Chloride Ions as a Beneficial and Essential Micronutrient Multifunctional, Role and Regulation in Plant Physiology: A Review

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ABSTRACT
Chloride occurs predominantly as Cl\(^{-}\) in soil, plant, and considering as a micronutrient largely excluded by plants due to its ubiquity and abundance in nature. It is an essential micronutrient of higher plants and participates in several physiological metabolism processes. Including osmotic and stomatal regulation, evolution of oxygen in photosynthesis, disease resistance and tolerance. Chloride (Cl\(^{-}\)) has traditionally been considered harmful to agriculture because of its toxic effects in saline soils and its antagonistic interaction with nitrate (NO\(_3\)^{-}), which impairs NO\(_3\)^{-} nutrition. It has been largely believed that Cl\(^{-}\) antagonizes NO\(_3\)^{-} uptake and accumulation in higher plants, reducing crop yield. However, we have recently uncovered that Cl\(^{-}\) has new beneficial macronutrient functions that improve plant growth, tissue water balance, plant water relations, photosynthetic performance, and water-use efficiency (WUE). Increasing plant biomass indicates in turn that Cl\(^{-}\) may also improve nitrogen use efficiency (NUE). Structure of water around the sodium and potassium ions is a key test of the quality of interaction potentials, and are not completely aligned toward their electric fields, but rather tilted. This tilt is more defined for potassium than it is for sodium. The hydration number of sodium is restricted to either five or six molecules, however for potassium has ranging from five to ten molecules. Most striking energetic difference between Na and K resides in the first shell. Water molecules have a very strong interact under such condition Na\(^{+}\) is more effect on the soil salinity than K\(^{+}\). However, an increase in Na\(^{+}\) content is always accompanied by Cl\(^{-}\) accumulation and K\(^{+}\) loss in plants exposed to salt (NaCl) stress. Considering that N availability is a bottleneck for the growth of land plants excessive NO\(_3\)^{-} fertilization frequently used in agriculture becomes a major environmental concern worldwide, causing excessive accumulation leaf NO\(_3\)^{-} in crops particularly in vegetables, that poses a potential risk to human health. New farming practices aimed to enhance plant nitrogen use efficiency (NUE), by reducing NO\(_3\)^{-} fertilization should promote a healthier and more sustainable agriculture. Given the strong interaction between Cl\(^{-}\} and NO\(_3\)^{-} homeostasis in plants, we have verified if indeed Cl\(^{-}\} affects NO\(_3\)^{-} accumulation and NUE in plants. For the first time to our knowledge, we provide a direct demonstration, which shows that Cl\(^{-}\} , contrary to impairing NO\(_3\)^{-} nutrition, facilitates NO\(_3\)^{-} utilization and improves NUE in plants. This is largely due to Cl\(^{-}\} improvement of the N–NO\(_3\) utilization efficiency (NUTE), having little or moderate effect on N–NO\(_3\) uptake efficiency (NUPE) when NO\(_3\)^{-} is used as the sole N source. Clear positive correlations between leaf Cl\(^{-}\} content vs. NUE / NUTE or plant growth have been established at both intra- and interspecies levels. Optimal NO\(_3\)^{-} versus Cl\(^{-}\} ratios become a useful tool for increasing crop yield and quality, sustainability of agricultural land and reducing negative ecological impact of NO\(_3\)^{-} on the environment and human health as well.

Keywords: Chloride, crop production, plant nutrition, chloride / nitrate interaction, physiological functions

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1. Introduction

Chloride (Cl) in natural soils mainly come from either rainwater, sea spray, dust, or air pollution. However, Irrigation systems and fertilization may contribute significantly to deposition of chloride in soil. The rates of deposition ranged from 1 to >1000 kg ha$^{-1}$, depending on location and cultural practices (White and Broadley, 2001). It is an essential micronutrient for higher plants and participates in several physiological processes, ascribing as a main resource of salinity stress. This review provides brief information on the major progresses of Cl nutrition of higher plants. The chloride (Cl$^-$) anion is the dominant form of the halogen element chlorine in soils. Especially in the agronomic context, Cl$^-$ has traditionally been considered a toxic anion rather than a plant nutrient Fig. (1).

**Fig. 1:** Regulation of root xylem chloride (Cl$^-$) translocation by the anion channels SLAH1 and SLAH3 according to environmental cues. Under favorable growing conditions (A), high Transcriptional activity of both SLAH1 and SLAH3 genes determines the formation of heteromeric SLAH1/SLAH3 complexes in the xylem-pole pericycle. The SLAH1 channel does not transport anions itself, but modifies the kinetic properties of SLAH3, which increases its Cl$^-$ conductance by seven times and mediates xylem translocation of both Cl$^-$ and nitrate (NO$_3^-$) anions. However, under abiotic stress conditions like water deficit or salinity (B), gene expression of AtSLAH1 is strongly inhibited by an abscisic acid (ABA)-dependent regulatory pathway. This favors the formation of SLAH3/SLAH3 homomers, which significantly reduces the Cl$^-$ conductance of SLAH3, decreasing xylem Cl$^-$translocation but maintaining xylem NO$_3^-$ translocation. The schematic representation describes the regulatory mechanism (Cubero-Font et al., 2016).

This is a consequence of two main reasons: Toxicity resulting from excessive Cl$^-$ accumulation in sensitive organs under salt stress conditions, and the widespread belief that Cl$^-$ and nitrate (NO$_3^-$) are antagonistic molecules. As a result, root Cl$^-$ uptake and accumulation occurs to the detriment of nitrate (NO$_3^-$) nutrition, an important source of nitrogen (N) for plants Fig. (2).

Both Nitrate and Chloride are belonged to essential nutrients element for plant growth. However, plants need chloride in small concentration for healthy growth of plants ranged between about 50 to 100 mol. in the nutrient solution and can be classified as a micronutrient (Broyer et al., 1954). Therefore, the role of effectiveness of chloride on plants, dealing with two extreme situations, either its function in plant physiology (as an essential micronutrient) or its toxicity particularly under salt stress circumstances. Recently, Cl$^-$ has been described as a beneficial element for the adequate development of plants when it is accumulated to macronutrient levels (Franco-Navarro et al., 2016; Wege et al., 2017). Many researches have reported that fertilization practices including chloride may increase yield crops production Xu, et al., (2000), until now the physiological processes affected, or to what extent the beneficial effects are Cl$^-$ specific or even associated with the accompanying cations was unclear. Therefore, it is necessary to expand our knowledge on: (A) The identification of the biological functions requiring macronutrient Cl$^-$ levels; (B) Degree of Cl$^-$ specificity in these processes; (C) Identification of genes encoding Cl$^-$ membrane transporters relevant for plant nutrition; (D) the signal-transduction pathways regulating Cl$^-$ nutrition processes and (E) Interaction between Cl$^-$ and NO$_3^-$ in vivo. Excellent reviews have been published regarding the origin and abundance of chlorine in the environment, its function as a mineral micronutrient for plants, the occurrence and effects of Cl$^-$ deficiency, its distribution in the plant, its toxicity under saline stress conditions, and the identification of genes involved in Cl$^-$ exclusion mechanisms (Raven, 2017; Marschner 2012). The review cover, new vision
the role of chloride as a beneficial macronutrient, for agriculture, besides the biological functions in which Cl⁻ is involved as a beneficial macronutrient and finally gene families incriminated in regulation of Cl⁻ transport, mainly in light of the recently identified genes, and their role in nutritional, biochemical, and stress-acclimatization functions.

Fig. 2: Illustrates effectiveness of Cl⁻ nutrition on plant growth, NO₃⁻ content, N uptake efficiency (NUPE), and N utilization efficiency (NUTE), in several species of agronomic interest. Plants were treated with two nutritional treatments: 5 mM Cl⁻ salts (CL) and a mixture of SO₄²⁻ + PO₄³⁻ salts (SP) containing the same cationic balance as in the CL treatment. Ratios of total biomass (A), NO₃⁻ content expressed as mg kg⁻¹ of fresh weight (B), NUPE (C), and NUTE (D) are presented considering the % of CL in relation to SP treatment and in contrast to leaf anion content in several species. Olive (Olea europaea L. ssp. europaea; bold cross), mandarin (Citrus reshni Hort. ex Tan; open triangles), tomato (Solanum lycopersicum L.; open circles), tobacco (Nicotiana tabacum L.; filled triangles), lettuce (Lactuca sativa L.; open diamonds); spinach (Spinacia oleracea L.; filled diamonds), and chard (Beta vulgaris L. ssp. vulgaris; gray-colored diamonds) After José et al., (2019).

1.1. Essentiality of chloride as micronutrient

Chloride is an essential cofactor for oxygen evolution of photosystem II (PSII) in the chloroplast, stabilizing the water splitting system at the oxidizing site of PSII. Two Cl⁻ molecules are required to maintain the coordination structure of the Mn Ca cluster (Raven, 2017). Kawakami, et al., (2009), Fig. 3.

Photosynthesis has a great potential in the field of Bioenergy (Conlan et al., 2007; Govindjee, et al., 2010). Hydrogen production from water splitting has been considered as an ideal fuel for the future (Pace 2005; Lewis, 2007). Water is the source of both oxygen and hydrogen, but energy is needed for hydrogen production by water splitting. Using sunlight, splitting of water into hydrogen and oxygen is one of the most important goals of some of the research on ‘artificial photosynthesis’ and this could be one of the solutions for future energy needs (Pace 2005; Daniel and Alessandro 2008). The development of a catalyst for water oxidation to evolve oxygen is an important key goal for a technology-based water splitting since the reaction involves a multi-electron transfer and is much more difficult due to thermodynamic and kinetic limitations (Bockris, 1977). Other strategies involve
attempts to employ not only energy obtained directly from the Sun, but also from the wind, ocean
currents, tides or waves for water splitting, some of which may turn out to be impractical or
uneconomical (Conlan et al., 2007). An efficient system for water oxidation has already evolved in
cyanobacteria, algae and plants (Allakhverdiev, 2011; Fromme and Grotjohann, 2011). The biological
water oxidation in the natural system is catalyzed by a CaMn$_4$O$_5$(H$_2$O)$_4$ cluster housed in a protein
environment in Photosystem II (PSII) that controls reaction coordinates, proton movement and water
access. The cluster is the only biological catalyst that could oxidize water to molecular oxygen, and it
appears that it has remained unchanged during 2 billion years of evolution (Allakhverdiev, 2011;
Renger 2007). In this review, we focus on the art of Nature to oxidize water for the production of
hydrogen. To evolve hydrogen in a sustainable manner, it is necessary to first synthesize a stable, low
cost, and efficient, environmentally friendly and easy to use catalyst for water oxidation (Bockris,
1977). The water oxidation half reaction in water splitting is both overwhelmingly rate limiting and
environmentally unacceptable for large-scale H$_2$ production as at this high voltage, other chemicals will
be oxidized (Pace 2005; Bockris 1977).

Thus, a significant challenge in the sustainable hydrogen economy is to design a ‘super anode’ for
water oxidation (Bockris, 1977). The role of such a super anode is not only for sustainable hydrogen
economy, but also for providing electrons for other reduction-reactions that are equally important in
artificial photosynthesis Pace (2005) Photosynthesis has a long history (Hill 2012; Rabino
witz 1945). Joseph Priestley (1733–1804) described the ability of plants to generate power to restore the air that had
been injured by the burning of candles. Carle Wilhelm Scheele (1742–1786) and Antoine Laurent
Lavoisier (1743–1794) identified this gas as oxygen. Chloride also regulates the activity of some enzymes such as the asparag
ine synthetase Rognes (1980), and the vacuolar proton-pumping ATPase (Churchill, and Sze, 1984).

Ge-Hong Sun-Wada et al., (2004), they reported that V-ATPase is structurally and evolutionarily
related to FATPase (ATP synthase), which is responsible for ATP synthesis in mitochondria,
chloroplast and bacteria, although the physiological roles of these two enzymes are completely different (Futai et al.,
1989). The V-ATPase consists of two major functional sectors known as V1 and Vo Fig. (4).

![Fig. 4: Subunit organization of V-ATPase and F-ATPase. The structures of V-ATPase and F-ATPase are schematically shown together with catalysis and proton transport. Membrane intrinsic (VO, FO), and peripheral (V1, F1) sectors, the catalytic hexamer, stalk regions and proton pathway are indicated.](image)

The V1 sector comprises at least eight different subunits (A–H). This sector contains three catalytic
sites for ATP hydrolysis formed from the A and B subunits. The VO sector containing up to five
subunits (a, c, c', cU and d) is responsible for proton translocation across the membranes (Anraku,
1996). A role of Cl$^-$ in regulating amylase activity has also been proposed (Metzler, 1979). To ensure
these cellular functions, only micromolar amounts of Cl$^-$ are required in glycophytic plants Fig. (5).
Franco-Navarro et al., (2016), reported that Cl\textsuperscript− is a strange micronutrient since actual Cl\textsuperscript− concentration in plants is about two orders of magnitude higher than the content required as essential micronutrient. This accumulation requires a high cost of energy, and since Cl\textsuperscript− is a major osmotically active solute in the vacuole, we propose that Cl\textsuperscript− plays a role in the regulation of water balance in plants. We show here that, when accumulated to macronutrient levels, Cl\textsuperscript− specifically regulates leaf cell elongation and water balance parameters, improving water relations at both the leaf tissue and the whole plant levels, increasing drought resistance in higher plants.

