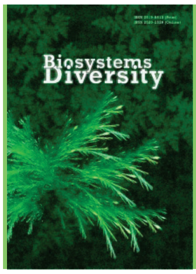

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Chlorine in plant life

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Chlorine is an essential nutrient, a deficiency of which reduces plant productivity. Chlorine-containing substances have been known and used for a long time. The most common chlorine compound, sodium chloride (table salt), has been in use since ancient times. It was used as early as 3000 BC and brine as early as 6000 BC. Cl substances are mentioned in ancient texts from different cultures. The discovery of chlorine was in 1774 by Carl Wilhelm Scheele. He obtained it by reacting pyrolusite (manganese dioxide, MnO_2) with hydrochloric acid (HCl, then known as muriatic acid). Scheele thought that the gas produced contained oxygen. It was Sir Humphry Davy's proposal and confirmation in 1810 that chlorine was an element, and he also named the element. Chlorine has been considered a biologically important element almost since its discovery. Research into the effects of chloride fertilisers was carried out in the second half of the last century. In 1949, Warburg argued that chloride was an important trace element for plant growth and showed that it was necessary for the water distribution system at the site of photosystem II oxidation. In the 1954 Broyer et al. finally demonstrated the biological importance of chlorine for plants. Chloride is the most abundant inorganic anion in plant cells, an element available in most agrophytocenoses. The average Cl$^-$ content in plants ranges from 2.0–20.0 mg/g DM, but for Cl-sensitive and Cl-tolerant glycophyte species, the critical (often toxic) Cl-content in tissues can be around 4–7 and 15–35 mg/g DM, respectively. Chlorine deficiency in plants has characteristic symptoms: wilting, numerous spots, and reduced productivity. Chloride performs a wide range of functions in plants, primarily forming turgor and osmoregulation, respectively, affecting transport processes on membranes (plasmalemma, tonoplast, etc.), water & nitrogen use efficiency (WUE & NUE), and affects the functioning of photosystem II, and is therefore an important part of agricultural plant productivity. Chloride stimulates the structural and functional role of the plasma membrane, sugar transport, as well as nitrogen fixation and assimilation in the plant. Nitrogen assimilation, and photorespiration become more efficient when fed with chloride. Recent studies have discussed the role of chlorine in nitrogen assimilation and photorespiration. It has been shown that Cl plays an important role in the oxygen-evolving complex by adjusting the affinity of different amino acid residues for manganese (Mn). Chlorine acts as a counterion, balancing the positive charges of potassium (K$^+$) and other cations in plant cells, which is essential for maintaining electrical neutrality and proper ionic balance in cells. Chlorine plays a significant role in soil salinity. Sources of chlorine in soil include mineral weathering, chlorine from marine species and anthropogenic pollution. Fertilisers such as potassium chloride help to increase the chloride content of the soil. Planting salt-tolerant crops can help maintain agricultural productivity on saline soils. The sensitivity of crops to chlorine varies according to the type of crop. Some crops can tolerate higher levels of chloride without adverse effects, while others are more sensitive and may show symptoms of toxicity or growth retardation when exposed to higher chloride concentrations. Understanding the response of specific crops to chloride is important for the development of nutrient systems and irrigation practices. Chloride increases plant resistance to diseases that require relatively large amounts of Cl$^-$. These doses are much higher than those required for its use as a trace element, but much lower than those required to induce salinity control effects. Most of the research on chlorine nutrition has been devoted to studying the effect of the element on the incidence of physiological leaf spot (PLS) in cereals. PLS form on the leaves of cereal crops when there is a lack of chlorine in the nutritional systems. The necrosis that develops in Cl-deficient plants is thought to be associated with the accumulation of H_2O_2 during the release of Cl from the Mn cluster of the oxygen-evolving complex. Physiological spotting in the form of completely/partially transparent dots/spots on the leaf was observed, which may indicate inhibition of chlorophyll synthesis rather than degradation. Given that chlorine at micromolar concentrations affects transport processes on membranes and that the element is easily leached through the soil profile, its deficiency occurs in the second half of the growing season, during the period of generative development, which may be the initial mechanism for the formation of PLS in the form of transparent/translucent leaf spots. The development of these spots in the generative period of development, during grain filling, can be significantly accelerated by high levels of actinic light and, accordingly, significantly limit the productivity of cereal crops and their quality. A possible component of chlorine deficiency and leaf damage in wheat and other cereals by PLS may be the application of phosphate fertilizers with high fluoride content, such as phosphate rock, etc. Therefore, in high productivity technologies, it is advisable to use phosphate fertilizers with a low fluoride content, such as potassium monophosphate. Therefore, the use of chlorine fertilisers, mainly potassium chloride in the basic application, ammonium chloride, calcium chloride, etc. in the foliar application, is important to provide plants with chlorine during the growing season to increase WUE & NUE, increase plant resistance to pathogens, control PLS, and increase productivity of cereals and other agricultural crops. Chlorine's role in increasing WUE & NUE is particularly important for the country's profitable crop production in the face of resource shortages.

Keywords: Cl$^-$ anion, mechanisms of biological activity, physiological leaf spots, productivity.

History of chlorine discovery

Chlorine as we understand it today was not recognised as a distinct element until the late 18th century. It is now generally accepted that chlorine anions are among the most important inorganic elements. Substances containing chlorine, such as sodium chloride (common salt) and other chlorine compounds, have been known and used for a long time. The most common chlorine compound, sodium chloride (table salt), has been known since ancient times. It was used as early as 3000 BC and brine as early as 6000 BC. These substances are mentioned in various ancient texts across different cultures. Salt is mentioned several times in the Bible and is often associated with various symbolic and practical applications. Salt in the Bible serves as a powerful symbol and practical element with various meanings and uses. It represents the enduring covenant between God and His people, purity, preservation, and the enhancement of life. Conversely, it can also signify judgment, destruction and desolation. The multifaceted role of salt in biblical texts underscores its importance in the cultural and spiritual life of the ancient world. Medieval alchemists knew of various chloride compounds, although they did not understand the chemical nature of chlorine. They often worked with hydrochloric acid (muriatic acid) and other chlorine-containing substances in their experiments. Chlorine as an element was not known in antiquity, but its compounds, particularly sodium chloride (common salt), are widely documented as having been used in various ancient cultures. References to salt and its use appear in biblical texts, ancient Egyptian writings, Greek and Roman literature, Indian Ayurvedic texts and Chinese medical writings. These historical references underline the importance of chlorine-containing substances in human history and their multiple uses long before the scientific understanding of chlorine. The ancient Egyptians used a naturally occurring mixture called natron, which contains sodium carbonate and sodium chloride, for mummification and cleansing purposes. This substance is mentioned in various funerary texts and inscriptions related to embalming practices. In ancient Greek and Roman texts Pliny the Elder, a Roman author and naturalist, mentioned salt and its various uses in his encyclopedic work "Natural History" (circa 77–79 AD). He described the extraction and uses of salt, including its importance in food preservation and medicine. The Greek physician Hippocrates (circa 460–370 BC) mentioned the therapeutic uses of salt in his medical texts. Salt was used for its antiseptic properties and in various treatments. In Ancient India, Ayurvedic texts refer to salt in various medicinal preparations. Salt is considered one of the essential tastes (Rasa) and is used in numerous therapeutic formulations. Ancient Chinese texts, such as The Yellow Emperor's Classic of Medicine, mention the use of salt in medicine and food preservation. Salt has been an essential component of Chinese medicine and culinary practices for millennia. The alchemist and physician Abu Bakr al-Razi (circa 865–925, Latin Razes) experimented with ammonia (ammonium chloride) around 900. Distilling this mixture with vitriol (hydrated sulfates of various metals) yielded hydrogen chloride. The gaseous by-products of early chloride salt research were not considered, and hydrogen chloride could be produced instead until its chemical applications were discovered. Initially, it was used to produce mercuric chloride, which was first described in the Arabic text De aluminibus et salibus (On Alum and Salts). Chlorine was a key ingredient in the experiments of medieval alchemists. The experiments involved heating chloride salts such as sodium chloride (table salt) and ammonium chloride (ammonia). One example of the use of ammonium chloride was when it was added to nitric acid, which led to the discovery of a powerful solvent that could dissolve gold (aqua regia, sometimes called 'royal water'). Royal water was an unstable mixture that emitted fumes, including gaseous free chlorine. However, it was not given the importance it deserved at the time (https://mobilephysiotherapyclinic.in/chlorine-cl).

The nature of free gaseous chlorine as a separate substance was recognised until around 1630 by the physician and scientist Jan Baptiste van Helmont. In the 1770s, the Swedish chemist Carl Wilhelm Scheele (1774), as a result of the reaction of manganese (IV) oxide with hydrochloric acid: $4\ HCl + MnO_2 \rightarrow MnCl_2 + 2\ H_2O + Cl_2$, discovered chlorine in its pure form and believed it to be an oxide of a new element. He did not go on to prove that chlorine was an element. Many chemists, including Lavoisier and Claude Berthollet, suggested that Scheele's compound might be a mixture of oxygen and the yet-to-be-discovered element hydrochloric acid. In 1789, French chemist Claude Berthollet discovered that chlorine gas had bleaching properties, and in 1799, Charles Tennant developed a solid form of chlorine, $Ca(OCl)_2$, a bleaching powder, by reaction of chlorine gas with lime. The use of chlorine as a bleaching agent has transformed the textile industry, reducing the time required to bleach fabric from weeks to hours. The main use of chlorine is to purify drinking water, killing bacteria and making it safe to drink [www.ista.ie/wp-content/uploads/2019/04/Chemistry-Spring-2019.pdf]. At the beginning of the 19th century, in 1809, chemists Joseph Louis Gay-Lussac and Louis-Jacques Tenard suggested that the gas could be an element. In 1810, Sir Humphrey Davy, working at the Royal Institution in London, repeated the experiment of the previous authors and showed that the gas did not react with hot carbon and did not contain oxygen, concluding that the substance was an element and not a compound. Because of its colour, he named the new element 'chlorine', from the Greek word χλωρος (chlōros, 'green-yellow', romanised: khlōrós, 'pale green'). The chemist Michael Faraday created liquid chlorine in 1823, proving that what was then called 'solid chlorine' was actually a chlorine hydrate structure ($Cl_2•H_2O$) (Porter, 1996; Wisniak, 2002).

