ROS affect the activity of important transporters, which in turn affects Cl^- transport within roots (Colmenero-Flores et al., 2019).

The transcellular pathway adds an additional layer of control, facilitating ion transport across cell membranes. This process requires transporter proteins, which mediate consecutive influx and efflux steps as Cl^- crosses various root cell layers, including the exodermis, hypodermis, cortex and endodermis, before reaching the stele (Andersen et al., 2018). The exodermis, a specialized hypodermis found in certain plant species, forms the second outermost root layer and shares structural similarities with the endodermis. Both layers act as selective barriers equipped with Casparian strips and suberin depositions that restrict the apoplastic flow (Damus et al., 1997). However, passage cells, which are unsuberized cells scattered throughout the exodermis and endodermis, allow for selective transcellular transport (Holbein et al., 2021). These passage cells, first described in the 19th century (Kroemer, 1903 reviewed in Holbein et al., 2021), are assumed to play distinct but overlapping roles in Cl^- transport. Endodermal passage cells, in particular, frequently co-localize with xylem poles, highlighting their role in ion transport (Hose et al., 2001). As Cl^- moves radially towards the stele, its transport through the transcellular pathway bypasses the hydrophobic barriers of suberized cell walls. This mechanism becomes particularly relevant in older root regions, where apoplastic diffusion is hindered. Instead, transcellular transport depends on PM-localized transporter proteins, whose expression may be concentrated in parenchyma cells (Ramakrishna & Barberon, 2019).

4. Chloride uptake during salt stress

When seedlings of *A. thaliana* are exposed to an external Cl^- concentration of 100 mM, Cl^- rapidly accumulates in root cells to cytosolic concentrations that exceed 50 mM (Lorenzen et al., 2004). However, the accumulation of Cl^- is inhibited by high extracellular Ca^{2+} concentrations, as well as by the application of 2 mM of the broad-range cation channel blocker La^{3+}. Roots thus seem to have an uptake system that enables rapid sequestering of Cl^- and is regulated by a Ca^{2+}-dependent mechanism.

It is likely that the same NPF transporters that are essential for the uptake of Cl^- at low concentrations (as explained above) also cause the rapid accumulation of Cl^- during salt stress. A main function of NPF transporters is the uptake of NO_3^-, which is essential for protein synthesis. Because of the limiting ability of NPF transporters to discriminate between Cl^- and NO_3^-, it is likely that Cl^- uptake is inevitable. Consequently, Cl^- toxicity can be reduced by high levels of NO_3^- (Bar et al., 1997) or by breeding plants with transporters that have a higher specificity for NO_3^- over Cl^- (Bazihizina et al., 2019).

The importance of NPF transporters in Cl^- tolerance was confirmed in a study on soybean (Wu et al., 2025). In a genome-wide association study, the *GmNPF*7.5 gene was found to be of major importance for salt tolerance. A point mutation in *GmNPF*7.5 reduced the Cl^- permeability of the transporter, while keeping the NO_3^- transport activity high and thus enhancing the ability to grow at high-Cl^- conditions.

5. Xylem loading of Cl^-

Once Cl^- has entered the root epidermal cells, or cortex cells, it is likely to move from cell to cell via plasmodesmata into the endodermis. The apoplastic movement of Cl^- into the endodermis is blocked by the Casparian strip, whose formation is enhanced during salt stress in maize plants (Karahara et al., 2004). In line with this important role, a lower salt tolerance has been found for mutants with defects in Casparian strip development (Wang et al., 2022).

Cl^- that has entered the endodermis is loaded into xylem vessels via a mechanism in which anion channels play a major role. The activation of anion channels depolarizes the PM of xylem parenchyma cells and enables the extrusion of both Cl^- and potassium into xylem vessels (Hmidi et al., 2024). Slow (S)-type anion channels are found to be active in xylem parenchyma cells (Köhler & Raschke, 2000; Wegner & Raschke, 1994), and in *A. thaliana*, the S-type channels SLAH2 and SLAH3 exert this function (Cubero-Font et al., 2016; Maierhofer et al., 2014). The SLAH3 channel interacts with the non-function SLAH1-subunit, which enhances the activity and Cl^- conductivity of SLAH3 (Cubero-Font et al., 2016). It is thus very likely that the SLAH3/SLAH1 heteromeric channels are important for the long-distance transport of Cl^- through the xylem. In addition, in maize, a rapid (R)-type anion channel was found to be prevalent, which suggests that these channels may also contribute to the translocation of Cl^- from roots to shoots (Gilliham & Tester, 2005).