Accordingly, it has been generally accepted that the minimum Cl\textsuperscript− requirement for adequate plant growth in most plant species is in the range of 0.2–0.4 mg·g\textsuperscript−1 dry weight (mg·g\textsuperscript−1 DW) (Broyer et al., 1954; Marschner 2012). Johnson et al., (1957), Chloride is sufficiently abundant in nature to fulfil these requirements (White and Broadley, 2001).

1.3. Chloride as a salutary nutrient to plants

Spite the putative low requirements; the average of chloride is higher than the requirements of micronutrient concentration in plants (Xu et al., 2000). It is actually the most abundant inorganic anion in plant cells when this nutrient is available at concentrations present in most environments (Marschner, 2012). Unexpectedly chlorides content coincide with those reported as toxic elements for frequent plant species (Xu et al., 2000; White, and Broadley 2001; Marschner, 2012). Although average of chloride concentration in plants varied between 2.0 to 20.0 ppm, the critical level concentration of chloride in tissue particularly as toxicity level is about 4–7 and 15–35 ppm for sensitive and tolerant of glycophytes species, respectively. Thus, according to this traditional vision of plant Cl\textsuperscript− homeostasis, adequate plant development requires micronutrient Cl\textsuperscript− contents. However, plants accumulate about 10 to 100 times higher concentrations despite being toxic to many species. This vision implies that plants are unable to adequately regulate optimal levels of Cl\textsuperscript− and, therefore, the dominant homeostatic strategy should be the exclusion of this element Fig. (6).

Munns, et al., (2020) stated that high-energy phosphate containing molecules other than ATP could be used for energizing processes. Vacular pyro phosphatases (Vacular H\textsuperscript+-PPase, EC.3.6.1.1) pump protons across the tonoplast into vacuoles Gaxiola et al., (2016); Schilling et al., (2017), using pyrophosphate (PPi) as an energy source (Fig. 3). They work together with vacuolar H\textsuperscript+-ATPases to acidify the vacuole (Kriegel et al., 2015; Schilling et al., 2017). Plants with high expression of vacuolar H\textsuperscript+-PPases have significant abiotic stress tolerance, including salinity tolerance (Gaxiola et al., 2016; Schilling et al., 2017. Vacular H\textsuperscript+-PPases may be particularly important when ATP supply is limited during abiotic stress. A significant portion of vacuolar acidification may be generated by non-ATPase pathways and used by Na\textsuperscript+/H\textsuperscript+ and Cl\textsuperscript−/H\textsuperscript+ antiporters to sequester Na\textsuperscript+ and Cl\textsuperscript− in the vacuole as part of a tissue tolerance mechanism (Li et al., 2006; Kriegel et al., 2015; Nguyen et al., 2016). H\textsuperscript+-PPases are dependent on potassium ions (K\textsuperscript+), thus K retention in the cytoplasm under salinity may be critical for their function (Shabala et al., 2014). H\textsuperscript+-PPases have been shown more recently to be involved with
rapid mobilization of sugars and carbohydrates from source to sink tissue Pizzio et al., 2015 Gaxiola et al., 2016, and in faster metabolism of sugars in cells (Ferjani et al., 2012. Both processes will contribute to enhancing a cell’s energy budget.

Fig. 6: A variety of roles for the vacuolar proton-pumping pyro phosphatase. A generic plant cell showing the variety of ways the vacuolar proton pumping pyro phosphatase (H+ - PPase) can provide an alternative source of energy during salinity stress. (1) Vacuolar acidification. Localized to the tonoplast, the vacuolar H+ - PPase (blue) will use energy released from the hydrolysis of PPI to orthophosphate (Pi) to pump protons (H+) into the vacuole. Along with vacuolar ATPases (purple), vacuolar H+ - PPases establish an electrochemical potential for H+ across the tonoplast, which is used by other vacuolar transporters (red and brown) to sequester Na+ and Cl_ into the vacuole. (2) Removal of inhibitory pyrophosphate (PPI). Vacuolar H+ - PPases regulate PPI concentrations in the cytosol. Accumulation of PPI in the cytosol, particularly in younger tissues, can inhibit PPI-dependent metabolic pathways, such as gluconeogenesis and the Smirnoff-Wheeler pathway. (3) Enhancing sucrose transport from source to sink tissues. In phloem companion cells, H+ - PPases are shown to be localize to the plasma membrane, where it is hypothesized they synthesize PPI from orthophosphate. This additional PPI is used to enhance sucrose metabolism in these cells, thereby generating more ATP to pump protons into the apoplast that can be used by sucrose transporters, ultimately enhancing sucrose transport into sieve elements. Not all of these processes will be occurring in all cells at all times, and some may be cell-type specific. After Khadilkar et al., 2016 and Schilling et al., 2017.

The common view that currently exists about management of chloride in agriculture. Recent researches reported that prolonged application of fertilizers containing chlorides as impurities as low as (4 - 5 mM Cl-) resulted an accumulation of chloride in leaf tissues by about 25 and 50 mg.g-1 DW in different plant species. Although these chlorides content sometimes exceed the critical toxicity values mentioned above, these plants develop normally and grow without apparent symptoms of stress (Franco-Navarro et al., 2016; Brumós et al., 2010; Cubero-Font 2016). Root Cl- uptake and long-distance transport require a considerable use of metabolic energy Brumós et al., 2010, Felle (1994), Britto, et al., 2006, clearly indicating that shoot Cl- accumulation to macronutrient levels responds to specific biological adaptations From the chemical and physical characteristic of both sodium and potassium, Timothy et al., (2020) reported that, water molecules in the first hydration shell were found to have the same intramolecular geometries and dipole moments as those of the bulk. Furthermore, their dipoles were not aligned to the electric field produced by the ion, but quite tilted. The hydration number for the sodium was found to be five or six water molecules, whereas the potassium’s hydration number had a probability distribution ranging from five to ten. From an analysis of the energetic contributions of each hydration shell to the total enthalpy of hydration, we propose that the hydrated ions have a distinct behavior. Sodium has a stronger interaction with its first hydration shell than potassium, giving the latter a more flexible structure. From the above results, we can see that there are differences between the hydration of sodium and potassium. First, losing the second hydration shell is more costly for the former. Also the fact that for the latter the average interaction energy of first shell waters with the ion is very similar to that with the solvent leads to an orientational freedom and explains why the hydration number has a distribution so close to a Gaussian one Fig (7).
Fig. 7: Represents RDFs of solvated sodium and potassium ions in water with the revPBE-D3 and SCAN functionals demonstrating that the SCAN functional reproduces the experimentally observed peak position (Na\(^+\), K\(^+\)) and peak shape (Na\(^+\)) much more accurately and number of water molecules in the first hydration shell of the ion \((n(H_2O))\) After Timothy et al., (2020).

Cation hydration structure depicts the radial distribution function (RDF) for the sodium and potassium ions utilizing both revPBE-D3 and SCAN functionals. Significant differences are apparent. For Na\(^+\) we compared with the rescaled Na\(^+\)–O peak extracted from XRD of NaCl at 6 M20 and for K\(^+\) we compare with the experimentally determined peak position. As described by Kim, et al., (1995), Metroplis et al., (1953).

Timothy et al., (2020) stated that the ability to reproduce the experimental structure of water around the sodium and potassium ions is a key test of the quality of interaction potentials due to the central importance of these ions in a wide range of important phenomena. Here, we simulate the Na\(^+\) and K\(^+\) ions in bulk water using three density functional theory functionals: (a) the generalized gradient approximation (GGA) based dispersion corrected revised Perdew, Burke, and Ernzerhof functional (revPBE-D3) (b) the recently developed strongly constrained and appropriately normed (SCAN) functional (c) the random phase approximation (RPA) functional for potassium. We compare with experimental X-ray diffraction (XRD) and X-ray absorption fine structure (EXAFS) measurements to demonstrate that SCAN accurately reproduces key structural details of the hydration structure around the sodium and potassium cations, whereas revPBE-D3 fails to do so. However, we show that SCAN provides a worse description of pure water in comparison with revPBE-D3. RPA also shows an improvement for K\(^+\), but slow convergence prevents rigorous comparison. Finally, we analyse cluster energetics to show SCAN and RPA have smaller fluctuations of the mean error of ion–water cluster binding energies compared with rev PBE-D3.

Such an agreement supports the reliability with which our model can be used to understand the molecular processes involved in the hydration, specifically the differences between the two ions. The hydration numbers for both ions are concentration dependent. Therefore, it is very important to know this value at infinite dilution. Bernal-Uruchurtu, and Ortega-Blake (1995), they found that waters around monovalent cations are not completely aligned toward their electric fields, but rather tilted. This tilt is more defined for potassium than it is for sodium. We also see that none of the ions induces a further modification of the water geometry or dipole moment relative to the bulk. The hydration number of sodium is restricted to either five or six molecules, whereas revPBE-D3 fails to do so. However, we show that SCAN provides a worse description of pure water in comparison with revPBE-D3. RPA also shows an improvement for K\(^+\), but slow convergence prevents rigorous comparison. Finally, we analyse cluster energetics to show SCAN and RPA have smaller fluctuations of the mean error of ion–water cluster binding energies compared with rev PBE-D3.

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Several researchers ( Munns and Tester, 2008; Horie et al., 2012; Deinlein et al., 2014; Maathuis, 2014; Wu et al., 2015; Hanin et al., 2016; Wu et al., 2018a). Reported that traditionally, adverse effects of soil salinity have been attributed to with Na+ toxicity, prompting the majority of studies on this topic. However, an increase in Na+ content ( Munns and Tester, 2008; Wu, 2018) is always accompanied by Cl− accumulation ( Tavakkoli et al., 2010) and K+ loss ( Wu et al., 2018b) in plants exposed to salt (NaCl) stress. K+ is the major inorganic nutrient cation in non-halophytes (Dreyer and Uozumi, 2011), and plays important roles in plant cell activities (Anschütz et al., 2014; Shabala and Pottosin, 2014; Wu et al., 2018c) and stress responses ( Wang et al., 2013). Cl− is a plant micronutrient and regulates leaf osmotic potential, and turgor, and stimulates growth in plants ( Franco-Navarro et al., 2016). However high Cl− solutions are toxic, and impair photosynthesis and growth ( Tavakkoli et al., 2010; Tavakkoli et al., 2011). Specific reasons for these detrimental effects are much less understood than those of Na+, but the excessive accumulation of Cl− in chloroplasts is one effect ( Seemann and Critchley, 1985; Geilfus, 2018b). In recent years, the role of Cl− in plant salinity stress tolerance has attracted more attention. ( Bazihizina et al., 2019) reviewed the role of Cl− in halophytes. They suggest that rather than targeting Cl− exclusion, a better way to breed salt tolerant crops would be to improve the selectivity of the broadly selective anion-transporting proteins. Cl− as an essential micronutrient and its beneficial role in plants ( Raven, 2017; Wege et al., 2017), the role of Cl− in organelle development ( Geilfus, 2018a; Geilfus, 2018b), and control of Cl− transport in plants ( Li et al., 2017b) have been recently reviewed. Moreover, it is suggested nowadays that Cl− is a beneficial macronutrient for plants ( Franco-Navarro et al., 2016; Franco-Navarro et al., 2019). Unlike the abovementioned recent reviews, the present mini review is focused on the main traits related to controlling Cl− transport, and its role in plant salt tolerance.

Effectiveness of salinity on root dissection and its impact on the energy needed for plant salt tolerance, since plants must exclude nearly all the salt in the soil solution while taking up water with at least uptake of Na+ and Cl− in low amounts ( Munns et al., 2020). Three aspects that affect the ability of roots to exclude salt while taking up water, without exhausting the energy budget of the plant. Root type systems and their postmortem was affected by salinity stress, most important aspect of roots compared to shoots is that the stele tissues (xylem, phloem, pericycle and parenchyma cells) are internal, surrounded by a cortex and an epidermis (Fig. 6a–d). Epidermal cells may differentiate into root hairs Fig. 5). Shoots has stele tissues distributed throughout parenchyma cells. Evidence has emerged that the root cortex must do the heavy lifting of excluding Na+ from shoots ( Munns et al., 2020). Within this context, the large differences between cortex and stele anatomies are intriguing ( Varney et al., 1991; Watt et al., 2008, 2009). Large variation depends on root type (axile versus branch types), age and soil (Fig. 6a–d). The single root model underlies our current framework for salinity tolerance mechanisms (e.g. Fig. 5). This is generally an axile root from the embryo (seminal; Fig. 6a) or stem (nodal; Fig. 6d); branch roots are rarely considered ( Faiyue et al., 2012). The single root model greatly underestimates pathways for salts and water from soil to shoot, based on distances and cell sizes. In wheat, within 10 d of germination, the plant develops a system of different root types with branch roots (termed lateral or fine roots) that have emerged from an axile root (Fig. 8a or d). The complexity of the system increases with time: by the time of flowering, wheat roots below the topsoil can be 90% branch roots with the fine structure and anatomy shown in Fig. 8(c) ( Watt et al., 2008). Soil conditions, including high salinity, strongly influence allocation between axile and branch roots ( Rich and Watt, 2013).