It is generally accepted that chlorine was discovered by Carl Wilhelm Scheele in 1774. He obtained it by reacting the mineral pyrolusite (manganese dioxide, MnO_2) with hydrochloric acid (HCl, then known as muriatic acid). Scheele believed that the resulting gas contained oxygen. Sir Humphry Davy proposed and confirmed chlorine as an element in 1810, and he also named the element (cited in Smeaton, 1992; Mingos, 2019; www.webelements.com).

Chlorine has been considered a biologically important element almost since its invention. Research into the effects of chloride fertilisers was carried out in the second half of the last century. The fact that chloride is essential for plants was demonstrated as early as 1862 by Nobbe and Siegert (Nobbe & Siegert 1862). They reported that buckwheat (*Fagopyrum esculentum* Moench) did not mature in the absence of Cl ions in the nutrient solution. They continued their research on buckwheat (*F. esculentum*) until 1866. In 1867, the British scientist Voelcker (1867), as well as other researchers in the following years, provided accurate information about the strong salt tolerance and insensitivity to Cl in a wide variety of varieties and lines of beet (*Beta vulgaris* L.). In 1919, Tottingham concluded that chlorides affected plant growth in a species-specific manner (Tottingham, 1919). Rudolfs (1921) and Schufan (1939; 1967) used chlorides on asparagus (*Asparagus officinalis* L.). Stoffert (1922) observed that when excessive amounts of chloride fertiliser were applied to *Ribes rubrum* L. (*R. nigrum* is more resistant to high doses of NaCl), the leaf tips first became rusty brown, dried out and discoloured, and then these symptoms spread to the entire leaf margin.

Later, in the 1930s, Lipman (1938) noted that chlorine was necessary for the growth of buckwheat (*F. esculentum*) and concluded that "if chlorine is not necessary, it is undoubtedly very useful". Eaton (1942) and Raleigh (1948) wrote about the effects of additional fertilisation with chlorine, reporting significant increases in yields of tomato (*Solanum lycopersicum* L.), cotton (*Gossypium hirsutum* L.) and beet (*B. vulgaris*). Warburg (1948) argued that chloride is an important trace element for plant growth and showed that it is necessary for the water distribution system at the site of photosynthesis of photosystem II (PSII). The fact that Cl is an important trace element in plant nutrition was not recognised until 1954 (Broyer et al., 1954).

Broyer et al. (1954) in their research on tomato (*S. lycopersicum*) plants showing symptoms of disease when treated with recrystallised KNO_3 found that the 'X element' of growth was lost. The purified salts were too low in 'element X' to meet the nutritional requirements needed to extend the life cycle of the tomato (*S. lycopersicum*) plant. They suspected, and then experimentally confirmed, that chlorine was the 'element X' – the missing growth factor. Chloride was needed in very small amounts.

Broyer et al. (1954), Ulrich & Ohki (1956), Johnson et al. (1957) and Broyer (1966) verified the accuracy of the results of previous studies. To obtain accurate results during the experiments, they cultivated plants under controlled conditions on a nutrient solution in filtered air without chlorine, which was a very important condition. In the leaves of tomato plants (*S. lycopersicum*) suffering from chlorine deficiency, the chlorine concentration was around 7 μM per gram of DM (250 ppm), symptoms of chloride deficiency appeared and the plant eventually began to die. Symptoms of deficiency included inhibition of growth of the youngest parts and wilting of the leaves, especially at the margins. Root growth was also inhibited.

In the 1950s, Broyer et al. (1954) finally proved the biological importance of chlorine for plants. They described the symptoms of chlorine deficiency, which were alleviated by the addition of chloride, and suggested that it should be considered a necessary microelement for plants.

Chlorine is a non-metal that is widely distributed in nature and occurs in soil and plants as the chloride anion (Cl^-), one of the most abundant anions in nature. Chlorides, salts of hydrochloric acid, are readily soluble and therefore easily mobile in soils, as they have a low binding strength to exchangers and are therefore easily leached under wet conditions, with a corresponding accumulation under dry conditions. Chlorides are adsorbed in very small amounts in soils with a pH above 5 (>5). At lower pH values (<5) the adsorption of iron oxides increases (Scheffer & Schachtschabel, 1989). The anionic form significantly influences the behaviour of chlorine in the environment and its functions in plants (Winterton, 2000). Chloride is formed from chlorine-containing minerals such as chlorapatite, from the air (sea salt, combustion residues, industrial emissions, etc.), the latter of which can amount to up to 20 kg chloride/ha per year. As sorption and deposition are negligible, this occurs almost exclusively in the soil solution.

Chloride anions are mainly found in oceans, seawater, salt lakes, rivers and surface waters. They are also found in large underground deposits of NaCl formed by the drying up of seas. It occurs in the form of minerals such as halite (rock salt), sylvinite (potassium chloride) and carnallite (hydrated double potassium chloride) (Bielański, 2002). Chloride anion in the soil solution can be actively taken up by the plant, i.e. by counter electrochemical concentration gradient (Gerson & Poole, 1972). Relatively small amounts of chlorine from air or precipitation can be absorbed by leaves (Johnson et al., 1957). Chloride is highly mobile in plants in both the acropetal and basipetal directions (Marschner, 1995). The rate of absorption of the element is usually high, and thus significant amounts of the anion can accumulate in the cytosol and vacuoles (Cram, 1984). Chloride activates the water balance of plants, since in the presence of its pools in plants, the osmotic pressure of the cells increases, with corresponding changes in ion transport, adapting the plant to salinity conditions.

The total chloride content of the soil is very variable. Areas with marine sediments and some arid soils may contain high concentrations (10–1000 ppm Cl in soil solution). Immobilisation in the soil is virtually absent. Nevertheless, it can be argued that all soils in Western and Eastern Europe can be characterised as sufficiently supplied with the element for the vital needs of plants. The balance of chloride in agrophytocenoses can be considered as low deficit, because despite high leaching rates and, in some cases, significant removal during harvesting, the supply by means of precipitation and the regular application of fertilizers containing chlorine bring the balance into equilibrium or lead to an excess of the element.

Chlorine determination methods

There are several methods for the detection of chlorine. It is important to note that the specific method selected for chlorine detection depends on the application, the concentration of chlorine being measured, and the sensitivity and desired accuracy level. Among the many methods for determining chlorine in plant, water, and soil samples from phytocenoses, ion-selective electrodes have been the most widely used, and a number of methods using ion or liquid chromatography with mass spectrometry or methods based on colourimetric reactions are now available.

Chloride concentrations (down to parts per million) in a large excess of chlorate or perchlorate can be quantified using chloride ion-selective electrodes (ISEs). Trace amounts of chloride in chromic acid solutions can be determined using a heterogeneous chloride ISE based on silicone rubber. However, homogeneous chloride ISEs are problematic for practical applications because their response in complex saturated solutions changes over time due to chemical attack on the membrane surface. It has been proposed to use both homogeneous and heterogeneous electrodes to monitor chloride ions (Subrananian et al., 1984). ISEs are used to measure the concentration of chloride (Cl^-) in aqueous samples. The concentration of the chloride ion gives a quick measurement of salinity of water samples. Chloride ISEs are suitable for measuring chloride levels in soil, in ocean saltwater, or salt in food samples (Liu et al., 2017; Sikder et al., 2018; Wilson et al., 2019).

Electrochemical sensors can be used to detect chlorine in water or air by measuring the electric current produced when chlorine reacts with an electrode. These sensors are commonly used in portable chlorine detectors (Okazaki et al., 2021). Spectroscopic techniques such as infrared spectroscopy and mass spectrometry can be used to detect chlorine based on its unique chemical properties and molecular structure. It is important to note that the specific method chosen for chlorine detection depends on the phytocenosis sample, the chlorine concentration being measured, and the level of sensitivity and accuracy required (Wilson et al., 2019).

To summarize, chlorine can be detected using colorimetric detection kits that change color in the presence of chlorine. These kits typically contain reagents that react with chlorine to produce a visible color change. Gas detection tubes can be used to measure the concentration of chlorine gas in the air. These tubes contain a reagent that changes color when exposed to chlorine gas, and the length of the color change is proportional to the concentration of chlorine present. Electrochemical sensors can be used to detect chlorine in water or air by measuring the electrical current produced when chlorine reacts with an electrode. These sensors are commonly used in portable chlorine detectors. Spectroscopic techniques, such as infrared spectroscopy and mass spectrometry, can be used to detect chlorine based on its unique chemical properties and molecular structure. Chlorine can also be detected through chemical titration methods, where a titrant solution is added to a sample containing chlorine until a color change or other indicator shows that the reaction is complete.

In addition, field detectors for the presence of chlorine in water are used in irrigation systems, the excess of which is often observed in vegetable crops when KCl is applied.

Chlorine can be detected using colourimetric kits that change colour in the presence of chlorine. These kits typically contain reagents that react with chlorine to produce a visible colour change (Lodge, 2017; Palladino et al., 2020).

Gas analysers can be used to measure the concentration of chlorine in air. These tubes contain a reagent that changes colour when exposed to chlorine gas, and the duration of the colour change is proportional to the concentration of chlorine present (Shin & Jung, 2006).

Chloride ion chromatography is a method used to separate and analyze ions, particularly chloride ions (Cl^-) in a sample (Fig. 1). It is a type of ion chromatography (IC), a widely used analytical method for determining the concentration of ions in solution.