In addition to anion channels, NPF transporters have also been found to play a role in xylem transport, with their activity regulated by salt stress (Shelke et al., 2019). The transporter *AtNPF2.4* is localized to the PM of cells in the root stele, and its overexpression increases shoot Cl^- levels by approximately 23%, underscoring its essential function (Geilfus, 2018a; Li et al., 2016), while silencing of *AtNPF2.4* in *A. thaliana* reduces shoot Cl^- by 20–30% (Li et al., 2016). A similar function was found for AtNPF2.5, closely related to AtNPF2.4 (Li, Qiu, et al., 2017a). While the authors suggest a role of *At*NPF2.4 and 2.5 in Cl^- extrusion (Li et al., 2016; Li, Tester, et al., 2017b), alternatively these transporters may facilitate Cl^- uptake in root cells, as suggested above. In this scenario, the high activity of NPF proteins would ensure high cytosolic Cl^- concentrations in root cells and an efficient loading of Cl^- into the root xylem. In maize, *ZmNPF6.4* has been identified as encoding a potential Cl^--selective transporter. This protein is localized in root cell membranes and likely employs H^+-coupled active transport. This process depends on an *ExxER/K* motif containing three proton-binding, chargeable residues in its first transmembrane helix.

Wen et al. (2017) reported that increasing the external Cl^- concentration from 0 to 10 mM enhanced Cl^- uptake by *ZmNPF6.4*.

6. Stomatal movements

The R- and S-type of anion channels were first identified in patch clamp experiments with guard cells (Keller et al., 1989; Linder & Raschke, 1992; Schroeder & Hagiwara, 1989) and later also in other cell types. In *A. thaliana*, the S-type anion channels are encoded by *SLAC*/*SLAH* genes (Geiger et al., 2011; Guzel et al., 2015; Negi et al., 2008; Vahisalu et al., 2008) and play a major role in regulating stomatal movements (Merilo et al., 2013). The S-type channels differ in the selectivity for the anions they conduct; SLAC1 conducts both NO_3^- and Cl^- (Geiger et al., 2011), SLAH3 has a preference for NO_3^- (Geiger et el., 2011; Hedrich & Geiger, 2017), while SLAH2 is selective for nitrate (Maierhofer et al., 2014). So far, no Cl^--selective SLAC1/SLAH channel has been identified, and it is thus likely that the extrusion of Cl^- by these channels is always linked to that of NO_3^-.

Stimuli that provoke stomatal closure, such as darkness, high atmospheric CO_2 concentrations, ABA and microbe-associated molecular patterns (MAMPs), all activate S-type anion channels (Guzel et al., 2015; Pei et al., 1997; Roelfsema et al., 2002; Roelfsema et al., 2004). Upon activation of these channels, Cl^- is released, causing a depolarization of the PM (Hmidi et al., 2024), which also supports K^+ release via the GORK K^+ channel (Ache et al., 2000; Hosy et al., 2003). The simultaneous efflux of Cl^- and K^+ lowers the osmotic content of guard cells, which leads to efflux of water and closure of stomata (Kollist et al., 2014).

In *A. thaliana*, the loss of the *aluminium-activated malate transporter* 12 (*ALMT*12) stimulates stomatal opening (Jalakas et al., 2021) and inhibits several stomatal closure responses (Meyer et al., 2010; Sasaki et al., 2010). It is likely that the *ALMT*12-encoded protein is related to R-type channels, since the loss of ALMT12 reduced the activity of these channels in guard cells (Meyer et al., 2010). Although the ALMT12 channel mainly seems to transport malate (Jaslan et al., 2023), R-type channels in *Vicia faba* guard cells also conduct Cl^- and NO_3^-, in addition to small organic anions like acetate and malate (Dietrich & Hedrich, 1998), and thus provide an alternative efflux pathway to S-type anion channels. Despite in-depth insights into the structure of ALMT12 (Qin et al., 2022), the properties of R-type channels are still under discussion, and these channels may be influenced by so-far-unidentified components (Jaslan et al., 2023).

7. Storage of Cl^- in the vacuole and other organelles

Vacuolar Cl^- accumulation in non-halophytes depends on external Cl^- availability, with vacuolar concentrations easily reaching 40 mM (Barbier-Brygoo et al., 2000; Fricke et al., 1994; Geilfus, 2018a). In root cortical cells of barley, vacuolar Cl^- concentrations under non-saline conditions range from approximately 2–9 mM, but increase significantly upon exposure to saline conditions, with concentrations of up to 290 mM (Flowers & Hajibagheri, 2001; Huang & Van Steveninck, 1989). Similarly, in maize, salinity induces an increase in the vacuolar Cl^- concentration from approximately 40 mM to as high as 1300 mM (Harvey, 1985). In the vacuoles of leaf epidermal cells of barley, Cl^- concentrations reach approximately 100 mM in response to salt exposure (Fricke et al., 1995), but also substantially higher levels of up to 400 mM are reported in the vacuoles of epidermal and mesophyll cells of barley and wheat following salt exposure (James et al., 2006).