Roots of wheat plant (durum) system were studied in a gradient of under salinity stress in order to imitator distribution under natural soil conditions ( Rahnama et al., 2011). Seminal axile root lengths in saline gradients were 25% less than those of the control, whereas branch length was 500% greater. The consequences of shifts to different root types could be large in terms of anatomy: in the saline gradient, 26% of total root length shifted to finer branch roots Fig. (6b,c), in addition, the branch roots emerged much closer to the axile tip (3 cm in saline conditions Compared with 20 cm in nonsaline). Salinity inhibits cell division in the primary roots of species including wheat and barley ( Rahnama et al., 2011; Shelden et al., 2013). Branch root initiation and extension was uninhibited by external salt ( Rahnama et al., 2011). Decreasing primary root length and allocating energy to the initiation of lateral roots may be linked to adaptation to salinity, a mechanism that also was seen by Zolla et al., (2010) in Arabidopsis. Branch roots arise from pericycle cells and water for elongation may come from the phloem ( Boyer et al., 2010). Salinity can promote differentiation of underlying xylem tissues in cotton ( Reinhardt and Rost, 1995).
Fig. 8: Changes in anatomy, root system and root cells that may be important in the energetics of salinity tolerance. (a–d) Cross-sections of wheat roots, all at the same magnification. Inset of (c), Arabidopsis primary root cross-section, shown at same magnification as wheat second-order branch root (bar, 50 μm; from Sotta and Fujiwara, 2017). (e) Speculation about importance of root hairs as a pidermal barrier to Na\textsuperscript{+} movement into the root. Left. Root hair of nodal root in (d) outlined to indicate surface area with soil. Right, enlargement of hair tip with hypothesized, drawn transport of vesicles (yellow) delivering Na\textsuperscript{+} to the outside of the cells (bar, 25 μm). Speculation and drawing based on the root hairs of sorghum, which can transport sorgoleone to the surface and to the soil in vesicles (see the subsection ‘Cell specializations at the epidermis including hair growth and functions’). (f) Schematic view of influence of salinity on a root system. Events: (1) shortening of primary root; (2) increased first-order branch root length; (3) branch root and xylem maturity closer to the tip; (4) increased rate of root aging. See text for references to original research for these events. (g) Modelled effects of either one or two cortical cell layers, and presence of an epidermal barrier, on energy costs of transmembrane transport. (h) Positive relationship between plasma membrane surface area outside an apoplastic barrier and energy cost. The Foster and Miklavcic (2017) model root geometry was adapted to simulate wheat roots with one or two cortical layers. For all simulations, the external medium contained 100mM NaCl, and a hydraulic pressure of 0.3 MPa was assumed at the top boundary of the root. The remaining simulation conditions were as described in Foster and Miklavcic (2017) for the nonuniformtransport scenario. C, cortex; PM, plasma membrane. After Munns et al., (2020).

Shabir et al., (2020) reported that both two ions Na\textsuperscript{+} and Cl\textsuperscript{−} are taken up by the outer root cells. At that time transported to the root xylem and finally from the root to the shoot, where they may be stored in vacuoles or in the apoplastic space, or possibly recirculated back to the root system or to older leaves that are less active Fig. (9).
Fig. 9: Summary of Na⁺ fluxes and transport in plants. Represented processes include those at the whole-plant level, in various tissues and tissues interfaces, as well as within cells. Ions, e.g. Na⁺, from the soil can enter the root cortex via apoplastic transport through cell wall spaces and intercellular cavities. Except for the branching zone (sites of lateral root formation) and the meristematic and elongation zones, the endodermis forms a barrier that stops the apoplastic flow of Na⁺ and forces all ions to move through the symplast into the xylem. With respect to root hairs, ions can enter the cytoplasm through specific channels and transporters, and are then transported via the symplast to the central vascular cylinder. Once loaded into the xylem, Na⁺ is transported to the shoot, where it is unloaded from the xylem into the shoot tissues and apoplast. It is still a matter of debated whether Na⁺ recycling can occur through the phloem back down to the root. Specific transport mechanisms are probably involved in excluding Na⁺ flow from the xylem towards back into cortex and from epidermis cells back into the soil. Redistribution of Na⁺ from young tissues and organs towards older ‘sinks’, organs that may be sacrificed, is another possibility that has been suggested as a salt tolerance mechanism. Processes or components are unproven at present are indicated by question marks.

Several researchers reported that, Cl⁻ may play definitive physiological appearance that increasing the dry matter and furthermore improving plant performance Franco-Navarro et al., (2016), Franco-Navarro et al., (2016). Broadley et al., (2012) stated that those elements that stimulate growth are defined as advantageous elements, but are not important in certain plant species, particularly under specific conditions. Where Chloride is not an important macronutrient, however it stimulates growth when accumulated to macronutrient levels. In addition, chloride has been defined as a beneficial macronutrient Franco-Navarro et al., (2016) Fig. (10).

Fig. 10: Represents chloride accumulated in tissues of tobacco plants subjected to level of chloride concentration, (A), distribution of chloride throughout the plant, reaching its maximum concentration in adult leaves, where it is stored in their large vacuoles. When subjected at lower concentrations, sufficient to meet micronutrient requirements but insufficient as a macronutrient (B), tobacco plants prioritize preferential the accumulation of chloride particularly in actively growing young leaves, indicating chloride has a biological role in plant cell growth. After José et al., (2019)

José et al., (2019) reported that, tobacco plants is gradually increased by application of chloride at a concentration of 50 mg.g⁻¹ DW which is represented a 5-fold of the critical toxicity threshold previously reported for this species (Xu et al., 2000). Therefore prolonged treatments below 5–10 mM Cl⁻ can determine high leaf accumulations with no stress symptoms and/or growth responses, shorter
salt stress treatments above 10–15 mM Cl- can produce symptoms of toxicity with relatively low leaf Cl- contents (Xu et al., 2000; Downton, 1985, Bar et al., (1997). This is indicative that moderate Cl-applications enable adequate transport and distribution of Cl- at the subcellular, organ, and whole-plant levels. Brumós et al., (2010), stated that different varieties of citrus treated with 4.5 mM chlorides particularly after 30 weeks were accumulated chloride between 150 and 425 mM in their leaf tissues with no symptoms of salt stress.

2. Movement of chloride in in relation to soil- water- root system

Cl moves through the soil system to root network systems up to xylem, symplastic transport is the key pathway for chlorides uptake, in a study involving the use of $^{36}$Cl as a tracer in the grapevine (Vitis vinifera) (Gong et al., 2011). The symplastic pathway has also been shown to dominate Cl transport in (CitrusBrumo’s et al., 2009). Once taken up into the cytosol, Cl follows its chemical gradient through the plasmodesmata from cell to cell towards the plasma membrane (PM) of the root xylem pole pericycle cells, which contribute to the xylem loading of Cl Cubero-Font et al., (2016) Fig. (11).

**Fig. 11:** Whole organism to single-cell multiscale view, emphasizing cell–cell connectivity via plasmodesmata (PD). a Schematic representation of Arabidopsis thaliana at flowering stage. b Two plant cells showing their cytoplasmic contents (nucleus in yellow, mitochondria in red, vacuole in grey, cell wall in coral, endoplasmic reticulum (ER) in blue, peroxisome in violet and ribosomes in purple, cytoplasm in light yellow, chloroplast in green) and displaying PD at their cell–cell interfaces. c PD are plasma membrane (PM; pink) lined, cell wall (coral) spanning pores that enable transport of molecules (red and light blue circles), mostly across the cytoplasmic sleeve (light yellow space). PD neck constriction via deposition of the wall polysaccharide callose (red) can reduce trafficking across the pores. The proposed model is through reduced ER–PM spacing (left side of panel c). Physical continuity of the endomembrane system (ER and PM) is also observable in panels’ b and c with the ER becoming highly constricted within PD and largely preventing lumenal transport of macromolecules. The colored arrows show examples of potential directional transports. Differences in PD densities between cellular interfaces (basal vs lateral sides in panel b) are also represented. Scale bars: 50 μm in (b) and 50 nm in (c). Abbreviations: ER, endoplasmic reticulum. PD, plasmodesmata. PM, plasma membrane.

Li et al., (2020) stated that plasmodesmata are intercellular pores connecting together most plant cells. These structures consist of a central constricted form of the endoplasmic reticulum, encircled by some cytoplasmic space, in turn delimited by the plasma membrane, itself ultimately surrounded by the cell wall. The presence and structure of plasmodesmata create multiple routes for intercellular trafficking of a large spectrum of molecules (encompassing RNAs, proteins, hormones and metabolites) and enable local signalling events. Movement across plasmodesmata is finely controlled in order to balance processes requiring communication with that necessitating symplastic isolation Fig. (12).
Fig. 12: Diagrams represent role of Chloride (Cl\textsuperscript{−}) (A), chloride move in root / shoot system (B) and its translocation in plant cells to a level that gradually significantly increases size of leaf cells, resulting in a reduction in stomatal density and, therefore, conductance (gs). At the same time, Cl improving mesophyll diffusion conductance to CO\textsubscript{2}, furthermore increasing surface area of chloroplasts exposed to the intercellular airspace. Higher mesophyll diffusion conductance compensates for the reduction in stomatal conductance, resulting in overall higher water use efficiency (WUE). After José et al., (2019).

We highlight the extensive and dynamic interactions that exist between the plasma/endoplasmic reticulum membranes, cytoplasm and cell wall domains, binding them together to effectively define plasmodesmata shapes and purposes.

After Cl is loaded into the xylem, it is pulled by the transpiration stream towards the aboveground organs (Köhler and Raschke 2000; Gilliham and Tester 2005). The protein-mediated uptake of Cl is influenced by the electrochemical gradient of Cl, which is composed of the Cl concentration gradient across the PM Sanders and Hansen (1981) and the difference in membrane potentials, which is negative on the inside, reaching -120 to -160 mV at the cytosolic side Sze et al., (1999) Fig. (13).

Figs. (13 a and b): Illustrate thermodynamics and mechanisms of Na\textsuperscript{+} and Cl\textsuperscript{−} transport at the soil-root and stellar cell–xylem-vessel interfaces in roots. Indicative cytosolic pH, ion concentrations, and voltages (Tester and Davenport, 2003; White and Broadley 2001. (a) Longitudinal section of wheat root provided by (Dr. Watt et al., 2008). The cells between the endodermis and the xylem vessel are not labeled, but include pericycle cells and xylem parenchyma (darker blue) as well as phloem parenchyma. The stele of dicotyledonous plants is more complex because it includes cambial vascular elements. The thermodynamics of ion movements are indicated by the arrow colors: Active transport is shown as a red arrow; passive transport is shown as a blue arrow. (b) The proposed mechanisms of passive and active Na\textsuperscript{+} and Cl\textsuperscript{−} transport at the two interfaces, mediated by ion channels and carriers (uniporters and H\textsuperscript{+}-coupled antiporters and symporters). Abbreviations: SOS1, salt overly sensitive mutant1; HKT, high-affinity K\textsuperscript{+} transporter (Munns and Tester, 2008).
Munns, and Tester, (2008), Stated that, thermodynamics of each of these processes for Na⁺ represented in Fig. (a), and molecular mechanisms are shown in Fig. (b). The thermodynamic analysis assumes that cytosolic Na⁺ concentrations of 30 mM and an electrical potential of 120 mM, but even if values differ by a factor of two, the principles remain unchanged. The xylem parenchyma, the efflux of Na⁺ from the cells would be active even if the xylem Na⁺ concentrations were nearly ten times lower than cytosolically were necessary with cytoplasmic free Na⁺ concentrations greater than approximately 100 mM (which, with an activity coefficient of 0.7, is a total concentration of around 140 mM). Another way to look at this is if the cytoplasmic free Na⁺ were 30 mM and the membrane potential difference were −60 mV, active influx would only be necessary with xylem apoplastic concentrations below 3 mM. Consideration of the thermodynamics of a Na⁺/H⁺ antiporter is simpler, because the electroneutral exchange this antiporters catalyzes is unaffected by membrane potential. Thus, the direction of Na⁺ movement is determined simply by the differences in free concentrations of Na⁺ and H⁺. A Na⁺/H⁺ antiporters could only work in the opposite direction to that indicated (i.e., it could only pump Na⁺ into cells) if, for a pH difference of one unit (xylem more acidic), the xylem concentration increased to 10 times that found in the cytoplasm (i.e., to over 300 mM for a cytoplasmic Na⁺ concentration of 30 mM). Alternatively, if the pH became more alkaline than pH 7.7, then the Na⁺/H⁺ antiporters could pump Na⁺ into xylem parenchyma cells from a free concentration of 10 mM. These conditions would rarely, if ever, occur, and thus, the Na⁺/H⁺ antiporter will mostly act to pump Na⁺ out of cells. The various processes of Na⁺ transport are each briefly considered here, but the reader is referred to the more extensive analysis of these processes in (Tester and Davenport, 127). Fluorescence resonance energy transfer based measurements obtained by using the recombinant anion indicator CLOMÈLEON have revealed cytosolic Cl concentrations of 10-15 mM in Arabidopsis root cells (Lorenzen et al., 2004; Saleh and Plieth 2013).