Chlorine ion chromatography works on the principle of ion exchange. A sample solution containing chloride ions is passed through a chromatography column filled with a stationary phase containing charged functional groups, typically quaternary ammonium groups (positively charged), attached to a resin matrix. When chloride ions are eluted from the column, they pass through a detector, such as a conductivity detector. The detector measures the conductivity of the eluent, which is directly proportional to the concentration of chloride ions present in the sample.

Chlorine ion chromatography is used to analyze chloride ions in environmental samples such as plants, surface and groundwater, soil extracts, and atmospheric aerosols. Monitoring chloride levels in these samples provides insight into contamination, salinity, and environmental impact assessment.

Chloride ion chromatography offers high sensitivity for the detection of chloride ions even at trace levels. The method offers excellent selectivity for chloride ions, enabling accurate quantification in complex sample matrices over a wide dynamic range of chloride ion concentrations, from ppm to %.

Thus, chlorine ion chromatography is a valuable analytical method for the separation and quantification of chloride ions in various samples. It finds applications in environmental analysis, food processing, water quality monitoring, and biomedical research. Due to its high sensitivity, selectivity, and versatility, chlorine ion chromatography plays a crucial role in quality assurance, safety, and environmental protection.

Other methods include the determination of chlorine (Cl^-) in plant leaves by destructive but time-consuming wet laboratory analysis (Miller, 1998), high-performance liquid chromatography (Goyal, 2002) and ICP mass spectrometry (ICP-MS) (de Gois et al., 2016; Horst et al, 2017); liquid chromatography with mass spectrometry (de Bruin-Hoegée et al., 2024). Non-invasive research methods are increasingly being introduced into plant production using portable leaf spectrometers operating at wavelengths in the visible near-infrared (Vis-NIR) range.

Fluorescent probes for detecting chlorides and chlorine compounds in plants and environmental objects are also being actively developed (Zhang et al., 2018; Lv et al., 2024).

Such instruments are used for rapid assessment of plant nutrient status, mostly for indirect estimation of leaf nitrogen content (LNC) by measuring the transmittance, reflectance and/or fluorescence properties of leaves (Padilla et al., 2018). Visconti & de Paz (2019) developed a regression model to estimate Cl^- from leaf reflectance, with best results for Cl^- concentrations above 1.5%, and found that the most important wavelengths for Cl^- estimation were from 390–472 to 690–692 nm. The Cl^- content of persimmon (*Diospyros kaki* L.) leaves was measured using a CI-710 portable miniature spectrometer (CID Bio-Science, Inc., Camas, WA, USA) in the visible near-infrared spectrum. The two wavelength intervals correspond to two Chl a absorption maxima in the visible spectrum. The high correlations indicate that Cl^- interacts with the main leaf pigment, possibly facilitating its degradation and thus allowing the use of Vis-NIR spectroscopy for its assessment.

Chlorine oxide ClO_2 can also be used in nutrient systems, so field instruments to determine chlorine oxide concentration are widely used to refine field/greenhouse doses of chlorine oxide (Zhong et al., 2021).

Fig. 1. Ion chromatograph Compact IC PRO 881 Metrohm with conductometric detector (range 0–15,000 µs/cm, Switzerland; Institute of Plant Physiology and Genetics, NAS of Ukraine): determination of the amount of inorganic anions and cations in plants was carried out on a Metrosep A Supp 5,250x4.0 mm column, eluent – carbonate buffer 3.2 mM Na_2CO_3 + 1.0 mM $NaHCO_3$ for determination of anion content and a Metrosep C2 150x4.0 mm column, eluent – nitric acid (50 mM) for determination of cation content (Merck reagents, Germany); plants were divided into separate organs, dried, and homogenized; each individual 50 mg plant material was dissolved in 10 mL (for anion determination) and 15 mL (for cation determination) of ultrapure water of class 1 (Ultra Pure Water System, Human Corporation, Korea/Left block) and extracted in a boiling water bath for 10 min, the extract was collected and repeated under the previous conditions three times; the total amount of water was 50 mL (1:1000 dilution) for cationic content and 30 mL (1:600 dilution) for anionic content; the extract was filtered (0.22 or 0.45 nm); primary data processing was performed using the Magic Net IC v. 1.1 program by Metrohm (Switzerland)

Regulatory documentation for the determination of chlorine in ecosystems

Methods for determination of free, total and residual active chlorine in water according to DSTU ISO 7393-1-2003, DSTU ISO 7393-2:2004, DSTU ISO 7393-3:2004, GOST 18190-72. The standards cover titrimetric, colourimetric and iodometric methods for the determination of chlorine content. Titrimetric method according to DSTU ISO 7393-1:2003, colourimetric method according to DSTU ISO 7393-2:2004, iodometric method according to DSTU ISO 7393-3:2004, determination of residual free chlorine by titration with methyl orange, method for separate determination of free chlorine combined with monochlorine and dichloramine. Methods for determination of chloride ions in aqueous soil extracts according to GOST 26425-85. The standard establishes three methods – argentometric, direct ionometry and ionometric titration – for the determination of chloride ions in aqueous extracts of saline soils. Scope of the standard: determination of chloride ion by argentometric method; determination of chloride ion by direct ionometry; determination of chloride ion by ionometric titration (Khilchevskyi et al., 2016).

For chlorine analysis, Common Anion Analysis by U.S. EPA 300.0 & 300.1 for the determination of inorganic anions in water or EPA Method 325A for the measurement of volatile organic compounds, including chlorine and other halogens, can be used. EPA Method 300.0 & 300.1 is a standard method used to analyze inorganic anions in drinking water, surface water, groundwater, and wastewater. The method utilizes ion chromatography to separate and quantify various inorganic anions such as chloride, bromide, fluoride, nitrate, nitrite, phosphate, and sulfate. EPA Method 325A is a standard method used for the measurement of volatile organic compounds (VOCs) from stationary sources such as industrial facilities, landfills, and other sources of air pollution. This method is specifically designed for the quantification of VOC emissions using sorbent tubes and gas chromatography-mass spectrometry (GC-MS) analysis.

Chlorine determination by 35, 36, and 37 isotopes

Chlorine is a chemical element with the atomic number 17. Cl is known to have 24 isotopes with mass numbers ranging from ^{28}Cl to ^{51}Cl. There are only two stable isotopes: ^{35}Cl and ^{37}Cl with proportions of 75.76% and 24.24%, respectively (Berglund & Wieser, 2011; Eggenkamp, 2014).

Chlorine-35 (^{35}Cl): this is the most abundant and stable isotope of chlorine. It has 17 protons and 18 neutrons. Chlorine-37 (^{37}Cl): has 17 protons and 20 neutrons. These isotopes are used in various applications, including tracer studies, environmental monitoring, and industrial processes

(Kaufmann et al., 1984; Bonifacie, 2018). Stable isotopes of chlorine (^{35}Cl i ^{37}Cl) are fractionated by diffusion and used to characterise diffusion-dominated systems, understand seawater intrusion and determine the origin of chlorine. A method for measuring α (Hasegawa & Nakata, 2018) has been proposed using through-diffusion, where a sample is poured into a tank from a high to a low concentration. Theoretically, the diffusion flux in a through-diffusion experiment should have a mass ratio fractionated by diffusion, and the difference between the δ^{37}Cl in the high and low concentration tanks approaches 103 ln α with time. A significant difference between the δ^{37}Cl in the high and low concentration systems was shown. The measured α ranged from 1.0015 to 1.0022. The proposed method can be applied to other isotopes to measure α, contributing to a more accurate quantification of Cl, and δ^{37}Cl profiles (Hasegawa & Nakata, 2018).

Chlorine isotopes can also provide information on plant physiology, soil-plant interactions and agrophytocenoses/ecosystems. Isotopic fractionation can occur during different physiological processes such as uptake, transport and metabolism. Fractionation factors can be influenced by environmental conditions, plant species and physiological state of the plant. Chlorine isotopes can be used to trace the sources of water and nutrients taken up by plants, helping to understand soil-plant interactions and nutrient cycling. Isotopic analysis can provide insight into environmental conditions that affect plant growth, such as soil salinity and the presence of anthropogenic pollutants. Techniques such as isotope ratio mass spectrometry (IRMS), a specialisation of mass spectrometry in which mass spectrometric methods are used to measure the relative abundance of isotopes in a given sample, are used to measure the ratio of chlorine-35 to chlorine-37 (^{35}Cl to ^{37}Cl) in plants and other samples. Inductively coupled plasma mass spectrometry (ICP-MS) can also be used to determine chlorine isotope ratios with high accuracy.

Chlorine isotopes can be used to study the effect of soil salinity on plant growth and to trace sources of salinity in agricultural fields. Isotopic analysis can help to identify sources of chlorine pollution, such as industrial emissions or the use of chlorinated fertilisers, and their effect on plant productivity potential. The study of chlorine isotope fractionation during various physiological processes can provide insight into plant metabolism and stress response. Thus, chlorine-35 and chlorine-37 isotopes in plants are an important tool for understanding various physiological and environmental processes. Isotopic analysis can provide insight into soil-plant interactions, nutrient uptake and the effect of environmental conditions on plant productivity potential. Analytical techniques such as mass spectrometry allow accurate and rapid measurement of chlorine isotope ratios, facilitating research in plant physiology, soil science and environmental monitoring.