The vacuolar membrane has an electrical potential that is negative at the cytosolic side, just as the PM. Anion channels thus can support the uptake of Cl^-, but because of the low potential (approximately −30 mV, Wang et al., 2015) it can only support an accumulation of up to threefold of the concertation of the cytosol. This mechanism is supported by a group of ALMT proteins that are localized to the vacuolar membrane (Sharma et al., 2016). ALMT9 is shown to encode a Cl^--permeable channel, which supports light-induced stomatal opening, suggesting that it enables the accumulation of Cl^- in vacuoles. The activation of cation channels, such as two-pore potassium (TPK) channels (Dabravolski & Isayenkov, 2023) and two-pore channel 1 (TPC1) (Jaslan et al., 2019), is likely to depolarize the vacuolar membrane and thereby enable the backflow of Cl^-, from the vacuole into the cytosol (Eisenach et al., 2017).

CLC proteins in plants, such as *AtCLCa*, are major contributors to nitrate storage in vacuoles (Geelen et al., 2000). In *A. thaliana* leaves, vacuoles serve as reservoirs, accumulating nitrate to concentrations as high as 30 mM, a significant enrichment compared to the 2 mM in the cytosol (Cookson et al., 2005). Barley root cells show a similar pattern, with cytosolic nitrate concentrations remaining relatively stable around 4 mM, while vacuolar concentrations increase significantly, up to 20-fold, depending on the external nitrate supply (van der Leij et al., 1998). A single amino acid modification in CLC proteins can shift selectivity from nitrate to Cl^-. The proline residue at position 160 (P160) in the selectivity filter of AtCLCa is critical for its ability to preferentially transport nitrate over Cl^-, a function essential for nitrate accumulation *in planta* (Wege et al., 2010). Mutation of this amino acid shifts the selectivity of the transporter and turns the preferentially nitrate-transporting AtCLCa into an efficient Cl^- transporter (Bergsdorf et al., 2009; Wege et al., 2010). Whole-vacuole patch-clamp analysis demonstrated that the mutation does not affect the stoichiometry of the anion–proton coupling, an uncoupling effect that is observed in animal CLCs (Wege et al., 2010). The selectivity motif (containing either a proline or a serine) of other CLC family members suggests that they likely function as $2Cl^-/H^+$ antiporters, such as AtCLCc and AtCLCd; this is supported by the phenotypic defects observed in CLCc loss-of-function mutants (Jossier et al., 2010). Plants lacking functional CLCs show reduced Cl^- uptake, decreased salinity tolerance and impaired stomata aperture regulation (Hu et al., 2017; Jossier et al., 2010). CLC proteins reside in endomembranes and are not present in the PM; the importance of endomembrane-localized Cl^- transporters strongly suggests that the anion has an important role here. This is also demonstrated by the severe phenotypic defects observed in plants lacking a different endomembrane-localized Cl^--permeable channel, cation chloride cotransporter (CCC) (McKay et al., 2022).

The first plant CCC characterized was from *A. thaliana* (AtCCC1) (Colmenero-Flores et al., 2007). The CCC genes encode putative Cl^- and potassium transporters (Negi et al., 2008). When expressed in Xenopus oocytes, AtCCC1 facilitated the cotransport of Cl^-, sodium and potassium. AtCCC1 is, however, localized in the trans-Golgi-network/early endosome (TGN/EE), where it also remains upon salt or osmotic shock exposure (McKay et al., 2022). Additionally, AtCCC1 is expressed in all cell types, and CCC1 loss-of-function plants show severe phenotypic defects under control non-stressed conditions, demonstrating a role of CCCs outside of salinity tolerance in both *A. thaliana* and rice (Chen et al., 2016; McKay et al., 2022). Saleh and Plieth (2013) discovered that internal calcium impacted the transport of Cl^-. A calcium-activated Cl^- channel (*CaCC*) blocker, anthracene-9-carboxylic acid (*A9C*), causes Cl^- accumulation (Saleh & Plieth, 2013) in root cells of *A. thaliana* during salt stress and is controlled by both internal and external calcium levels.

In some halophytes, epidermal bladder cells have been proposed to contribute to salt tolerance by sequestering excess Cl^- and sodium (Kiani-Pouya et al., 2017; Shabala et al., 2014). However, another study challenges this. While wild-type epidermal bladder cells store substantial amounts of Cl^- under saline conditions, the ebcf mutant shows no such enrichment, indicating that Cl^- retention in EBCs is not essential for salt tolerance (Moog et al., 2022).