Teakle and Tyerman (2010) have reported that chloride concentrations were range from (5 to 20) mM, seems to be valid for many glycophytic plants not stressed excessively by Cl salts. Thus, under non-saline conditions, the chloride concentration in the cytosol is considered to be higher than that in the soil solution (i.e. 0.06- 0.25 mM; Parker et al., 1983), Brucher (2007) or in the apoplastic fluid (Shahzad et al., 2013). This phenomenon has led to the assumption that, under non-stressed conditions, Cl uptake is active because it accumulates against both the electrical and the chemical component of its electrochemical gradient. According this basis, chloride influx in barley roots Hordeum vulgare; Jacoby and Rudich (1980) and the common coral weed Chara coralline, Sanders and Hansen (1981) has been predicted to be actively mediated by co-transport with H⁺. Finally, Felle (1994) has presented electrophysiological evidence that the influx of Cl into the root hair cells of white mustard (Sinapis Alba) is energetically coupled to the movement of protons down their gradient, i.e. from the acid apoplast into the neutral cytosol. This symport functions with an estimated coupling ratio of (1 Cl : 2 H⁺). The gradient of pH across the membrane is maintained by the extrusion of H⁺ via the PM H⁺-ATPase ultimately energizing the activity of this Cl/2H⁺ symporters (Felle, 1994). Notably, the molecular details of this symporters have not been adequately resolved and the functional annotation of the underlying genes lags behind the electrophysiological evidence. Key message: symplastic transport is a relevant pathway for the radial movement of Cl across the root towards the xylem. Under non-Cl salinity conditions, Cl uptake into root cells (i.e. the root hair or root cortical cell) is active, because Cl accumulates against both components of its electrochemical gradient. A Cl/2H⁺ symport facilitate Cl influx Fig. (14).
2.1. Passive chloride influx under salinity conditions.

Uptake of chloride caused a kinetics change in their entirety particularly under conditions of salinity when external Cl concentrations exceed the cytosolic Cl concentration. This occurs because an increase of the Cl concentration outside the PM results in a membrane potential that is less negative than the Cl equilibrium potential, authorizing a passive Cl influx Tyerman and Skerrett (1998) Fig. (15). During this process Chloride influx into root cells is passive, being facilitated by anion channels (Hedrich 1994; Barbier-Brygoo et al., 2000. Skerrett and Tyerman (1994) have described an anion channel that mediates passive inward fluxes of Cl into the protoplasts of the root cells of wheat (Triticum aestivum) particularly under salinity conditions. This favors a rapid increase in cytosolic Cl. The molecular identity of this channel remains to be elucidated.

6-Control of Cl Xylem under loading and unloading process

The loading and unloading of the xylem with Cl is the rate limiting process that determines the Cl concentration in the shoot, including under salinity (Li et al., 2017). The plant endeavors to maintain the shoot Cl content below toxic concentrations by restricting xylem-driven root to shoot transport. This can be achieved either (i) by increasing the Cl efflux from the root cells back into the soil solution Sun et al., (2009), Li et al., (2016b) or (ii) by reducing the xylem loading and thus restricting acropetal transport (Teakle et al., 2007; Broumo’s et al., 2010; Li et al., 2016a). Research into Cl-sensitive glycophytes showing damage associated with the accumulation of excessive Cl, namely Lotus corniculatus Sanchez et al., (2011), Citrus rootstocks Broumo’s et al., (2010) and grapevine (Vitis vinifera) Henderson et al., (2014), clearly shows that reduced net Cl loading into the root xylem is the critical step that allows the better performance of crops in salt-affected soils.
Fig. 15: Diagram of Kinetics influx of chloride at two different NaCl concentrations. Flow through in vivo experiments with Arabidopsis thaliana plants grown on full MS were performed in a buffer system consisting of 5 mM MES/KOH (pH = 6.0). 100 mM and 150 mM NaCl were applied after 120 min.; Curves are averages from 4 independent experiments. The data were normalized by the mean of the time interval 5 min ≤ t ≤ 15 min. Error bars represent StDv. The red lines at the bottom indicate the two distinct phases: depolarization phase (DP, full line), saturation phase (SP, dotted line).

2.2. Chloride efflux from roots

The extrusion of Cl out of the root cells into the external solution, as described for the roots of the desert poplar Populus euphratica; Sun et al., (2009) and Arabidopsis Lorenzen et al., (2004), is an efficient mechanism for the avoidance of Cl accumulation in the plant during Cl salinity. Cl efflux is positively correlated with salt tolerance in many glycophytes, because the net uptake of Cl can be minimized by Cl efflux (Britto et al., 2004; Sun et al., 2009; Li et al., 2016b). A member of the nitrate excretion transporter (NAXT) subfamily, namely the nitrate transporter 1/peptide transporter (NRT1/PTR) family 2.5 protein (NPF2.5), has been found to be expressed in the PM of the root cortical cells, being up-regulated by NaCl (Li et al., 2016b). Arabidopsis T-DNA knockout mutants show a reduced Cl efflux from the root but accumulate more Cl in shoots during salt stress. This strongly suggests an involvement of NPF2.5 activity in plant salinity tolerance via the excretion of Cl out of the root cells into the soil solution. Further indications for candidate genes that are involved in Cl stress responses can be derived from transcriptomic studies on Citrus (Bruomo’s et al., 2009) and grapevine (Vitis spp.) genotypes that contrast in their degree of Cl accumulation in the shoot (Henderson et al., 2014). The putative grapevine anion channels VvNRT1.5 and VvNAXT1, which are both NPF proteins, are thought to be involved in Cl efflux from roots, because their transcripts are more abundant in the Cl-excluding rootstocks (Henderson et al., 2014). Recent results demonstrate that the Arabidopsis NRT1.5 protein is actually a proton-coupled H+/K+ antiporter that mediates K+ release from root parenchyma cells into the xylem (Li et al., 2017) Fig. (16).

Tegeder and Masclaux-Daubresse (2018), reported that nitrogen is an essential nutrient for plant growth. Worldwide, large quantities of nitrogenous fertilizer are applied to ensure maximum crop productivity. However, nitrogen fertilizer application is expensive and negatively affects the environment, and subsequently human health. A strategy to address this problem is the development of crops that are efficient in acquiring and using nitrogen and that can achieve high seed yields with reduced nitrogen input. Review integrates the current knowledge regarding inorganic and organic nitrogen management at the whole-plant level, spanning from nitrogen uptake to remobilization and utilization in source and sink organs. Plant partitioning and transient storage of inorganic and organic nitrogen forms are evaluated, as is how they affect nitrogen availability, metabolism and mobilization. Essential functions of nitrogen transporters in source and sink organs and their importance in regulating nitrogen movement in support of metabolism, vegetative and reproductive growth are assessed. Finally, plant engineering, demonstrating that nitrogen transporters are effective targets to improve crop
productivity and nitrogen use efficiency. Varieties of inorganic and organic N transporters with different substrate affinities and specificities have been identified in roots are presented in Fig. (16), illustrated that the presence of highly diverse of the uptake systems allows the plant root to adjust to fluctuations in soil N compositions and concentrations during the growing season, including under nutrient stress conditions. Different transporters as well as downstream N assimilatory processes are coordinated are currently unclear, but it may involve the presence of common regulatory systems.

Fig. 16: Schematic representation of nitrogen Chloroplast (N) root uptake and partitioning from root to leaves. Inorganic N transporters for nitrate (NO₃⁻; arrow with orange circle) and ammonium (NH₄⁺; green circle), as well as organic N transporters for amino acids (AA; yellow circle) and ureides (Ur; blue circle) are shown at root, nodule, xylem, phloem and source leaf levels. Arrows for the respective transporters either refer to uptake of N from the apoplast and import into the cell, or indicate cellular efflux. Listed are characterized Arabidopsis nitrate, ammonium, amino acid and ureide transporters that are involved in N (1) root uptake, (2) root efflux, (3) movement from the root or nodule to the xylem and xylem loading, (4) root reimport, (5) xylem removal and xylem–phloem transfer, (6) import into leaf mesophyll cells, (7) import into leaf vacuoles or (8) exchange across the chloroplast envelope. Question marks indicate unknown transporters. For details, see Sections I, II and III in the main text. The nomenclature of the nitrate transporter genes has recently changed (Leran et al., 2014); both the terms Nitrate Transporter/Peptide Transporter Family (NPF) and Nitrate Transporter1 (NRT1) are used in the figure. AAP, Amino Acid Permease; AMT, Ammonium Transporter; AVT, Amino Acid Vacuolar Transporter; CLC, Chloride Channel; DiT, Dicarboxylate Transporter; LHT, Lysine/ Histidine-like Transporter; NAXT, Nitrate Excretion Transporter; ProT, Proline and Glycine Betaine Transporter; UmamiT, Usually Multiple Acids Move In and Out Transporter; UPS, Ureide Permease. After Tegeder and Masclaux-Daubresse (2018)
2.3. Uptake of nitrate and ammonium by root

Low- and high-affinity nitrate transporters (Fan et al., 2017) mediate nitrate uptake from the soil. Nitrate transporters of the Nitrate Transporter1 (NRT1) family, recently renamed the Nitrate Transporter1/Peptide Transporter Family (NPF) (Leran et al., 2014), are low-affinity systems, with the exception of Arabidopsis NPF6.3/NRT1.1 and rice (Oryza sativa) NRT1.1B, which have both a low and high affinity for nitrate (Wang et al., 1998; Huang et al., 1999; Liu et al., 1999). Of 53 NPF/NRT1 proteins in Arabidopsis, 16 have been characterized so far. High-affinity nitrate transporters belong to the NRT2 family, seven of which have been studied (Fan et al., 2017). Members of the NPF/NRT1 and NRT2 transporter families are proton-coupled importers, except for the bidirectional NPF7.3/NRT1.5 transporter (Lin et al., 2008), and NPF2.7/Nitrate Excretion Transporter1 (NAXT1), which mediates nitrate efflux (Segonzac et al., 2007). Additional nitrate transporters are found within the Chloride Channel (CLC) family, which consists of either anion channels or anion proton exchangers (De Angeli et al., 2006) Fig. (17).

In Arabidopsis, at least six transporters are involved in root nitrate uptake Fig. (18). NPF6.3/NRT1.1 (also called Chlorate resistance Protein 1 (CHL1)) and NPF4.6/NRT1.2 mainly operate under high nitrate supply, while NRT2.1, NRT2.2, NRT2.4 and NRT2.5 function under nitrate starvation (Tsay et al., 1993; Huang et al., 1996, 1999; Liu et al., 1999; Cerezo et al., 2001; Filleur et al., 2001; Little et al., 2005; Orsel et al., 2006; Remans et al., 2006; Kiba et al., 2012; Lezhneva et al., 2014).

The four NRT2 transporters take up c. 95% of the total nitrate under limited N supply, with NRT2.1 and NRT2.2 being the main contributors (Lezhneva et al., 2014). Based on expression and localization studies, NRT2.4 and NRT2.5 seem mainly to be involved in direct nitrate acquisition from the soil via the epidermis and cortex at the root hair zone, while NRT2.1 and NRT2.2 additionally import apoplastic nitrate into cortical and endodermal cells (Kiba et al., 2012; Lezhneva et al., 2014). Nitrate transporters have also been functionally characterized in tomato (Solanum lycopersicum), rice and maize (Zea mays).
Because excess ammonium is toxic to plant cells, its uptake and assimilation are tightly regulated. Saturable high-affinity (i.e. Ammonium Transporters (AMTs)) and nonsaturable low-affinity uptake systems (i.e. aquaporins or cation channels) control ammonium transport and homeostasis in plants. Six AMT genes were found in Arabidopsis (Gazzarrini et al., 1999), 10 in rice (Sonoda et al., 2003), 14 in poplar (Populus trichocarpa) (Couturier et al., 2007) and 3 in pine (Pinus pinaster) (Castro-Rodriguez et al., 2016). In Arabidopsis, four AMTs function in ammonium root acquisition, with AMT1;1, AMT1;3 and AMT1;5 being involved in direct soil uptake via the epidermis (Loque et al., 2006; Yuan et al., 2007a). AMT1;2 is expressed in cortical and endodermal cells and mediates apoplastic absorption of ammonium. Collectively, AMT1;1, AMT1;2 and AMT1;3 import up to 95% of the ammonium. In rice, OsAMT1;1, OsAMT1;2 and OsAMT1;3 play a role in root ammonium uptake (Li et al., 2016). OsAMT1;1 and OsAMT1;2 expression is up-regulated in response to high ammonium concentrations, whereas OsAMT1;3 is expressed under N deprivation, suggesting its function in rice adaption to low-ammonium environments (Ferreira et al., 2015).

**Fig. 18:** Summary of spatiotemporal functionality of nitrate transporters/channels and nitrate transport routes in Arabidopsis. Nitrate is taken up by roots, loaded/unloaded by xylem and phloem, and transported to leaves, shoots and seeds. Arrows indicate the directions of nitrate movement. Transporters and channels are depicted according to their localization. After Guan (2017).