Chlorine-36 detection can also be used in ecosystem and plant research. Chlorine-36 is a radioactive isotope of chlorine with a half-life of about 301,000 years. It is much less abundant than the stable isotopes 35 and 37, but can still be found in the environment, including in plants. Chlorine-36 is formed in the atmosphere when cosmic rays interact with argon-36. It then falls to the Earth's surface as precipitation. Chlorine-36 can be formed *in situ* in rocks and soils as a result of fission reactions involving cosmic rays and elements such as potassium and calcium. Atmospheric nuclear testing, nuclear reactor operation, and nuclear accidents have also contributed to the presence of chlorine-36 in the environment. Chlorine-36, like stable isotopes, is absorbed by plants from the soil in the form of chloride ions. The absorption process is passive and occurs mainly through the roots. Once absorbed, chlorine-36 is transported throughout the plant by the same routes as stable chloride ions through the xylem and phloem. Plants growing in areas with groundwater containing chlorine-36 can absorb and assimilate it, providing information on the sources and dynamics of water movement in the aquifer. Studying the presence and movement of chlorine-36 in plants can help to understand nutrient cycling and chloride ion dynamics in the soil-plant system.

Chlorine-36 contamination studies can be used to monitor and study the spread of contamination from nuclear activities or industrial processes. Plants can serve as bioindicators for the presence of chlorine-36 in the environment. The classical method for the determination of the chlorine-36 isotope is Alpha Magnetic Spectrometer (AMS is a TeV precision, multipurpose particle physics magnetic spectrometer): AMS is a highly sensitive method used to measure low concentrations of chlorine-36 in environmental samples, including plants. It can be used to distinguish chlorine-36 from much more abundant stable isotopes.

The presence of chlorine-36 in plants can therefore provide information on the impact of natural and anthropogenic activities on the environment. This can help to track the movement and accumulation of radioactive substances. Understanding the uptake and distribution of chlorine-36 in plants is important for assessing the potential health risks associated with the consumption of contaminated plant material. Until recently, experimental studies on the dynamics of chlorine and chlorine-36 in terrestrial ecosystems (field crops, vegetables, grasses) had been limited compared to forests, for which there are numerous data (Le Dizès & Gonze, 2019).

Chlorine content in plants

Chlorine in plants occurs as the chloride anion (Cl$^-$). Chloride is the most abundant inorganic anion in plant cells and is present in most agricultural environments (Marschner, 1995). Note that chlorine is the most abundant element in the world's oceans: 19400 ppm (James & Lord, 1992, cited by MacMillan's chemical and physical data, 1993). The source of chloride is usually soil solution, which is absorbed by plant roots. After absorption, chloride is transported through the xylem with an upward transpiration flow from roots to shoots and can accumulate in leaves and fruits (White & Broadley, 2001; Raveh, 2005; Li et al., 2017). Optimal nutrient concentrations are required for normal plant growth and development. Nutrient concentrations below or above the optimal range cause deficiencies or ionic toxicity, which affect plant growth (Munns, 2002). The uptake of nutrients by plants is directly affected by salinity, e.g. sodium (Na^+) reduces potassium (K^+) uptake and calcium (Ca^{2+}) availability, and chloride (Cl$^-$) reduces nitrate (NO_3^-) uptake. On the other hand, salt-tolerant plants grown under saline conditions show reduced leaf salt damage and growth reduction (Cai et al., 2014). Plants tolerate salinity by eliminating ions, maximising root Na^+ efflux, maintaining a high cytosolic potassium to sodium ratio (K^+:Na^+) or accumulating compatible solutes (Tester & Davenport, 2003). Plant species or cultivars respond differently to salinity.

Chlorine is mainly present as a highly mobile anion, with concentrations in plant shoots ranging from 28 μm to 1.68 mm (Broadley et al., 2012). The critical level for chlorine deficiency is 0.2% of the DM of the plant. The average concentration of chlorine in plants is 2–20 mg/kg DM. The actual requirement of the plant organism for this element is 1–2 orders of magnitude lower. According to Xu et al. (1999, 2012) and White & Broadley (2001), tissue chlorine levels of ~4–7 mg/g DM are toxic to Cl$^-$ sensitive species, while up to 15–33 (up to 50 for *B. vulgaris*) mg/g can be toxic to Cl-tolerant species, indicating a much greater ability to translocate in tolerant species than in sensitive ones. At high concentrations, Cl$^-$ can be toxic, manifested by chlorosis and necrotic lesions, symptoms of scorched leaves (Geilfus, 2018a). Due to the increase in irrigated land and climate change, soil salinity is increasing, which threatens agricultural production and is a problem for the whole world (Van Zelm et al., 2020). In barley (*Hordeum vulgare* L.), the concentration of vacuolar (Cl$^-$) can reach 50–150 mm and in the phloem up to 120 mm (Xu et al., 1999; Broadley et al., 2012). However, under certain conditions, plants accumulate up to 1.5–5.0% Cl$^-$ in the leaves (Xu et al., 1999). Excessive Cl$^-$ in leaves damages photosynthetic activity (Geilfus, 2018a). Although plants buffer the cytosolic concentration of Cl$^-$ by pumping this ion into cell vacuoles through transport proteins (Li et al., 2017; Geilfus, 2018a), this process requires energy and disrupts the growth and development of crops. The effect of phytotoxicity often progresses unnoticed, and by the time it finally manifests as leaf chlorosis and even necrosis, it is too late to avoid visually visible damage and yield loss (Geilfus, 2018b).

In Lohry's research, chlorine is called an unusual nutrient. Its normal concentration in plants, observed in most fields, is more typical of micronutrients, and at the same time, the high levels of chlorine required by plants for growth and development allow us to attribute chlorine to macronutrients. The author presents the following data: the range of chlorine concentrations is 10–80,000 ppm, while the anion concentration of 100 ppm is sufficient for most cases (Lohry, 2007).

While the concentrations of chloride normally present in soils are higher than those needed to meet micronutrient requirements but not sufficient to cause toxicity (1–5 mM) (Franco-Navarro et al., 2016), plants accumulate Cl^- to macronutrient levels (Wege et al., 2017) because it is widely available in nature and is actively taken up by higher plants, contributing to plant productivity. For example, Franco-Navarro et al. (2016; 2019) found that Cl^- in tobacco (*Nicotiana tabacum* L.) plants plays a role in regulating leaf osmotic potential and turgor, allowing plants to improve leaf water balance parameters. At the plant level, Cl^- alters water relations by reducing plant transpiration, resulting in lower stomatal conductance, which leads to less water loss, higher photosynthetic efficiency and water use efficiency for increased plant productivity. The effect of chloride on stomatal activity was also studied in coconut (Braconnier & d'Auzac, 1990).

Chlorine is classified as a micronutrient, although its concentrations in tissues are usually significantly higher than those of macronutrients (>1000 mg/kg DM). Under production conditions, increased plant productivity is often observed at chlorine concentrations above 1000 μg/g DM (Kopsell & Kopsell, 2015).

Cl^- levels can reach toxic levels for many plant species, especially for sensitive species (Xu et al., 1999; White & Broadley, 2001; Marschner, 2011). During prolonged treatment with a nutrient solution containing low concentrations of Cl^- (4–5 mM Cl^-), different plant species accumulated Cl^- in leaves from 25 to 50 mg/g DM. Despite the fact that this Cl^- content exceeds critical toxicity levels, the plants developed normally and grew without obvious stress symptoms (Brumós et al., 2010; Cubero-Font et al., 2016; Franco-Navarro et al., 2016; Cubero-Font, 2017; Rosales et al., 2020). Root Cl^- uptake and long-distance Cl^- transport require the use of considerable metabolic energy (Britto & Kronzucker, 2006; Brumós et al., 2010), which indicates that shoot Cl^- accumulation is at the level of basic (NPK) macronutrients and corresponds to specific biological adaptations. Under these conditions, a specific physiological role of Cl^- is to increase dry biomass and improve plant productivity (Franco-Navarro et al., 2016; 2019).

Today, the level of chlorine supply to plants is considered an integral element in the formation of crop productivity (Schwartau et al., 2023). At the same time, chlorine deficiency in plants has characteristic manifestations.

Chlorine deficiency

Typical signs of chlorine deficiency are wilting and chlorosis of young leaves at the margins, small surface chlorosis spots that resemble brown spots but are smaller in size and do not have a characteristic border around the spots, and premature leaf senescence. Chlorine deficiency can cause a variety of symptoms in plants ; multiple manifestations of chlorosis, from yellowing to albicillation, with abnormalities in leaf morphology, with a corresponding delay in plant growth and development, reduced seed set and fruit set. Chlorine deficiency results in increased susceptibility to disease. At high chlorine concentrations, the element iron is known to be phytotoxic, with negative effects on the growth and productivity of a number of plant species (Schwartau et al., 2023). Thus, chlorine plays a vital role in several physiological processes in plants, and its deficiency or excess can have a significant impact on plant growth and productivity.

In our long-term experiments on winter wheat (*Triticum aestivum* L.) varieties, the following symptoms of chlorine deficiency were identified. Symptoms of chlorine deficiency in wheat can include leaf chlorosis (yellowing), which begins at the tips and margins of older leaves. Leaf necrosis (death of leaf tissue) progresses from the leaf margins inwards. Stunted growth and reduced tillering are also observed, as well as deterioration in grain quality, such as reduced protein content and increased susceptibility to disease.

Lohry identifies the following symptoms of chlorine deficiency, which include: a blue-green shiny appearance of young leaves; wilting, followed by chlorosis; excessive branching of lateral roots; bronzing of leaves; chlorosis and necrosis in tomatoes and barley (Lohry, 2007). It should be noted that changes in the development of the root system were observed mainly in the second half of the wheat growing season with sufficiently high levels of precipitation during the growing season.

Early symptoms are similar to those of magnesium deficiency, with pronounced interveinal chlorosis. Damage is mainly to old leaves and, over time, spots may appear on young leaves. Some leaves wilt and die. The location, colour and size of the spots are often similar to the early symptoms of leaf blight. Leaf spots known as multiple PLS are associated with chlorine deficiency in cereals. The reduction in the photosynthetic surface area of plants, particularly during the period from flowering to grain filling, caused by PLS, which occurs in the second half of the growing season, significantly reduces the productivity and quality of grain seeds.