8. The role of chloride transport in ion homeostasis

In recent years, it has become more and more clear that the investigation of individual transport events is insufficient for explaining the complex interconnectivity of ions and therefore nutrient homeostasis. Looking at transporter networks is a more promising approach (Blatt, 2024; Dreyer, 2021). The smallest unit of a transporter network is a so-called homeostat, as described, for example, for potassium by Dreyer et al. (2024). Potassium, similar to Cl^-, is a monovalent ion that is not metabolized, and it functions primarily as an osmoticum and, importantly, as a positive charge that can be shuttled across membranes without major disturbances of metabolic reactions. This potassium shuttling is crucial for maintaining cellular function and does not constitute futile cycling (Dreyer, 2021).

A similar situation might exist for Cl^- transport and might explain further the preference of plants for Cl^- over nitrate as a non-metabolized anion. A constant cycling of the anion across cellular membranes might be crucial for the capacity of cells to dynamically respond to external changes, as well as maintaining internal nutrient concentrations. Nitrate, as a similarly sized monovalent anion, can replace Cl^- in this role; a constant cycling of the metabolized ion might yet have several disadvantages, and plants that show a defect in vacuolar nitrate storage show increased amino acid catabolism (Hodin et al., 2023). Cycling across the PM holds the potential risk of nitrate loss to the environment. This loss has been observed, for example, in rice (Kurimoto et al., 2004), where up to 40% of the nitrate taken up by the plants is effluxed back in media without added Cl^-. Interestingly, in carrot (*Daucus carota*), Cl^- uptake from external media is faster than nitrate uptake in root tissue starved for both ions (Cram, 1973). This very interesting observation might be explained by the need for a 'cycling' anion. Cl^- is taken up faster and first, compared to nitrate, so that the subsequent potential loss of nitrogen can be minimized and nitrate can be directly and efficiently imported into the vacuole for storage and subsequent nitrate reduction. Higher cytosolic Cl^- concentrations compared to nitrate also point in this direction.

Cl^- transport across intracellular membranes might play equally important roles. CCC proteins in the TGN/EE are part of the pH regulatory circuit in this major cellular trafficking hub, which likely explains the multitude of phenotypic defects observed in *CCC* loss-of-function plants in rice and *A. thaliana*. These plants show defects in almost all parts, including reduced root and root hair lengths, malformed leaves, disturbed phyllotaxis and a strongly reduced fertility, in both rice and *A. thaliana* (Chen et al., 2016; Colmenero-Flores et al., 2007; McKay et al., 2022). Similarly, double knockout plants lacking the activity of both TGN/EE-localized AtCLCd and AtCLCf cannot be isolated (Scholl et al., 2021), suggesting that putative $2Cl^-/H^+$ antiporters are crucial for plants to complete their life cycle. CLCs might fulfil similar yet different roles as H^+/K^+ antiporters at endomembranes. Different from the large group of likely electroneutral K^+/H^+ antiporters in plants (NHXs, KEAs, CHXs), CLCs mediate electrogenic transport with a stoichiometry of $2Cl^-:1H^+$ (De Angeli et al., 2006; Wege et al., 2010). This has a major impact on Cl^- homeostats and indicates that the homeostats either function differently and/or that not all components are yet known.

In addition, Cl^- transport across the PM has been shown to directly impact potassium transport, further strengthening a connection of the homeostats of the two non-metabolized ions. Plants with the loss of the Cl^- channel SLAC1 also show decreased potassium movement across the PM, although SLAC1 does not transport potassium (Jezek & Blatt, 2017; Wang et al., 2012).

9. Conclusion

Cl^- plays a dual role in the plant, functioning as a micronutrient (i.e. essential) and a beneficial (i.e. non-essential) osmoticum and negative charge. Despite its importance, similar to other nutrients, excessive Cl^- concentrations are toxic and become a substantial challenge for many crops. While considerable progress has been made in understanding Cl^- transport and homeostasis, key gaps remain in identifying the full range of Cl^- transporters, elucidating their regulatory networks. Addressing these gaps is critical for improving stress resilience and productivity, particularly in the context of increasing soil salinity and changing climate conditions.

Future research should focus on identifying and characterizing Cl^- transporters that remain unknown, particularly in halophytes, where they play a critical role in enabling growth under saline conditions. Investigating the mechanisms of Cl^- regulation in chloroplasts, including its influence on photosynthetic efficiency, will also enhance the understanding of how halophytes thrive in high-salt environments. Additionally, research into the role of Cl^- in enzyme regulation and cellular activities can reveal broader physiological impacts and potential applications. Finally, integrating Cl^- transporters into the broader framework of ion transport networks will be key to uncovering how plants coordinate ion cycling to maintain cellular homeostasis and adapt dynamically to environmental changes.

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