### 2.4. Uptake of amino acids by plant roots

Although peptides, proteins and other N-compounds are also taken up, research on root uptake of organic N has focused on amino acids Rentsch et al., (2007); Komarova et al., (2008); Paungfoo-Lonhienne et al., (2008). In Arabidopsis, over 100 putative amino acid transporters have been identified belonging to the Amino acid Polyamine Choline (APC) transporter superfamily, and the Usually Multiple Acids Move In and Out Transporters (UmamiT) family Rentsch et al., (2007); Pratelli and Pilot (2014). Most of the amino acid transporters characterized to date are localized to the plasma membrane and function in cellular import of a broad range of amino acids in co-transport with protons. Root uptake systems belong to three families within the APC group: the Amino Acid Permeases (AAPs), Lysine /Histidine-like Transporters (LHTs) and Proline and Glycine Betaine Transporters (ProTs). Generally, substrate specificity and affinity vary between and within the different transporter families. AAPs are considered moderate-affinity systems with broad substrate specificity. Arabidopsis AAP1 is involved in root uptake of glutamate and neutral amino acids Lee et al., (2007); Perchlik et al., (2014), and AAP5 functions in acquisition of basic amino acids Svennerstam et al., (2008), (2011). LHTs are assumed high-affinity transport systems. LHT1 imports neutral and acidic amino acids into the root Chen and Bush, (1997); Hirner et al., (2006); Svennerstam et al., (2007); Ganeteg et al., (2017), but also seems to transport the ethylene precursor 1-aminoacyclopropane-1-carboxylic acid (Shin et al., 2015). LHT6 acquires acidic amino acids, glutamine and alanine from the rhizosphere (Perchlik et al., 2014). Finally, Arabidopsis ProT2 functions in import of proline and glycine betaine, and root expression of ProT2 is up-regulated in salt stressed plants, suggesting increased uptake of the
compatible solutes under water deficiency Rentsch et al., (1996); Grallath et al., (2005); Lehmann et al., (2011) Fig. (19).

Fig. 19: Diagram illustrates proline metabolism in higher plants, which solid lines represents biosynthetic pathways while catabolic pathways are shown with dashed lines. BAC, basic amino acid transporter (for arginine and ornithine exchange); Glu, glutamate; G/P, mitochondrial glutamate/proline antipporter; KG, α-ketoglutarate; P, mitochondrial proline transporter; Pi, inorganic phosphate; ProT, proline transporter. After Szabados and Savoure (2010).

Shamsul Hayat, (2012) reported that under stressful conditions, plants may accumulate an array of metabolites, particularly amino acids, which traditionally been considered as precursors to and constituents of proteins, and play an important role in plant metabolism and development particularly under stress condition either salinity or drought. There is a positive correlation between proline accumulation and plant stress. Proline, an amino acid, plays a highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelators, an antioxidative defense molecule and a signaling molecule. Stressful of environment resulting an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants.

Several researchers Shamsul Hayat, (2012) Vogel and Davis (1952), reported that plants, proline is synthesized by two pathways viz. glutamate pathway and ornithine pathway. Glutamate pathway accounts for major proline accumulation during osmotic stress. The synthesized proline is from glutamic acid via intermediate Δ'-pyrroline-5-carboxylate (P5C). The reaction is catalyzed by Δ'-pyrroline-5-carboxylate synthetase (P5CS) and Δ'-pyrroline-5-carboxylate reductase (P5CR) as presented in Fig. (1). Aarzoo Qamar et al., (2015), reported that P5C, an N-substituted imino acid containing imino and carboxyl functional groups (IUPAC, 1997), is an intermediate not only in proline biosynthesis but also in its catabolism Fig. (20) P5C is synthesized from glutamate by pyrroline-5-carboxylate synthase P5CS; Hu et al., (1992) and then converted to proline by pyrroline-5-carboxylate reductase P5CR; Szoke et al., (1992); Hare and Cress, (1997) in cytosol and plastids. Proline is transported into mitochondria by membrane located transporters for its catabolism. Proline dehydrogenase (ProDH) catalyzes conversion of proline to P5C, which is then converted to glutamate by pyrroline-5-carboxylate dehydrogenase (P5CDH) in mitochondria Elthon and Stewart, (1981); Hare and Cress, (1997). In addition to proline catabolism by ProDH Boggess et al., (1978); Kiyosue et al., (1996), catabolism of arginine to ornithine by arginase ARG; Goldraij and Polacco, 2000). Later transamination of ornithine by delta-ornithine amino transferase (δOAT) also synthesizes P5C, Delauney et al., (1993); Roosens et al., (1998); Sekhar et al., (2007); Funck et al., (2008); Stránská et
al., (2008). P5C remains in rapid equilibrium with glutamate semi-aldehyde GSA; Vogel and Davis, (1952). This equilibrium is pH dependent and P5C form is favored over GSA at physiological pH of around 7.0 (Lewis et al., 1993; Bearne and Wolfenden, 1995).

Two genes encode Sekhar et al., (2007) P5CS whereas P5CR is encoded by only one in most plant species. Strizhov et al., (1997) Armengaud et al., (2004) Verbruggen et al., (1993) Proline catabolism occurs in mitochondria by means of the chronological action of proline dehydrogenase or proline oxidase (PDH or POX) producing P5C from proline and P5C dehydrogenase (P5CDH) which converts P5C to glutamate. Two genes encode PDH, whereas a single P5CDH gene has been identified in Arabidopsis and tobacco (Nicotiana tabacum) (Deuschle et al., 2001; Ribarits et al., 2007). PDH transcription is activated by rehydration but repressed by dehydration, thus preventing proline degradation during abiotic stress. Kiyosue et al., (1996), Verbruggen et al., (1996), an alternative pathway, proline can be synthesized from ornithine, which is transaminated to P5C by ornithine-δ-aminotransferase (Verbruggen et al., 1996).

**Fig. 20:** Model showing genes and pathways possibly involved in synthesis and catabolism of P5C in plant cell and their regulation in response to pathogen infection. Pyrroline 5-carboxylate (P5C) is the intermediate product of both biosynthesis and catabolism of proline. It is synthesized in mitochondria during catabolism of proline by enzyme proline dehydrogenase (ProDH1/2). We speculate that like their counterparts from bacteria and yeast, this enzyme reduces FAD+ to FADH and increases electron flow in mitochondrial electron transport chain (mETC). Arginine is converted into ornithine by arginase (ARG) enzyme. Another enzyme delta-ornithine amino transferase (δOAT) convert ornithine to P5C in mitochondria. P5C is catabolized by pyrroline 5-carboxylate dehydrogenase (P5CDH) in mitochondria into glutamate. In addition, P5C is synthesized in cytosol and chloroplast, from glutamate by pyrroline 5-carboxylate synthase 1 and 2 (P5CS1, P5CS2) and converted to proline by pyrroline 5-carboxylate reductase (P5CR). P5C and glutamate semi aldehyde (GSA) are non-enzymatically inter-convertible forms. Virulent pathogen infection in plants increases transcript accumulation of ProDH1. Avirulent pathogen infection increases transcript accumulation of P5CS2 and ProDH1. Non-host pathogen infection increases transcript accumulation of δOAT as well as ProDH1 and P5CS2. Three transporters located on plasma membrane are ProT1, ProT2 and ProT3. Arginine is imported into mitochondria directly and/or in exchange of ornithine by basic amino acid carrier (BAC1) and BAC2. P5CDH gene is down regulated post transcriptionally by natural siRNAs from similar to RCD one-5 (SRO5) genes. No direct evidence available for this pathway shown in green color during plant–pathogen interaction and this information is speculated based on evidence available under salt stress. Compounds shown in rectangle are important component of metabolism of P5C and line arrows indicates the direction of synthesis. Curved arrows show the transport of compounds. Transporters shown in circle are present on membrane. Block thick arrows show upregulation of genes, in which white arrow indicates virulent pathogen, dark arrows represent avirulent pathogen and striped arrows indicate non-host pathogen. ROS, reactive oxygen species. This model is predominantly based on information from Arabidopsis thaliana literature. After Aarzoo et al., (2015)
It has been suggested that the ornithine pathway is important during seedling development and in some plants for stress-induced proline accumulation. Armengaud et al., (2004) Roosens et al., (1998), Xue et al., (2009) Accumulation of proline has been suggested to contribute to stress tolerance in many ways. As proline acts as the molecular chaperon, it is able to maintain the protein integrity and enhance the activities of different enzymes. (Rajendrakumar et al., 1997). Numerous studies have reported that proline as an antioxidant suggesting its role as ROS scavenger and singlet oxygen quencher (Matysik et al., 2002; Smirnoff et al., 1989). Exogenous proline application reduces ROS levels in fungi and yeast, thus preventing programmed cell death, Xue et al., (2009) and prevents lipid peroxidation in algae cells exposed to heavy metals. Mehta et al., (1999) Pretreatment of proline also mitigated Hg²⁺ toxicity in rice (Oryza sativa) through ROS scavenging, such as H₂O₂ (Wang et al., 2009). Damaging effects of ROS on Photosystem II (PSII) can be reduced by proline in isolated thylakoid membranes (PSII). Alia PPS, Mohanty (1997). Internal proline content can be determined by biosynthesis, catabolism and transport between cells and different cellular compartments. The biosynthetic enzymes (P5CS1, P5CS2 and P5CR) are predicted to be localized in the cytosol whereas a mitochondrial localization is predicted for the enzymes involved in proline catabolism (such as PDH1/ERD5, PDH2, P5CDH and OAT). Shamsul Hayat, (2012) Intercellular transport of proline occurs between cytosol, chloroplasts and mitochondria as implied by compartmentalization of proline metabolism (Fig. 1). It has been reported that uptake of proline in mitochondria is an active process suggesting the existence of specific amino acid transporters, (Yu et al., 1983). These transporters have been identified in Arabidopsis thaliana Rentsch et al., (1996), in addition, in tomato pollen. Chen et al., (2001). At least three transporters (Pro T1, Pro T2 and AAP6) of proline were identified in Arabidopsis thaliana based on C-DNA technology, Rentsch et al., (1996).

These transporters belong to the amino acid permease (AAP) family and are expressed during stressful conditions. Pro T1 expresses ubiquitously but in Arabidopsis thaliana plants exposed to salinity stress, higher levels of Pro T1 were recorded in roots, stems and flowers. Young flowers showed highest expression, particularly in floral stalk phloem. Under water or salinity stress, strong expression of Pro T2 was recorded whereas, AAP6 transcripts were detected mainly in sink tissues (roots, cauline leaves) (Rentsch et al., 1996). In the halophyte species Limonium latifolium, proline was sequestered to vacuoles in non-stressed plants, whereas, high proline content was detected in the cytosol of salt-stressed plants, suggesting the importance of de novo proline biosynthesis as well as transport for proline accumulation (Gagneul et al., 2007). Proline metabolism has been studied for more than 40 y in plants, but little is known about the signaling pathways involved in its regulation. The proline biosynthetic pathway is activated and its catabolism repressed during dehydration, whereas rehydration regulates in the opposite direction. Strizhov et al., (1997), Deuschle et al., (2001) Verbruggen et al., (1993) Xue et al., (2009) Yoshiba et al., (1995) Chen et al., (2001) indicated that proline accumulation in detached rice leaves upon exposure to excess Cu was due to proteolysis and increased activities of Δ1-pyrroline- 5-carboxylate reductase or ornithine-ơ-amino transferase, which are enzymes of proline metabolism. It has also been revealed that Cu-induced proline synthesis and accumulation in detached rice leaves was mediated by ABA Chen et al., (2001) Zhang et al., (2008), reported that Cu-induced proline synthesis is associated with NO generation. In this study, the authors reported that exposure of Chlamydomonas reinhardtii to increasing concentration of Cu resulted in an increased synthesis of proline and a concomitant increase of intracellular NO levels. The authors argued that this intracellular NO generation was involved in Cu-induced proline accumulation and signaling and this theory was largely based on the fact that the application of sodium nitroprusside (a potent NO donor) increased the activity and transcript amount of P5CS (a key enzyme of proline biosynthesis) in Cu-treated algae which was blocked if a NO scavenger instead of NO donor was used (Zhang et al., 2008). Furthermore, it was reported in scenedesmus that the exogenous proline acts by detoxifying the ROS generated in response to the heavy metal (Cu or Zn) treatment rather than by improving the antioxidative defense system. (Tripathi and Gaur, 2004). Similarly, Wang et al. also demonstrated that the protective effect of proline against Hg toxicity in rice was through detoxifying ROS generated in response to metal treatment.