In plants with chlorine deficiency, the formation of lateral thickened roots increases and unnatural wilting of the plants is observed. Such symptoms are similar to those caused by excess nitrogen and salts. Pronounced chloride deficiency in plants is quite rare and may occur in cereals, especially in barley (*H. vulgare*) and certain varieties of wheat (*T. aestivum*) on sandy soils during periods of heavy rainfall. Chloride deficiency in sugar beet (*B. vulgaris*) was first reported by Ulrich and Ohki (1956). In 1957, Johnson et al. (1957) demonstrated acute chlorine deficiency or restricted growth in eight plant species (lettuce (*Lactuca sativa* L.), tomato (*L. esculentum*), cabbage (*Brassica oleracea* var. *capitata* L.), carrot (*Daucus carota* L.), sugar beet (*B. vulgaris*), barley (*H. vulgare*), lucerne (*Medicago sativa* L.), buckwheat (*F. esculentum*), maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.).

The peculiarities of the effect of chlorine deficiency on red clover (*Trifolium pratense* L.) were studied in model experiments of aquaculture. The first visible symptom was leaf curling, started on the youngest leaves. A few days later, some of the newly unfolded leaves were completely wrinkled, and the older leaves showed spots of brown necrosis starting at the tip and spreading from the apex to the petiole along the leaf margins. This was followed by general stunting of growth and death of some leaves. It was found that the critical concentration of chlorine in the leaves + petioles of red clover was within 200 μg/g dry weight (Whitehead, 1985).

Excessive chlorine can be toxic to plants, which is manifested by a decrease in height and general coarsening of the plant, stems become hard and leaves are small, dull green, with manifestations of chlorosis and necrotic lesions, symptoms of burnt leaves (Geilfus, 2018a).

Since the 1980s and in more recent studies, the sensitivity of some crops, including cereals, to chlorine fertilisers has been widely recognised (Christensen et al., 1981; Fixen et al., 1986a, 1986b; Fixen, 1993; Xu et al., 1999). Engel et al. (1997, 2001), Montana, USA, found chlorotic and necrotic spots on the leaves of some wheat (*T. aestivum*) cultivars and attributed them to chloride deficiency. A few years later, they were able to reproduce such symptoms of chloride deficiency in culture on nutrient solution. Symptoms of chloride deficiency on leaves and roots of kiwifruit (*Actinidia deliciosa* or *Actinidia chinensis* Planch.) plants were observed at much higher levels of chloride supply (Smith et al., 1987).

Excess chloride caused chlorosis and, more commonly, necrosis of leaf tips ('tip burns') and margins. At high Cl levels, leaves were smaller, curled and dropped prematurely. The cause of these symptoms was the destruction of chlorophyll by excess chlorine. At very high soil Cl levels, root uptake of nitrate ions was inhibited (Bergmann, 1993). It can therefore be argued that excess chloride may correspond to the symptoms of salt stress.

Nobbe & Siegert (1862) were the first to suggest that chlorine was necessary for the growth of buckwheat (*F. esculentum*). Mazé (1915; 1919), and Lipman (1938) concluded that chlorine was probably an essential element for higher plants. However, the symptoms caused by chlorine deficiency were confirmed much later in experiments with tomato (*S. lycopersicum*) vegetation (Broyer et al., 1954). This was followed by the observations of Ulrich & Ohki (1956) on sugar beet (*B. vulgaris*), and Gausmann et al. (1958) on potato (*Solanum tuberosum* L.). Johnson et al. (1957) found an increase in the effect of chloride deficiency on bean (*P. vulgaris*), maize (*Z. mais*), buckwheat (*F. esculentum*), lucerne (*M. sativa*), barley (*H. vulgare*), sugar beet (*B. vulgaris*), carrot (*D. carota*), cabbage (*B. oleracea*), tomato (*S. lycopersicum*) and lettuce (*L. sativa*). Hannemann (1964) studied the effect of chloride on grapevine. Although there are few reports of chlorine deficiency in grapevines (Gong et al., 2011), there has been much debate as to whether potassium should be applied as sulphate or chloride.

Ozanne (1958) described the symptoms of chlorine deficiency in *Trifolium subterraneum* (subterranean clover) and Venema (1959) in sugar cane (*Saccharum officinarum* L.). Leh (1977) classified various trees according to their susceptibility to NaCl salts used in de-icing as very sensitive: maple (*Acer platanoides* L.), lime (*Thilia cordata* Mill.), beech (*Fagus* *sylvatica* L.), elm (*Ulmus laevis* Pall.), moderately sensitive: plane (*Platanus orientalis* L.), poplar (*Populus pyramidalis* Rozier), ash (*Fraxinus excelsior* L.), and less sensitive: birch (*Betula pendula* Roth.), acacia (*Robinia pseudoacacia* L.), oak (*Quercus robur* L.). Studies commissioned by Kyivzelenbud (department of Kyiv City State Administration) in 2005–2008 showed that the use of calcium chloride in winter to combat ice and snow is preferable to the use of potassium chloride in terms of phytotoxicity to the city's green spaces. Tree species can also be used for phytoremediation of volatile anions (Sklyarenko & Bessonova, 2018). The research has shown sufficiently high ability of the plane-trees to air cleansing from compounds of sulfur, fluorine, chlorine and phenols (Kapelyush & Bessonova, 2007).

Geissler (1953) studied the effect of chloride and sulphate fertilisers on vegetable yields. Baumeister & Burghardt (1972) divided vegetables into chloride-loving: spinach (*Spinacia oleracea* L.), Swiss chard (*Beta vulgaris* var. *cicla* (L.) W. D. J. Koch), sugar beet (*B. vulgaris* subsp. *vulgaris* var. *vulgaris*), cabbage (*B. oleracea*), celery (*Apium graveolens* L.), and beetroot (*B. oleracea* & *B. vulgaris*), and chlorine-sensitive: tomato (*S. lycopersicum*), potato (*S. tuberosum*), cucumber (*Cucumis sativus* L.), radish (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin), and onion (*Allium cepa* L.). Later, Siegel & Bjasrch (1962), and Loch & Pethö (1992) confirmed this information also for tomato (*S. lycopersicum*).

Most publications on the correct determination of Cl deficiency have been made in studies with cereals, mainly barley (*H. vulgare*) and wheat (*T. durum* & *T. aestivum*) (Schwenke et al., 2015), while the Cl requirements of crops are usually met by precipitation and chlorine in potash or compound fertilisers. Therefore, Cl deficiency in soil-grown crops is not common, according to several authors, and the symptoms of deficiency resulting from this deficiency are still not well described and difficult to identify.

Biological significance of chlorine

Chloride is a trace/micro element for higher plants, available to plants in the form of the chloride ion Cl^- (Marschner, 2011; Cakmak et al., 2023), and at the same time is seen as a biologically useful macronutrient (Franco-Navarro et al., 2016; Raven, 2017; Colmenero-Flores et al., 2019). Chloride anions, together with potassium cations, are involved in cell osmoregulation and plant turgor regulation (Flowers, 1988; Flowers et al., 1991); they increase plant biomass and elongate leaf and root cells (Chen et al., 2016; Franco-Navarro et al., 2016). Chlorides can be structural components of polypeptides, are important for the activity of certain enzymes, and can also affect the absorption and assimilation of nitrogen, phosphorus, potassium, calcium, magnesium, iron, and copper. According to modern concepts, Cl has a wide range of functions in plants, mainly turgor formation and osmoregulation, affecting transport processes across membranes (plasmalemma, tonoplast, etc.), WUE & NUE, affecting the functioning of PSII, and is therefore an important component of agricultural plant productivity (Wege et al., 2017; Franco-Navarro et al., 2021; Lambers, 2023; Peinado-Torrubia et al., 2023).

It is described that chloride uptake by the parenchyma of oat seedling coleoptiles occurs through combined influx and efflux transport systems (Babourina et al., 1998a). There are also reports of phytohormonal effects on chloride transport (Cram, 1983). Thus, auxin stimulates chlorine uptake by oat coleoptiles (Babourina et al., 1998b). The importance of the presence of chloride pool in stimulation of auxin-induced growth of coleoptile segments of maize seedlings (Burdach et al., 2014).

Previous studies have shown that Cl deficiency does not affect photosynthesis, but reduces the efficiency of ATPase and the conversion of starch to sugars (Fixen, 1993). In wheat (*T. aestivum*), it stimulates the structural and functional role of the plasma membrane, sugar transport, and nitrogen fixation and assimilation in the plant (Colmenero-Flores et al., 2019; Lucas et al., 2024).

Recent studies have discussed the role of chlorine in nitrogen assimilation and photorespiration (Lilay et al., 2024). Chlorine plays a crucial role in the process of photosynthesis in plants. Chloride is an important cofactor in the evolution of PSII oxygen in the chloroplast, stabilising the water splitting system. One of the main functions of this element is to participate in the splitting of water at the sites of PSII oxidation, it acts as an important co-factor in the release of oxygen by PSII in chloroplasts (Hill reaction) (Chen et al., 2010; Rivalta et al, 2011), and also regulates enzyme activity, transport processes of plasma membrane ion channels and other membrane structures of plant cells, in the process of plant growth and development, osmotic and stomatal regulation, as well as the formation of disease resistance (Chen et al., 2010; Xu et al., 1999; White & Broadley, 2001; Franco-Navarro et al., 2016; Raven, 2017).

Aerobic life requires oxygen, which is produced by the photosynthetic oxidation of water in plants, algae, and cyanobacteria. A key component in the evolution of oxygenic photosynthesis was a metal complex that could accumulate oxidation equivalents to facilitate the four-electron oxidation of two water molecules to dioxygen, while releasing electrons for reductive carbon fixation reactions. The oxygen evolution complex (OEC) consists of an oxo-bridged structure with four Mn atoms and one Ca atom. Oxygen itself is a by-product of the photosynthetic oxidation of water (Yano & Yachandra, 2014). Wincencjusz et al. (1997) showed that Cl^- is necessary for the functioning in the redox cycle of the oxygen-evolving complex (OEC). As for the mechanisms, the energy states of ionized water molecules in the network of H-bonds near the Ca^{2+} and Cl^- binding sites in the photosystem are discussed (Saito et al., 2020).

Imaizumi & Ifuku (2022) proposed individual roles for the two chlorides bound to the Mn cluster of the OEC. It has been shown that the release of Cl^- from its binding sites in the complex known as the water-oxidizing complex (WOC; or oxygen-evolving complex OEC, or oxygen-producing complex OPC) enhances H_2O_2 formation (Arató et al., 2004; Pospíšil, 2016; Pospíšil et al., 2022). Kawakami et al. (2009) determined the localization and function of chloride in the EOS. Vinyard et al. (2019) showed that Cl plays an important role in the photoassembly of the OEC by modulating the affinity of different amino acid residues for manganese (Mn). Imaizumi & Ifuku (2022) proposed individual roles for two chlorides bound to the Mn cluster of the OEC. Release of Cl^- from its binding sites in the OEC has been shown to enhance H_2O_2 formation (Arató et al., 2004; Pospíšil et al., 2022).

It is now generally accepted that chlorine nutrition is a component of increasing the efficiency of nitrogen assimilation and photorespiration. Recently, it has been shown that Cl^- supplied with other macronutrients improves NUE. The biochemical mechanisms by which Cl^- nutrition improves plant NUE are still under discussion. It shows that chlorination at favourable macronutrient levels does not affect nitrate uptake efficiency, maintaining the same NO_3^- content in root and xylem sap. The NO_3^- content in leaves was also significantly reduced by 6 mM Cl treatment, in parallel with the increase in NO_3^- use and NUE. Chloride supply increased the accumulation of transcripts and the activity of most of the enzymes involved in the assimilation of NO_3^- into amino acids, together with a greater accumulation of organic nitrogen (mainly in the form of proteins). A decrease in the glycine/serine ratio and a higher accumulation of ammonium indicates a higher activity of photorespiration in the leaves of chloride-treated plants. Chloride, in turn, contributed to an increase in the level of transcripts of genes encoding photorespiratory enzymes. In addition, microscopic observations indicate a strong interaction between different cellular organelles involved in photorespiration. Thus, it is shown that the higher NO_3^- utilisation and increased NUE induced by chlorine nutrition are mainly related to the stimulation of NO_3^- assimilation and photorespiration, which may contribute to the production of ammonia, reducing agents and intermediates that optimise C-N recycling and plant growth. This work demonstrates new functions of Cl^- and indicates its relevance as a potential tool for manipulating NUE in plants (Peinado-Torrubia et al., 2023).

Chlorine is involved in redox reactions that help maintain the balance of oxygen and renewable energy in plant cells. It is required for the synthesis of glucose and other organic compounds. Chlorine (Cl) is required for the synthesis of certain amino acids, such as glycine and tyrosine, which are components in the synthesis of proteins. Chlorine is involved in the formation of plant cell walls, particularly in the synthesis of cellulose and other polysaccharides. Chlorine helps to regulate the water balance in plants by controlling stomatal aperture and transpiration rate and is therefore an important element in achieving high WUE values. Chlorine also has antioxidant properties, helping to protect plants from oxidative stress caused by free radicals. Chlorine can help plants defend themselves against pathogens by activating defence mechanisms and participating in the synthesis of phytoalexins, which are toxic to certain microorganisms.

Chlorine is involved in methylation reactions necessary for the synthesis of various plant metabolites such as hormones and alkaloids. It is also required for the synthesis of vitamins such as vitamin E and K, which are important for plant growth and development. Micromolar amounts of chloride are involved in the regulation of the activity of enzymes such as asparagine synthetase (Rognes, 1980), vacuolar proton pump ATPase (Churchill & Sze, 1984) and affect the activity of amylase (Metzler, 2003). The minimum chloride requirement for normal growth of most plant species is in the range of 0.2–0.4 mg/g DM (Broyer et al., 1954; Johnson et al., 1957; Marschner, 2011), which corresponds to the content of the trace element. There is sufficient chloride in nature to meet the needs of plants (White & Broadley, 2001).

High concentrations of Cl^- are phytotoxic, leading to an imbalance in cellular metabolism and a decrease in potassium uptake, and an increase in potassium uptake leads to a decrease in leaf Cl^-.

The use of moderate amounts of Cl^- ensures (normal) adequate transport and distribution of Cl^- at subcellular, organ and whole plant levels (e.g. optimal root uptake rates, translocation in the xylem, accumulation in shoots and intracellular compartmentalisation).

The presence of chlorine pools in plants is a component of membrane transport in plants. Up to 30% of the metabolic energy of a plant cell can be used for ion transport across plant membranes. Accordingly, membrane transport determines virtually all aspects of plant life: from the transport of nutrients to growth, development and the course of the generative period of plant development. The study of the effect of chlorine and a number of other elements on the functioning of guard cells of stomata has become a mainstream in biological research (Blatt, 2024).

To elucidate the mechanisms of chlorine nutrition, the authors identified and characterized the genes encoding carriers responsible for the transport of the element into the cell (ZmNPF6.4 & ZmNPF6.6), its leakage from the cell (AtSLAH3 & AtSLAH1), as well as genes regulating chlorine compartmentation (AtDTX33, AtDTX35, AtALMT4 & GsCLC2). These transporters are important for nutrient delivery, long-distance transport, and Cl-compartmentation, and for regulating cell turgor and plant stress and disease tolerance (Colmenero-Flores et al., 2019).

Chloride is involved in several biochemical functions in plants (Fixen, 1993). It acts as a counterion for cation transport, and as a hoeing agent (Flowers, 1988), and plays a role in regulating stomatal movement (Talbott & Zeiger, 1996). Chloride is involved in many plant's metabolic processes (Schilling et al., 2000). It is a cofactor of manganese protein in the release of O_2 in chloroplasts. It stimulates ATPases in the tonoplast, which pumps H^+ ions from the cytoplasm to the vacuole, maintaining the proton gradient to the cytosol. Cl plays an important role in the opening of stomata in plants whose guard cells do not contain chloroplasts (palm (*Cocos nucifera* L.), kiwi (*A. deliciosa*), onion (*Allium cepa*)).

Chlorine is now considered as important for plants as sulphur.

Photosynthetic O_2 evolution under the influence of Cl^- anions

In photosynthesis, chloride is an important cofactor for the activation of the oxygen-releasing enzyme associated with PSII (Arnon & Whatley, 1949; Izawa et al., 1969). Chloride can bind (Baianu et al., 1984) to polypeptides associated with the water-splitting complex of PSII and can stabilise the oxidised state of manganese by acting as a bridging ligand (Critchley, 1985; Coleman et al., 1987; Homann, 1988).

Chlorine is essential for the water splitting reaction, or Hill reaction, in PSII. In chloroplasts of spinach (*S. oleracea*) panicles with reduced chlorine content, photosynthetic O_2 production increased with an increase in external Cl supply (Renger, 2012). Chloride plays a fundamental role in the water splitting system of PSII. In plants, chlorine accumulates mainly in the chloroplasts and is essential for photosynthetic function.

PSII performs water oxidation in photosynthesis under the influence of solar irradiation. The site of action is the Mn_4CaO_5 cluster. PSII degrades and resynthesizes the OEC every 20–40 minutes. The presence of chlorine and a few metals is important for the assembly process, but the exact mechanism remains unknown. It is possible that the amino acids are chelants in the OEC to bind/mobilize metals. Photoassembly yield was higher when the solvent was changed from H_2O to deuterated water, D_2O. It is believed that chloride and D_2O increase the affinity for protons in important amino acid residues. These residues in the site adjust the binding affinity of $Mn^{2+/3+}$ and promote the deprotonation of water to form the intermediate μ-hydroxo bridged Mn^{2+}/Mn^{3+} (Vinyard et al., 2019).

The oxidation of water in photosynthesis under the influence of light takes place in the OEC of PSII. The review considers the individual roles of the two chlorides that are attached to the Mn cluster of the OEC. Chloride ions are essential for oxygen production in PSII, and two Cl^- ions have been found to bind specifically near the Mn_4CaO_5 cluster in the OEC. The retention of these Cl^- ions in the OEC is critically supported by some PSII subunits located outside the membrane. The functions of these two Cl^- ions and the mechanisms of their retention remain to be fully elucidated. However, recent intensive research has deepened our understanding of the functions of these Cl^- ions, and the structures of PSII from different species have been described, which has helped to interpret previous results on Cl^- retention by the outer subunits (Imaizumi & Ifuku, 2022).

The main reactions of chlorine in photosynthesis are as follows: chloride ions are important components of the OEC in PSII. The OEC is responsible for the photolysis of water, the process by which water molecules are split into oxygen, protons, and electrons.

PSII is the thylakoid-membrane-embedded complex that catalyzes the oxidation of water and the reduction of plastoquinone in plastids and cyanobacteria (Mabbitt et al., 2014; Müh & Zouni, 2020). The oxidation of water actually takes place in PSII, which initially transfers the electrons to plastoquinone (PQ) and thus is a water:PQ oxidoreductase. PSII is a multi-subunit membrane protein complex situated in the thylakoid membrane, and it releases the reduced PQ (plastoquinol, PQH2) into that membrane, where it diffuses to another membrane protein complex (cytochrome b6f) to deliver the electrons. The oxidation reaction of water is catalyzed by a protein-bound manganese (i.e., Mn4CaO5). In recent years, much effort has been put toward unraveling the structure and inner workings of the OEC (Yano & Yachandra, 2014; Junge, 2019; Lubitz et al., 2019).