3. Chlorides improve nitrates uptake and (NUE) in plant

Nitrogen (N) is the main limiting nutrient for plants and classified as an essential macronutrient. Nitrate (NO₃⁻) represents the major N source and a signal molecule involved in the control of many
physiological and developmental processes, strongly improving crop yield (Frink et al., 1999; Wang et al., 2012; Krapp et al., 2014; Guan, 2017). The decisive role of N in crop yield has led to excessive use of NO$_3^-$ in agriculture over decades generating serious environmental problems like water pollution, which is harmful to people and nature (Nitrate Directive, 1991; Kant et al., 2011). In addition, when the application rate of NO$_3^-$ exceeds the plant growth needs, over accumulation of NO$_3^-$ in leaves reduces the nutritional quality of crops (Prasad and Chetty, 2008; Xing et al., 2019). Many large-leaved plants such as beets, cabbage, celery, lettuce, or spinach tend to store huge amounts of NO$_3^-$ MAFF, (1998), posing a serious risk to human health. When ingested, NO$_3^-$ is rapidly converted to nitrite and N-nitrous compounds as nitrosamines or nitric oxide causing methemoglobinemia or “blue baby syndrome” in infants and gastric cancer among other pathological disorders (Comly, 1945; Santamaria et al., 1999; Mensinga et al., 2003). Considering that the growing world population is predicted to reach 9.8 billion in 2050, global efforts are being made to increase food resources by improving crop or agronomic practices (Tilman et al., 2002; Godfray et al., 2010). Since plants use only 30–40% of the N applied to soil, a greater N use efficiency (NUE) could improve the yield and quality of crops, reducing economic costs as well as decreasing environmental degradation (Baligar et al., 2001). NUE can be defined as the vegetative or reproductive biomass yield per unit of N available in the soil (Moll et al., 1982; Woodend and Glass, 1993; Rios et al., 2010). This concept has many variants that can be split into two main elements: (a) N uptake efficiency (NUPE), defined as the capacity of plant roots to take N from soil, and (b) N utilization efficiency (NUTE), defined as the fraction of plant acquired N to be converted to total biomass or grain yield (Xu et al., 2012). Both are considered important traits in agriculture to reduce the abusive use of N fertilizers or when low N availability constrains plant growth, with substantial benefits for farmers and to the environment (Baligar et al., 2001; Han et al., 2016). Crops with higher NUE promote greater yields under limited N in soil, or require lower N to produce the same yield as those with lower NUE capacity (Ruiz et al., 2006; Kant et al., 2011; Rubio-Wilhelmi et al., 2012). Therefore, when NUE is increased, both crop-production costs and the harmful input of NO$_3^-$ into ecosystems are reduced.

Chloride (Cl$^-$) considered an essential micronutrient for plants (White and Broadley, 2001; Broadley et al., 2012). It has been uncovered as beneficial when accumulated to macronutrient levels in plant tissues Franco-Navarro et al., (2016); Raven, (2017); Wege et al., (2017); Colmenero-Flores et al., (2019), with new biological functions that improve tissue water balance, whole-plant water relations, photosynthesis performance, and water-use efficiency (Franco-Navarro et al., 2016,2019; Nieves-Cordones et al., 2019). Chloride represents the dominant inorganic anion in the vacuole, promoting cell osmoregulation, turgor-driven processes, leaf cell elongation, and a reduction in stomatal conductance (Franco-Navarro et al., 2016). In addition, Cl$^-$ specifically increases mesophyll diffusion conductance to CO$_2$ (gm) as a consequence of the greater surface area of chloroplasts exposed to the intercellular airspace of mesophyll cells, which in turn points towards Cl$^-$ playing a role in chloroplast conductance (Franco-Navarro et al., 2019). Thus, Cl$^-$ specifically reduces gas and water loss through transpiration without affecting the photosynthetic capacity due CO$_2$ (gm) stimulation, resulting in overall higher water-use efficiency (Franco-Navarro et al., 2016, 2019; Maron, 2019). Nitrate and Cl$^-$ are the most abundant inorganic anions, having similar physical and osmoregulatory properties and sharing transport mechanisms (Colmenero-Flores et al., 2019). This is probably the reason why NO$_3^-$ and Cl$^-$ show strong dynamic interactions in plants Wege et al., (2017), a phenomenon that has been described as a competitive interaction between these two monovalent anions. Different studies have reported a negative effect of Cl$^-$ on root NO$_3^-$ uptake and accumulation (Siddiqi et al., 1990; Cerezo et al., 1997; Xu et al., 2000). For this reason and because of the toxicity generated by excessive Cl$^-$ accumulation in sensitive crops under salt–stress conditions Li et al., (2017); Geilfus, (2018), Cl$^-$ has been considered detrimental to agriculture. Overall, Cl$^-$ is believed to reduce NUE by limiting NO$_3^-$ uptake and accumulation in plant tissues, reducing in turn its availability for plant metabolism (Xu et al., 2000; Anjana and Iqbal, 2007; Wege et al., 2017). However, Cl$^-$ is a non-metabolized anion readily accumulated in plant tissues, whose vacuolar sequestration requires a lower energy cost than the accumulation of NO$_3^-$ (Wege et al., 2017). Thus, considering the close interactions between these two anions, it has been hypothesized that preferential Cl$^-$ compartmentalization may reduce vacuolar NO$_3^-$ storage in leaves Flowers, (1988), allowing higher NO$_3^-$ availability for plant metabolism and, consequently, promoting more efficient use of this N source, and higher NUE (Colmenero-Flores et al., 2019).
3.1 Sequestration and partitioning of chloride under salinity condition

Under high chloride content, shoot chloride must be away from dividing cells and sites of primary photosynthesis, because cells cannot tolerate high concentrations of Cl (Tavakkoli et al., 2010; Teakle and Tyerman 2010). This can be achieved by inter- and intracellular compartmentation (Fricke et al., 1996; Teakle and Tyerman 2010). The intracellular sequestration of Cl into the vacuoles of roots Storey et al., (2003) or leaves is a strategy for controlling cytosolic Cl concentrations under conditions of Cl salinity (Britto et al., 2004; Li et al., 2017). Glycophytes can easily accumulate up to 40 mM Cl in the vacuole (Barbier-Brygoo et al., 2000; Wege et al., 2017). In this regard, a Cl/H+ antiport has been demonstrated at the tonoplast (Nakamura et al., 2006; Isayenkov et al., 2010; Nguyen et al., 2016). The soybean CLC family member GmCLC1 encodes an anion transporter that has putative Cl/H+ antiporter activity. This anion transporter has been localized to the tonoplast where it may sequester Cl into the vacuoles (Li et al., 2006). The activity of the GmCLC1 protein delays the accumulation of Cl in the cytosol under conditions of Cl toxicity (Wong et al., 2013). The overexpression of GmCLC1 in the hairy roots of soybean enhances salt tolerance because less Cl accumulates in the shoot because of its sequestration into the root vacuoles (Wei et al., 2016). AtCLCa is a further vacuolar anion/H+ antiporter protein that has been localized to the mesophyll tonoplast. AtCLCa is able to transport nitrate and Cl from the cytosol to the vacuolar lumen with high selectivity for nitrate over Cl (De Angelis et al., 2006; Wege et al., 2010). Despite the preference for nitrate, the AtCLCa protein activity may be relevant for vacuolar Cl compartmentalization under conditions when cytosolic Cl concentrations increase during Cl salinity. This might be the case because nitrate is not so abundant in the cytosol, immediately reduced to nitrite for metabolic assimilation, whereas Cl abundance is increasing in the cytosol during salinity (Lorenzen et al., 2004). Another member of the Cl channel family, AtCLCg, is located at the vacuolar membrane of mesophyll cells and contributes to an accumulation of Cl in vacuoles of NaCl-stressed leaves (Nguyen et al., 2016). AtCLCc is the third member of the CLC protein family in Arabidopsis but this protein has been localized to the tonoplast of guard cells. Unlike other guard cell anion channels such as SLAC1 and SLAH3 that exhibit a high permeability for nitrate over Cl Geiger et al., (2011), AtCLCc has a high selectivity for Cl over nitrate. During stomatal opening, AtCLCc is involved in vacuolar chloride accumulation in guard cell vacuoles in which Cl acts as an osmotically active anion facilitating guard cell swelling. Since it has not been localized in the mesophyll Kusumi et al., (2017), its role in the tissue sequestration of Cl is questionable. Nevertheless, the protein AtCLCc has been shown to be essential for stomatal movement and salt tolerance by regulating Cl homeostasis (Jossier et al., 2010).

The protein ALMT9, a member of the aluminum-activated malate transporter protein family, is located at the tonoplast of the leaf mesophyll, the leaf vasculature Barbier-Brygoo et al., (2011), the endodermis, the pericycle, the guard cells and the root vasculature Eisenach and De Angeli (2017), and it mediates Cl flux to the vacuolar lumen during salt stress. By these means, it helps in the maintenance of cytosolic ion homeostasis (Baetz et al., 2016). Therefore leaf partitioning is a strategy for keeping Cl below toxic levels at sites that expand or at the primary sites of photosynthesis (Bourrier and L’a’uchli, 1989). James et al., (2006) have found that Cl can accumulate in the cells of the epidermis, thereby avoiding high Cl concentrations in the mesophyll. In sorghum (Sorghum bicolor), Cl can be partitioned via the phloem in the leaf sheath in order to keep these toxic ions away from the photosynthetically active leaf blades (Bourrier and L’a’uchli, 1989). However, tissue sequestration or vacuolar ion inclusion are associated with the problem that, under conditions of incessant Cl uptake, internal storages sites may become filled. With regard to sodium toxicity, some evidence exists for a mechanism to recirculate sodium from the shoot back to the root via the phloem. This process includes phloem loading and unloading as mediated by the Arabidopsis high-affinity potassium transporter (HKT) protein 1;1 (AtHKT1;1) (Berthomieu et al., 2003; Tester and Davenport, 2003). The export of sodium from leaves via the phloem correlates with tolerance to salinity. However, in comparison with the transpiration-driven uptake of sodium, its re-translocation is relatively small (Munns, 2002). In contrast to sodium, no indication has been found that such a mechanism exists for Cl, although this cannot be excluded because thorough tests have not been carried out. A study on barley has indicated that phloem sap contains only 1/10th of the Cl that was delivered through the xylem, implying that phloem-based basipetal re-translocation is not effective in keeping shoot Cl concentrations low (Munns and Fisher, 1986). Furthermore, studies involving the use of 36Cl as a tracer have failed to demonstrate that Cl from the shoots of barley can be released into the external solution of their roots (Greenway et al.,
36Cl efflux from the roots into the external solution is known to be very low (Lessani and Marschner, 1978). Plant endeavors to maintain the shoot Cl concentration below toxic levels. The vacuole acts as a storage compartment to keep the cytosolic Cl concentration low. For some plants, the accumulation of Cl in the cells of the epidermis or leaf sheaths is a mechanism for avoiding high Cl concentrations in the mesophyll of photo synthetically active cells.

3.2. Anion competition with chloride under salinity stress and their physiological effects

The way in which high concentrations of Cl impede growth and cause toxicity has only been sparsely studied. Antagonistic Cl anion-anion competition with regard to the cellular uptake of nitrate and phosphate is considered to hamper growth and yield Fig. (21).

Fig. 21: Schematic of energy gain and energy use by a crop plant and performance of crops under salinity stress. (A) The proportion of energy used for maintenance, growth and stress defense is illustrated. The relative proportions will change depending on the developmental stage of the plant and exposure to salt stress maintenance costs will be greater when plants are larger. Total energy gain will decrease with greater salinity by decreasing photosynthetic rate following induced closure of stomata and damage to cellular and photosynthetic machinery. Stress tolerance mechanisms represent additional costs to the plant required to deal with the salt load in the soil (for example, but not limited to, greater costs in ion exclusion or compartmentation, maintaining ion homeostasis and reactive oxygen species (ROS) detoxification). At high salinity, there will be zero growth, as the total costs to the plant equal energy gain; when costs exceed energy gained, then tissue will senesce. (B) Response of a sensitive and a tolerant crop to soil salinity. Both crop types display response to salinity that can be grouped in phases: homeostasis maintains high growth rate, eustress elicits defense gene expression and dysstress causes stagnation and death. The salinity range is narrow in sensitive crops and broad in tolerant ones. The induction of defense in sensitive crops occurs early, with less magnitude. After Zörb et al., (2019).

Zörb et al., (2019) reported that thirty crop species provide 90% of our food, most of which display severe yield losses under moderate salinity. Securing and augmenting agricultural yield in times of global warming and population increase is urgent and should, a side from ameliorating saline soils include attempts to increase crop plant salt tolerance. It provides an overview of the processes that limit growth and yield in saline conditions. Yield is reduced if soil salinity surpasses crop specific thresholds, with cotton, barley and sugar beet being highly tolerant, while sweet potato, wheat and maize display high sensitivity. Apart from Na+, also Cl-, Mg2+, SO42- or HCO3- contribute to salt toxicity. The inhibition of biochemical or physiological processes cause imbalance in metabolism and cell signalling and enhance the production of (ROS) reactive oxygen species interfering with cell redox and energy state. Plant development and root patterning is disturbed, and this response depends on redox and reactive oxygen species signalling, calcium and plant hormones.

Under conditions of Cl salinity, proteins for cellular nitrate or phosphate uptake are thought to leak Cl because they cannot discriminate between Cl and nitrate (or phosphate). This missing selectivity for nitrate or phosphate over Cl may lead to a reduced uptake of nitrogen or phosphorus when external Cl concentrations increase excessively. Finally hampering growth and yield, as shown for tomato (Solanum lycopersicum; Papadopoulos and Rendig 1983) or wheat (Hu and Schmidhalter 1997), with the exception of maize (Zea mays L.) (Hu’tsch et al., 2016).