Chlorine ions are essential for the optimal functioning of the manganese cluster in the OEC, which is directly involved in the Hill reaction/water splitting. This reaction releases oxygen (O_2) as a by-product and provides electrons for the photosynthetic electron transport chain.

Chloride helps to stabilise the structure of the OEC and maintain the correct conformation of the protein components involved in water splitting. The electrons released during water splitting are transferred along the electron transport chain in the thylakoid membrane of chloroplasts. Chloride ions play an important role in maintaining the integrity of this chain by ensuring the efficient flow of electrons. The electron transport chain creates a proton gradient across the thylakoid membrane, which stimulates the synthesis of ATP by ATP-synthase. Electrons are also used in the reduction of $NADP^+$ to NADPH.

In PSII, water oxidation occurs at a Mn4CaO5 cluster and results in production of molecular oxygen. The Mn_4CaO_5 cluster goes through five different oxidation states, which are called Sn states. As a result, protons are released at the metal cluster and transferred through a 35 Å hydrogen-bonding network to the lumen. It is considered probable that an internal hydronium ion and chloride play a direct role in an internal proton transfer event during the S1-to-S2 transition (Brahmachari et al., 2017; 2018; Pilepić et al., 2022).

Regulation of stomatal function

Chlorine plays an important role in the regulation of stomatal function. The opening and closing of stomata are mediated by fluxes of potassium and the associated anions malate and chloride (Lee & Assmann, 1991). In plant species such as *A. cepa*, chloride is essential for stomatal function and in the absence of chloride, stomatal opening is inhibited. In coconut (*C. nucifera*), there is a close correlation between potassium and chloride fluxes during stomatal opening into closing cells and vice versa during stomatal closure; in chloride-deficient plants, stomatal opening is delayed by about 3 hours (Marschner, 1995). The disruption of stomatal regulation in palms is considered to be the main factor responsible for the development of stress and wilting symptoms in chloride-deficient plants (Von Uexkull, 1985). The proper functioning of stomata ensures an adequate supply of CO_2 for the Calvin cycle, which is necessary for the synthesis of glucose and other carbohydrates during photosynthesis.

Chlorine deficiency can impair the function of the OEC of PSII, reducing the efficiency of water splitting. This leads to reduced oxygen production and a limited supply of electrons for the electron transport chain. Reduced electron flow can lead to reduced production of ATP and NADPH, which are critical for the Calvin cycle and overall photosynthetic efficiency. Chlorine deficiency can affect stomatal regulation, resulting in less efficient stomatal opening and closing. This can lead to reduced CO_2 uptake, limiting the availability of substrate for the Calvin cycle. Inhibition of stomatal function can also lead to water stress, which further affects photosynthetic efficiency and overall plant health.

Thus, foliar chlorination can be a factor in improving water use efficiency, which may be important not only for cereal crops, but also for sunflower (*Helianthus annuus* L.) and other crops.

The decrease in leaf transpiration after Cl^- treatment in tobacco (*N. tabacum*) was due to a decrease in stomatal density and an increase in leaf cells (Franco-Navarro et al., 2019). Cl^- simultaneously stimulates growth and reduces water consumption, leading to improved water use efficiency (Franco-Navarro et al., 2016; 2019). Chlorine (chloride anion) is essential for the proper functioning of the photosynthetic apparatus, especially in the oxygen complex of PSII. It plays an important role in water splitting, electron transport and stomatal regulation, which are essential for efficient photosynthesis. Chlorine deficiency in plants can significantly impair photosynthesis, resulting in reduced growth and productivity. Proper management of chloride levels through soil testing, balanced fertilisation and appropriate irrigation is essential to ensure optimal photosynthetic performance and overall plant growth and development.

Correlations have also been found between root hydraulics (water flow) and K^+ (Wegner & Zimmermann, 2009) or NO_3^- (Gloser et al., 2007; Orieux et al., 2018) ion homeostasis. It should be added that studies comparing citrus genotypes that differ in chlorine uptake have found a correlation between water uptake and chlorine transport (Moya et al., 2003; Brumós et al., 2009). At the same time, the mechanism of the selective effect of chlorine on root hydraulic conductivity through the regulation of aquaporin-mediated water transport through the plasmalemma of root cells is still unknown. Among the main osmoregulators, the importance of potassium and chloride in the formation of xylem currents and root pressure has been reported (Marschner, 2011). It should be added that it has been proposed to consider the role of chloride in the loading and unloading of sugars in phloem sap (Fromm & Eschrich, 1989; Broadley et al., 2012). AtALMT9, a malate-activated vacuolar chloride channel required for stomatal opening, was studied in Arabidopsis plants (De Angeli et al., 2013). MtNPF6.5 mediates chloride uptake and nitrate preference in the roots of *Medicago truncatula*, and encodes three orthologues of *Arabidopsis thaliana* AtNRT1.1/NPF6.3: MtNPF6.5, MtNPF6.6, & MtNPF6.7. MtNPF6.5 functions as a nitrate and chloride uptake carrier when expressed in *Xenopus laevis* oocytes, but is chloride selective. MtNPF6.5 is expressed in roots and is a plasma membrane localization protein (Xiao et al., 2021).

Chlorine as an osmotic agent

Chlorine helps to maintain osmotic pressure in plant cells, which is essential for regulating water uptake and movement. It helps to increase turgor pressure, which is essential for cell expansion and overall plant growth. Chlorine ions are involved in the opening and closing of stomata on the leaf surface. Proper stomatal function is essential for gas exchange (CO_2 uptake and O_2 release) and transpiration, which are essential for photosynthesis and plant cooling.

Chlorine acts as a counterion, balancing the positive charges of potassium (K^+) and other cations in plant cells, which is essential for maintaining electrical neutrality and proper ionic balance in cells. As an osmotic agent, chlorine is involved in the regulation of osmotic potential, helping cells to maintain turgor pressure, which is essential for maintaining the structural integrity of plants. Chlorine affects the flow of water from the soil to the roots and the movement of water throughout the plant, facilitating the transport of nutrients and maintaining cell hydration. As such, chlorine's role as an osmotic agent is essential for maintaining proper water balance, nutrient transport and plant productivity, in addition to its other physiological functions.

Cl^- is essential (in conjunction with K^+) for the proper functioning of plant stomata, thus controlling internal water balance, etc. Potassium is the major cation in plant cells and plays a crucial role in osmoregulation. By influencing the osmotic potential, potassium helps to regulate the uptake and retention of water in cells. Potassium ions are a major contributor to turgor pressure. This pressure is essential for maintaining cell structure, stimulating cell expansion and maintaining overall plant rigidity/efficiency and growth. Potassium ions are key regulators of stomatal movement. The guard cells surrounding the stomata absorb potassium ions, creating an osmotic gradient that draws water into the cells, causing them to swell and open the stomata. Conversely, the release of potassium ions causes the stomata to close. The correct functioning of stomata is essential for gas exchange (absorption of CO_2 for photosynthesis and release of O_2) and transpiration, which cools the plant and facilitates the transport of nutrients through the xylem. Potassium acts as an activator of several enzymes involved in photosynthesis, protein synthesis and other metabolic processes. Its role as an enzyme activator is partly related to its osmotic functions, helping to maintain the ionic environment necessary for optimal enzyme activity. Optimal potassium nutrition is particularly important when plants are exposed to stressful conditions (especially drought and heat), as the demand for the osmotic agent potassium increases. Low potassium levels in plant tissues are known to exacerbate the effects of drought and stress by disrupting osmoregulation and photosynthetic carbon metabolism. Cakmak & Rengel (2024) proposed a new potassium-related mechanism for alleviating drought stress, according to which optimal potassium nutrition can promote the breakdown of carbohydrates in stem tissues and the subsequent mobilisation of these carbohydrates into the grain during grain growth.

Chloride is an important anion in maintaining osmotic balance in plant cells. The highly mobile Cl^- anion is important for balancing the electrical charges of biologically important cations, mainly potassium (K^+), calcium (Ca^{2+}) and protons (H^+), which play an important role in stabilising the electrical potential of cell membranes and regulating pH gradients and electrical conductivity (White & Broadley, 2001; Hänsch & Mendel, 2009). Vacuoles accumulate high concentrations of Ca^{2+} and Na^+, with Cl^- acting as the major counterion. Chloride ions promote cell turgor by maintaining osmotic potential, thereby supporting cell expansion and overall plant growth. Chlorine ions, together with potassium, play an important role in regulating the opening and closing of stomata. They help to create the osmotic gradient necessary for the movement of water in and out of stomata. By regulating stomatal movement, chloride helps to control the exchange of gases necessary for photosynthesis, thus affecting overall photosynthetic efficiency and plant productivity. Chloride helps to maintain electrical neutrality in cells by balancing the charges of other ions, particularly potassium and sodium. This balance is essential for maintaining cellular homeostasis and preventing the toxic accumulation of certain ions.