Chloride is the most recent addition to the list of essential elements. Many people make the common mistake of confusing the plant nutrient chloride (Cl-), with the toxic form chlorine (Cl). Chlorine is not the form that plants use. Chlorine exists either as a gas, or dissolved in water, such as bleach, and is not found in fertilizer. The gas form of chlorine is commonly used in municipal water treatment systems.
As chlorine gas reacts with water, hypochlorous acid (HOCl), hydrogen (H\(^+\)), and chloride (Cl\(^-\)) are formed. This reaction lowers the pH of the water. The change in pH depends on how much chlorine gas is injected and on the buffering capacity of the water. Chlorine gas is 100% available active chlorine. Only 1 pound (lb) of chlorine gas (Cl\(_2\)) is required to provide a 1-ppm concentration of Cl\(_2\) to 1,000,000 lb (120,000 gallons) of water. Similarly, an injection of 1 lb of chlorine gas per hour will provide a 1-ppm concentration of Cl\(_2\) to a water supply with a flow rate of about 2,000 gallons per minute (gpm). Although Chloride is classified as a micronutrient, plants may take-up as much Chloride as secondary elements such as Sulfur.

34. Function
Chloride is essential for many plant functions. Some of them are
- It is essential (working in tandem with K\(^+\)) to the proper function of the plants stomatal openings, thus controlling internal water balance.
- It also functions in photosynthesis, specifically the water splitting system.
- It functions in cation balance and transport within the plant.
- Research has demonstrated that Cl diminishes the effects of fungal infections in an, yet undefined, way.
- It is speculated that Cl competes with nitrate uptake tending to promote the use of ammonium N. This may be a factor in its role in disease suppression, since high plant nitrate have been associated with disease severity.

3.5. Factors affecting availability
Most soil chloride is highly soluble and is found predominantly dissolved in the soil water. Chloride is found in the soil as the Chloride anion. Being an anion it is fully mobile except where held by soil anion exchange sites (Kaolinite clays, Iron and Aluminum Oxides). In areas where rainfall is relatively high and internal soil drainage is good, it may be leached from the soil profile. In addition, where muriate of potash fertilizer is not regularly applied Chloride deficiencies can occur. Atmospheric Chloride deposition tends to be rather high along coastal regions and decreases as you progress inland. Chloride, nitrate, sulfate, boron, and molybdenum are all anions in their available forms, and in that form, they are antagonistic to each other. Therefore, an excess of one can decrease the availability of another. Little information is available on other specific interactions that may occur.

4. Deficiency and toxicity symptoms
Alfalfa, broccoli, brussel sprouts, cabbage, cauliflower, lettuce, oil palm, potato, small grains, sugar/table beets, and tomatoes. Wilting restricted and highly branched root system, is often with stubby tips. Leaf mottling and leaflet blade tip wilting with chlorosis has also been observed. Chloride insufficiency in cabbage is marked by an absence of the cabbage odor from the plant Fig. (22) illustrates deficiency symptoms in some plants.

Toxic symptoms are similar as is found with typical salt damage. Leaf margins are scorched and abscission is excessive. Leaf/leaflet size is reduced and may appear to be thickened. Overall plant growth is reduced. Chloride accumulation is higher in older tissue than in newly matured leaves Fig. (23). In conifers, the early symptoms are a yellow mottling of the needles, followed by the death of the affected needles Fig (24).

5. Chloroplastidial Cl homeostasis
Chloride toxicity-induced chlorosis and necrosis can be derived from results obtained with the common bean (Phaseolus vulgaris); Seemann and Critchley 1985) and field bean Vicia faba; Slabu et al., (2009), which accumulate Cl in the chloroplast Fig. (24).
Fig. 22: Illustrates some deficiency symptoms in some plants
Fig. 23: Illustrates some toxicity symptoms in some plants
Fig. 23: Illustrates some toxicity, and deficiency in conifers, trees the early symptoms are a yellow mottling of the needles, followed by death
This accumulation is correlated with a reduction in Chl content, possibly based on Chl degradation, which is presumably responsible for the reduced photosynthetic quantum yield Slabu et al., (2009), Tavakkoli et al., (2010). The degradation of Chl may explain the formation of chlorosis or necrosis. Studies on spinach (Spinacia oleracea) suggest that plants seek to control Chloroplastidial Cl homeostasis by excluding Cl, the major anion in the chloroplast of spinach, from being excessively taken up into the chloroplast when Cl Robinson and Downton 1984, Schröpple and Kaiser 1988 stresses the plant. Efforts by the plant to control Cl homeostasis in the chloroplast of spinach have also been observed under Cl deficiency. In spinach (Teardo et al., 2005) and Arabidopsis (Marmagne et al., 2007), the Cl channel AtCLCe is known to be anchored in the thylakoid membrane. It has been suggested to function in the homeostatic control of Cl (Herdean et al., 2016a). The loss of the homeostatic control, i.e. the excess accumulation of Cl in the chloroplast, is anticipated to cause multiple.

5.1. Suitability of chloride for plant production.

The idea that only small amounts of Cl are required for optimal plant growth and that naturally occurring Cl-levels amply meet crops requirements still underlies the agronomic and even the scientific fields (Geifus, 2018). However, according to the findings described above, crops could benefit from Cl fertilization more broadly than is generally believed. The amount of Cl fertilization required to ensure beneficial macronutrient requirements would depend on the levels naturally present in the soil and on the specific necessity of the cultivated crop. In inland regions, far from the ocean, the low deposition of Cl, a highly mobile molecule subject to leaching in the soil, can limit the yield of crops (Fixen, 1987). Substantial responses to Cl-containing fertilizers have been reported for different crops in many parts of the world (Xu et al., 2000; Chen et al., 2010). However, most of these studies did not clarify to what extent plant yield enhancement was due to the accompanying cations, or whether other anions could replace Cl in such a growth-promoting effect. It has been recently proven that a number of physiological disorders impairing the growth and yield of durum wheat under field conditions are specifically due to soil Cl deficiency (Schwenke et al., 2015). For reasons still unknown, some plant species such as kiwi fruit Smith et al., (1987) and palm trees Bracconier (1990) have higher Cl requirements, which cannot be alleviated through NO₃ addition. These plants can be valuable models for better understanding of the regulation of Cl homeostasis in higher plants (Wege et al., 2017). For example, coconut plants appear to have greater dependence on Cl for proper regulation of stomatal function, since stomatal opening is delayed by about 3 h in Cl-deficient plants (Xu et al., 2000).

Interestingly, guard cells of another palm tree, Phoenix dactylifera, release Cl rather than NO₃ during stomatal closure, while NO₃ is required as a signal molecule to trigger the abscisic acid (ABA)-dependent response (Mueller et al., 2017). This clearly demonstrates that full understanding of Cl-homeostasis in higher plants requires going beyond of model plant species. Watanabe et al., (2007) reported leaf Cl concentrations for 670 species from 138 families of terrestrial seed plants collected from their natural habitats. The most frequent plant Cl content reported was around 5 mg g⁻¹ DW, which is below the beneficial range of Cl nutrition. This suggests that plants might frequently benefit from Cl fertilization in many environments. In the agronomic context, Cl-deficient soils can be identified in terms of plant growth for important crops like coconut, oil palm, wheat, durum wheat, and maize, (Xu et al., 2000 and Raven 2017). Therefore, these and most probably other species are favored by Cl-
fertilization, which is expected to improve plant performance and crop yield. In addition, given the close correlation between Cl- homeostasis and NUE adequate management of optimal NO$_3$/Cl$^-$ ratios in different agriculture systems could reduce NO$_3$ input rates without compromising plant performance (Inal et al., 1998). Chloride-dependent reduction of plant NO$_3$ accumulation in vegetables could also be used as a strategy to decrease excessive NO$_3$ content. Vegetables are classified as NO$_3$ accumulators Maynard et al., (1976) and the NO$_3$ metabolic derivatives nitrite and nitrosamines are well-known risk factor for human health (European Food Safety Authority, 2008).

5.2. Ion transport, stomatal response, and water use efficiency (WUE).

There is evident that stomatal movements of seed plants, including crop plants, arise from the transport, accumulation, and release of osmotically active solutes. A very large body of experimental evidence supports the collective role of ion transport across the plasma membrane and tonoplast in both stomatal opening and closing Willmer and Fricker, (1996); Blatt, (2000); Chen et al., (2012c); Hills et al., (2012) Fig (25).

The primary inorganic species transported are K$^+$ and Cl$_2$, which, with the organic anion malate22 (Mal) and Suc, comprise the bulk of solute that drives water flux and guard cell turgor (Willmer and Fricker, 1996; Roelfsema and Hedrich, 2005; McAINsh and Pittman, 2009). Because mature guard cells lack functional plasmodesmata Wille and Lucas, (1984), these solutes must be transported across the plasma membrane. Much of this solute uptake must also be transported across the tonoplast. The guard cell vacuole, like that of most mature plant cells, makes up the bulk of the cell volume and, hence, plays a very important role as a “repository” for osmotically active solutes (Gao et al., 2005; MacRobbie, 2006; Chen et al., 2012c). Mal metabolism (notably its synthesis within the guard cell cytosol) makes a substantial contribution to the osmotic content of the guard cell, while Mal loss during stomatal closure occurs largely via efflux across the plasma membrane (Willmer and Fricker, 1996; Wang and Blatt, 2011). Stomata determining plant productivity and water use efficiency.

Stomatal regulation consists of much more than guard cell signaling, however. It also involves tissue- and leaf-scale biophysical factors that translate guard cell function into changes in stomatal conductance Fig. (26). For example, the water potential of guard cells may be affected by vapor exchange with relatively dry air within the stomatal pore channel (Peak and Mott, 2011), or with relatively moist air in the airspaces between sun-warmed mesophyll cells (Pieruschka et al., 2010). Water status may be actively sensed in guard cells Bauer et al., (2013), or in other tissues such as mesophyll (McAdam and Brodribb, 2018) or phloem companion cells Endo et al., (2008), which experience different degrees of water stress. Understanding of stomatal function in intact leaves thus rests not only on guard cell biology, but also on features of leaf and plant biophysics such as finescale gradients in temperature and water potential. (Buckley, 2019). Stomatal responses to humidity, soil moisture and other factors that influence plant water status are critical drivers of photosynthesis, productivity, water yield, Ecohydrology and climate forcing, yet we still lack a thorough mechanistic understanding of these responses. Here I review historical and recent advances in stomatal water relations. Clear evidence
now implicates a metabolically mediated response to leaf water status (‘hydro active feedback’) in stomatal responses to evaporative demand and soil drought, possibly involving abscisic acid production in leaves. Other hypothetical mechanisms involving vapor and heat transport within leaves may contribute to humidity, light and temperature responses, but require further theoretical clarification and experimental validation. Variation and dynamics in hydraulic conductance, particularly within leaves, may contribute to water status responses. Continuing research to fully resolve mechanisms of stomatal responses to water status should focus on several areas: validating and quantifying the mechanism of leaf-based hydro active feedback, identifying where in leaves water status is actively sensed, clarifying the role of leaf vapor and energy transport in humidity and temperature responses, and verifying foundational but minimally replicated results of stomatal hydromechanics across species. Clarity on these matters promises to deliver modelers with a tractable and reliable mechanistic model of stomatal responses to water status.

Fig. 26: Stomatal conductance is regulated not only by guard cell biology, which governs guard cell osmotic content, but also by numerous biophysical factors that influence guard and epidermal cell water potentials and link these cells to other tissues across the leaf and plant. Abscisic acid and other signaling compounds may be synthesized in guard cells or synthesized elsewhere and transported to guard cells (Sections III.1, III.2), but may have little impact on stomata in some species (Section III.3). The potential ABA source tissues are located at different positions along the soil–plant–atmosphere continuum, and are thus differentially sensitive to soil drought and evaporative demand (Section III.5). Guard cells may or may not exchange vapor with air in the stomatal pore channel and liquid water with epidermal cells (Section III.4), and heat and liquid water may move in either direction between the mesophyll and epidermis (Sections III.4, III.5). After Buckley, (2019).

The balance between these two processes depends on stomatal responses to environmental and internal cues and the synchrony of stomatal behavior relative to mesophyll demands for CO₂. Here we examine the rapidity of stomatal responses with attention to their relationship to photosynthetic CO₂ uptake and the consequences for water use. We discuss the influence of anatomical characteristics on the velocity of changes in stomatal conductance and explore the potential for manipulating the physical as well as physiological characteristics of stomatal guard cells in order to accelerate stomatal movements in synchrony with mesophyll CO₂ demand and to improve water use efficiency without substantial cost to photosynthetic carbon fixation.
We conclude that manipulating guard cell transport and metabolism is just as, if not more likely to yield useful benefits as manipulations of their physical and anatomical characteristics. Achieving these benefits should be greatly facilitated by quantitative systems analysis that connects directly the molecular properties of the guard cells to their function in the field. In order for plants to function efficiently, they must balance gaseous exchange between inside and outside the leaf to maximize CO$_2$ uptake for photosynthetic carbon assimilation (A) and to minimize water loss through transpiration. Stomata are the “gatekeepers” responsible for all gaseous diffusion, and they adjust to both internal and external environmental stimuli governing CO$_2$ uptake and water loss Fig. (27). The pathway for CO$_2$ uptake from the bulk atmosphere to the site of fixation is determined by a series of diffusional resistances, which start with the layer of air immediately surrounding the leaf (the boundary layer). Stomatal pores provide a major resistance to flux from the atmosphere to the substomatal cavity within the leaf. Further resistance is encountered by CO$_2$ across the aqueous and lipid boundaries into the mesophyll cell and chloroplasts (mesophyll resistance). Water leaving the leaf largely follows the same pathway in reverse, but without the mesophyll resistance component. Guard cells surround the stomatal pore. They increase or decrease in volume in response to external and internal stimuli, and the resulting changes in guard cell shape adjust stomatal aperture and thereby affect the flux of gases between the leaf internal environment and the bulk atmosphere. Stomatal behavior, therefore, controls the volume of CO$_2$ entering the intercellular air spaces of the leaf for photosynthesis. It also plays a key role in minimizing the amount of water lost. Transpiration, by virtue of the concentration differences, is an order of magnitude greater than CO$_2$ uptake, which is an inevitable consequence of free diffusion across this pathway. Although the cumulative area of stomatal pores only represents a small fraction of the leaf surface, typically less than 3%, some 98% of all CO$_2$ taken up and water lost passes through these pores. When fully open, they can mediate a rate of evaporation equivalent to one-half that of a wet surface of the same area (Willmer and Fricker, 1996). Early experiments illustrated that photosynthetic rates were correlated with stomatal conductance (gs) when other factors were not limiting (Wong et al., 1979). Low gs limits assimilation rate by restricting CO$_2$ diffusion into the leaf, which, when integrated over the growing season, will influence the carbohydrate status of the leaf with consequences for crop yield. Stomata of well watered plants are thought to reduce photosynthetic rates by about 20% in most C3 species and by less in C4 plants in the field (Farquhar and Sharkey, 1982; Jones, 1987). However, even this restriction has been shown to impact substantially on yield. For example, Fischer et al., (1998) demonstrated a close correlation between gs and yield in eight different wheat (Triticum aestivum) cultivars. Those studies highlighted the effects gs can have on crop yield, not only through reduced CO$_2$ diffusion but also through the impact on water loss and evaporative cooling of the leaf. Indeed,
enhancing photosynthesis yields by only 2% to 3% is sufficient to substantially increase plant growth and biomass over the course of a growing season (Lefebvre et al., 2005; Zhu et al., 2007).

There are several approaches for improving carbon gain and plant water use efficiency (WUE) that focus on stomata. It is possible to increase or decrease the gaseous conductance of the ensemble of stomata per unit of leaf area (gs) through the manipulation of stomatal densities (Büssis et al., 2006). In addition, there is potential to alter the stomatal response or sensitivity to environmental signals through the manipulation of guard cell characteristics that affect stomatal mechanics (e.g. OPEN STOMATA [ost] mutants; (Merlot et al., 2002). Such approaches have produced an array of mutant plants with altered characteristics and varying impacts on CO₂ uptake and transpiration. An intuitive measure of the efficacy of such manipulations is the WUE, commonly defined as the amount of carbon fixed in photosynthesis per unit of water transpired. In general, higher WUE values have been observed in plants with lower gs, but these gains are usually achieved together with a reduction in A and slower plant growth. Plants with higher gs have greater assimilation rates and grow faster under optimal conditions, but they generally exhibit lower WUE. An approach that has not been fully explored or considered in any depth is to select plants for differences in the kinetics of stomatal response or to manipulate stomatal kinetics in ways that improve the synchrony with mesophyll CO₂ demand (Lawson et al., (2010, 2012). To date, the majority of studies assessing the impact of stomatal behavior on photosynthetic carbon gain have focused on steady-state measurements of gs in relation to photosynthesis. These studies do not take account of the dynamic situation in the field. As we discuss below, a cursory analysis of stomatal synchrony with mesophyll CO₂ demand suggests that gains of 20% to 30% are theoretically possible.

Turgor plays an essential role in regulating plant morphology, architecture, and movement, generating tension within the rigid cell wall by appressing the plasma membrane and maintaining stable internal pressure at the cellular level. Changes in the turgor of guard cells are responsible for stomatal movements (Heath, 1947; Daloso et al., 2016 a,b, De Angeli et al., 2013). Turgor changes involve the loss or accumulation of K+, accompanied by a parallel exchange of anions (e.g., Cl⁻ and malate) and organic solutes (e.g., sucrose) (Willmer and Fricker, 1996; Lee et al., 2008; De Angeli et al., 2013). Metabolism of carbohydrates in plants and fungal cells plays an important role in maintaining turgor pressure through the production of osmoactive molecules (Talbott and Zeiger, 1993; Kelly et al., 2013; Daloso et al., 2016 a,b).

Metabolomic analyses indicate that the treatment of Arabidopsis guard cells with abscisic acid (ABA) results in a significant decrease in the concentration of signaling-related metabolites, such as sucrose and malate Kelly et al., (2013); Three distinct osmoregulatory pathways are thought to be involved in the opening of stomata: uptake of K⁺ and Cl⁻ coupled with the biosynthesis of malate, accumulation of sucrose from the breakdown of starch and accumulation of the products of photosynthetic carbon fixation within the guard cells (Amodeo et al., 1996; Talbott and Zeiger, 1996; Lawson et al., 2014). Malate plays an important role in stomatal opening, and ABA mediated stomatal closure is often accompanied by a decrease in malate concentration within the guard cells (Dittrich and Raschke, 1977). In daylight, most malate in guard or mesophyll cells is produced via carboxylation of phosphoenolpyruvate (PEP). Specifically, light-stimulated PEP carboxylase catalyzes the carboxylation of PEP to produce oxaloacetate (OAA) in the cytosol, which is then further converted to malate via the action of NADP-dependent malate dehydrogenase (NADP-MDH or NAD-MDH) (Tcherkez et al., 2005). AtALMT9 (aluminum-activated malate transporter 9) is a malate-activated vacuolar chloride channel required for stomatal opening in Arabidopsis (De Angeli et al., 2013). Another ion channel, AtALMT4, mediates malate₂⁻ release from the vacuole, and is required for stomatal closure in response to ABA Eisenach et al., (2017). Sucrose and malate are important substrates for energy production through glycolysis and the tricarboxylic acid (TCA) cycle. Malate also functions as an essential carbon storage molecule (Martinoia and Rentsch, 1994; Fernie and Martinoia, 2009). No other metabolic pathways are known to supply osmolytes for cellular osmoregulation Fig. (28). Danilo et al., (2017) reported that Stomata are leaf epidermal structures consisting of two guard cells surrounding a pore. Changes in the aperture of this pore regulate plant water-use efficiency, defined as gain of C by photosynthesis per leaf water transpired. Stomatal aperture is actively regulated by reversible changes in guard cell osmolyte content. Despite the fact that guard cells can photosynthesize on their own, the accumulation of mesophyll-derived metabolites can seemingly act as signals that contribute to the regulation of stomatal movement.
During stomatal opening, the accumulation of malate inside the guard cell occurs mainly due to the influx from the apoplast through the AtABCB14 (1), guard cell photosynthesis, mitochondrial activity and possibly from blue-light-induced starch and lipid breakdown. Cytosolic light-induced PEPc can catalyze the carboxylation of PEP yielding OAA, which is further reduced to malate via NAD+ -malate dehydrogenase (NADP-MDH). Malate is additionally transported into vacuoles through the channels AtALMT6 (2), AtALMT9 (3) and possibly through the transporter AtDT (4). In the guard cells, malate acts as an osmoregulator and counter ion for K+ allowing the water inlet, and finally the stomatal aperture. Furthermore, cytosolic malate can increase the Cl- currents through AtALMT9 (3), which is permeable to both Cl- and malate. By contrast, during stomatal closing, the malate previously accumulated is released to the apoplastic space through AtQUAC1 (5), or it can be metabolized via the decarboxylation by NADP+-malic enzyme (NADP+-ME) or via NADP+-MDH yielding pyruvate, which can be further metabolized through the TCA cycle and oxalacetate that can be partially converted into starch (not presenting osmotic activity) via the gluconeogenesis pathway. ALMT, Aluminium-activated malate transporters; CBC, Calvin–Benson cycle; Hex-P, hexose phosphate; Mal, malate; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxalacetate; PEP, phosphoenolpyruvate; PEPc, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; QUAC1, quick activating anion channel; Suc, sucrose; TAG, triacylglycerols; tDT, tonoplast dicarboxylate transporter. After Danilo et al., (2017).

It has been shown that malate can act as a signalling molecule and a counter-ion of potassium, a well-established osmolyte that accumulates in the vacuole of guard cells during stomatal opening. By contrast, their efflux from guard cells is an important mechanism during stomatal closure. It has been hypothesized that the breakdown of starch, sucrose and lipids is an important mechanism during stomatal opening, which may be related to ATP production through glycolysis and mitochondrial metabolism, and/or accumulation of osmolytes such as sugars and malate. However, experimental evidence supporting this theory is lacking. Here we highlight the particularities of guard cell metabolism and discuss this in the context of the guard cells themselves and their interaction with the mesophyll cells.

6. Conclusion

The levels at which Cl- accumulates in plants, typical of a macronutrient, and the consequent improvement of plant performance have led to its designation as beneficial macronutrient. Tobacco plants with macronutrient Cl- levels display more effective use of water, nitrogen, and carbon/energy. Significant WUE improvement results from concurrent stimulation of growth and reduction of water use. Despite the Cl- -dependent improvement of water balance and water relations, a specific consequence of Cl- on root hydraulic conductivity is an issue yet unresolved. WUE enhancement by macronutrient Cl- nutrition probably increases the ability of plants to withstand water deficit, a hypothesis that must be explored. Although increased dry biomass of Cl- -treated plants clearly points to a more efficient use of N, direct evidence is still required to confirm whether Cl- improves NUE. Cl- must also clarify it if NUE is favored by an efficient compartmentalization of NO3- or because of NO3- replacement in the vacuole. The positive effect of Cl- on chloroplast performance may be due to various factors like the regulation of thylakoid swelling, improved photosynthetic electron transport, and photo
protective mechanisms. More research is required to clarify these issues, as well as whether Cl\(^{-}\) stimulates chloroplast biogenesis. Chloride promotes cell elongation because of better and "cheaper" osmoregulatory and turgor-generating ability. That is apparently the reason why auxin stimulates the influx of Cl\(^{-}\) into plant cells and why ABA may have the opposite effect. It is therefore important to confirm these points and to accurately determine the signaling pathways that regulate these processes, as well as the inhibition by ABA of ion xylem translocation. We expect that many crops could benefit from Cl\(^{-}\) fertilization to a higher extent than previously believed, resulting in the improvement of crop performance, stress resilience, and yield. Further research is also required to clarify the role of Cl\(^{-}\)- in vesicular trafficking and to confirm whether Cl\(^{-}\) homeostasis regulates the plant immunity response. The recent identification of transporters involved in cell Cl\(^{-}\) influx will give a decisive boost to a better understanding of Cl\(^{-}\) nutrition. For this reason, it will be very important to identify which residues determine Cl\(^{-}\) selectivity in the substrate-binding pocket of NPF proteins.

Natural variability of the Cl\(^{-}\) inclusion/exclusion rates exhibited by different plant species and varieties suggests the occurrence of an array of genes and alleles responsible for different NO\(^{3}\) /Cl\(^{-}\) selectivities (as observed for different members of the NPF and CLC families). Differential NO\(^{3}\) /Cl\(^{-}\) selectivities are expected to occur at different levels: The root soil, the symplast–xylem, and the cytosol vacuole interfaces. Characterization of Cl\(^{-}\) channels implicated in releasing Cl\(^{-}\) to the rhizosphere, which fine-tunes net Cl\(^{-}\) uptake in the root, is another issue that requires attention. Genes encoding these channels, as well as R-type channels involved in root-to-shoot Cl\(^{-}\) translocation and PM transport proteins involved in Cl\(^{-}\) allocation through the phloem, remain unexplored so far. The function of NPF transporters from the NAXT subfamily involved in Cl\(^{-}\) excretion from plant cells could be relevant under specific physiological conditions, possibly complementing those of S\(^{+}\) and R-type channels. However, this hypothesis requires further research. New experiments are also required to better understand the localization and biological function of CCC transporters in order to clearly establish their biological functions. This and other endomembrane Cl\(^{-}\)- transporters like AtALMT9, GmCLC1, GsCLC2, and possibly GmSALT3/CHX1 involved in intracellular Cl\(^{-}\) homeostasis have been proposed to regulate shoot Cl\(^{-}\) accumulation and salinity tolerance. In particular, intracellular Cl\(^{-}\) compartmentalization in vascular cells apparently plays a crucial, but still obscure, role in controlling whole-plant ion (e.g., Cl\(^{-}\) and Na\(^{+}\)) distribution. Finally, more research on hormonal regulation and signal transduction processes that control Cl\(^{-}\) nutrition at the whole-plant level is urgently required.

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