Chloride also plays an important role in signal perception and transduction, as such diverse signals (light, pressure, elicitors) cause membrane depolarisation by stimulating anion leakage. Cl^- is thought to have a non-specific osmotic function, whereas other anions may provide osmoregulation of plant vacuoles or balance positive charges. There is evidence that Cl^- is a quantitatively and qualitatively superior osmotic agent in plants and cannot be sufficiently replaced by other anionic micronutrients. The efficiency of chlorine anion accumulation in tobacco (*N. tabacum*) shoot tissue was four times higher than that of nitrate anions and three times higher than the accumulation of the sum of sulfate + phosphate anions. This resulted in an increase in negative osmotic potential and higher turgor (Franco-Navarro et al., 2016; Wege et al., 2017). The involvement of Cl^- in osmoregulation and water balance is also relevant for plant cells. As the increase in osmotic pressure caused by mineral fertilisers can inhibit the development of the root system of cultivated plants, the presence of chlorine in soil solutions is important for the positive effect of mineral fertilisers on the development of the plant root system, especially at the beginning of the growing season. Turgor pressure is an important part of plant life; under turgor pressure, the intracellular concentration of an element(s) is higher than the extracellular concentration. The main site of chlorine accumulation is the hypertonic vacuole. This organ is the basis of the processes of cell turgor formation, water absorption and accumulation. Therefore, the removal of chlorine from plant cells occurs mainly through vacuolar compartmentalization. This may be due to the selective stimulation of the vacuolar V-type proton ATPase by chlorine (Sze, 1985), whereas the plasma membrane H^+-ATPase is stimulated by monovalent cations, mainly K^+ (Marschner, 2011). Therefore, chlorine anion fluxes across the plasmalemma and tonoplast are also important factors in the regulation of intracellular osmotic potential, turgor, and cell volume regulation in plants (Zonia et al., 1981; 2002). Since the cytosolic chlorine concentration is always higher than the extracellular concentration, the equilibrium potential for Cl^- ions is usually positive. Activation of Cl^- channels promotes an intense release of Cl^- anions from plant cells with a corresponding depolarization of the plasma membrane.

Osmoregulation of water accumulation, and turgor under the influence of chlorine causes elongation of plant cells. When the chlorine content is at the level of macronutrients, the anion is distributed throughout the plant. The maximum concentrations are observed in formed leaves with vacuolar compartmentalization. At lower concentrations of the element, minimally sufficient for a microelement, chlorine accumulates in tobacco (*N. tabacum*) plants, mainly in young leaves, indicating its importance for plant cell growth. Microconcentrations of chlorine are also important for the rate of cell division (Terry, 1977).

In rapidly expanding tissues, such as elongated root and shoot cells, chloride accumulates in the tonoplast and acts as an osmotically active solute (Maas, 1986; Hager & Helmle, 1981). This transport of chloride into the tonoplast occurs in conjunction with the activity of the tonoplast proton pump ATPase, which is specifically stimulated by chloride (Churchill & Sze, 1984). This osmoregulatory function in certain tissues requires chloride concentrations that are atypical for micronutrients (Gerson & Poole, 1972; Flowers, 1988). Chloride accumulation in plant cells increases tissue hydration (Heckman, 1990) and turgor pressure (Christensen et al., 1981). This osmotic function of chloride interacts closely with potassium to promote cell elongation and growth. The importance of this osmoregulatory role of chloride in plants depends on the growing conditions and the availability of alternative anions, such as nitrate, which can act as substitutes for chloride.

When chloride is supplied at concentrations above the micronutrient requirement and below the toxicity threshold (1–5 mM Cl^-), Cl^- plays a role in osmoregulation and cell turgor by stimulating cell size and water balance in leaves. The result of increased leaf cell size is a decrease in stomatal density, which in turn reduces stomatal conductance and water use. In addition, Cl^- increases the diffusive conductance of the mesophyll to CO_2 (gm), which allows the photosynthetic capacity of plants to be maintained despite the reduction in stomatal conductance, leading to an overall higher WUE in well-watered plants (Franco-Navarro et al., 2014, 2019).

The critical level of Cl deficiency is about 2 mg/kg DM. However, Cl concentrations in plants generally exceed this critical deficiency level by two orders of magnitude and become important for osmotic regulation and water metabolism in plants (Marschner, 1995). In this concentration range, chlorine becomes the dominant inorganic anion in the vacuole. The concentration of chlorine in phloem sap can reach 120 mM and is thought to play a role in the loading and unloading of sugars in the phloem (Fromm & Eschrich, 1989; Moran, 2007). Chloride, together with potassium (K^+), has a specific osmoregulatory function in the tapetum of cereals (Heslop-Harrison & Reger, 1986). The bracts of cereals such as *Pennisetum americium* L. often elongate within minutes during flowering due to cell stretching, and this is mediated by the rapid transfer of K^+ and Cl^- from the surrounding tissue to the primordium.

Cl^- thus has important functions in osmoregulation at different levels. At high chloride concentrations in the plant (50–150 mM Cl^-), it is the main osmotic agent in the vacuoles of the main tissues, together with potassium. At low concentrations (1 mM Cl^- or less), these osmoregulatory functions of chlorine are probably restricted to specialised tissues or cells, such as the branching zones of roots and shoots, generative organ cells and stomata, where chloride concentrations can be much higher (Marschner, 1995; Xu et al., 1999). In summary, potassium and chloride often act together/complementarily or synergistically to regulate osmotic balance and turgor pressure. Their combined presence ensures that cells maintain the correct ionic and osmotic conditions necessary for growth and function. Both ions are involved in stomatal regulation, where they help to control the osmotic gradients that drive the movement of water in and out of stomatal cells, facilitating the opening and closing of stomata. Potassium and chloride ions complement each other in the processes of nutrient uptake and transport. For example, chloride can act as a counterion to potassium, facilitating the efficient transport of nutrients in the plant. Adequate but balanced levels of potassium and chloride in the soil are essential for optimum plant growth and productivity. Excess or deficiency of either nutrient can disrupt the osmotic balance and adversely affect plant productivity. Potassium and chlorine play an important role as osmotic agents in plants, contributing to osmoregulation, maintenance of turgor pressure and general cellular homeostasis. They are essential for stomatal function, nutrient transport and various metabolic processes. Proper management of potassium and chloride levels through balanced fertilisation and irrigation is essential to optimise plant growth, development and productivity.

Redistribution of Cl

During plant growth, chloride moves from the root to the shoot through the xylem, and redistribution to the aboveground levels of the plant occurs through the phloem. Long-distance transport in the phloem occurs in sieve tubes, and the sieve tube/satellite cell complex is a functional component of the transport mechanisms of angiosperms (Van Bel, 2003). Cl^- loading from the phloem occurs mainly in the stem and leaves of different layers and is transported along sieve tubes under the influence of the osmotic potential gradient (Marschner, 2011; Liesche & Schulz, 2013). In the phloem, chloride is relatively mobile (Lessani & Marschner, 1978), and its recycling in plants (recycling rate is defined as the ratio of nutrient fluxes in the phloem and xylem) is about 20%, and the concentration of the anion in the phloem is directly correlated with the concentration of chlorine in the nutrition system (White & Broadley, 2001). It is also thought that chlorine in phloem sap is involved in loading and unloading sugars in the phloem (Fromm & Eschrich, 1989; Moran, 2007).

Chloride – nitrate – counter ions

Both chloride and nitrate are important nutrients, but nitrate is needed in much larger quantities as it is the main macronutrient, while chloride is mainly classified as a micronutrient. Chloride and nitrate have similar physical and chemical properties that allow them to interact in cellular processes such as electrical balance and osmoregulation. As both anions share common transport mechanisms, Cl^- is considered an antagonist of nitrate (Rosales et al., 2020). Chloride is mainly involved in osmoregulation, stomatal function and photosynthesis. Nitrate serves mainly as a nitrogen source for the synthesis of proteins and nucleic acids, stimulating plant growth and metabolic processes. Nitrate deficiency has a more serious and widespread effect on plant growth and development because of its role in fundamental processes such as protein synthesis and metabolism. Chloride deficiency affects water balance, stomatal function and photosynthesis, but is less common and usually less severe. Plants usually require nitrates in greater amounts than chlorides. Nitrates are macronutrients and their requirement is relatively high to support rapid growth and development, whereas chloride is required in smaller amounts. So while both chloride and nitrate are essential for plant health, they have different functions. Chloride is essential for maintaining osmotic balance, ionic equilibrium and promoting photosynthesis, while nitrate is essential for providing the nitrogen needed to synthesise important biomolecules, support growth and metabolic processes.

Chloride is the most abundant anion in plants, an important macro- or microelement, and an osmoregulator. Nitrate is an important source of nitrogen. Interactions between chloride and nitrate are important during active plant growth (Cubero-Font et al., 2016; Colmenero-Flores et al., 2019). Nitrate and chloride form a charge balance and regulate the amount of turgor (Cubero-Font, 2017; Geilfus, 2018a; Colmenero-Flores et al., 2019). Nitrate and chloride have similar physical properties in solution and enter/exit via common anion transport mechanisms with low selectivity for these anions. Nitrate is absorbed and rapidly incorporated into nitrogen metabolism, whereas chlorine is absorbed and accumulates in plants. Therefore, it was previously thought that the interaction between nitrate and chloride was mainly antagonistic. For example, at high levels of chlorine accumulation, nitrate content decreases and vice versa (Xu et al., 1999; Anjana et al., 2007). It has also been shown that in the presence of external nitrate pools, chlorine uptake by plant roots is inhibited (Glass & Siddiqi, 1985; Iglesias et al., 2004). On the contrary, high levels of chlorine inhibit the supply and accumulation of nitrate in plants. This suggests that the transport mechanisms may be largely identical for both anions (Xu et al., 1999; Teakle & Tyerman, 2010).

An antagonistic interaction between chloride and nitrate has been observed in many crops, indicating that (high) chloride concentrations negatively affect nitrate uptake and mobilisation and thus NUE (Buwalda & Smith, 1991; Cerezo et al., 1997; Xu et al., 1999). Therefore, chloride has been considered a toxic agent because of its toxic effects on saline soils and its pronounced antagonistic interaction with nitrate. For many years, chloride was thought to inhibit nitrate uptake by higher plants, thereby reducing NUE and productivity. More recently, chloride is now considered to be a critical determinant of WUE & NUE and productivity. At the same time, its role in inhibiting nitrate supply remains in the background.

Chloride accumulates rapidly in plant tissues, and its accumulation in vacuoles requires significantly less energy than the accumulation of nitrate. Considering this interaction, it can be hypothesised that chloride compartmentalisation can reduce nitrate accumulation in vacuoles, leading to higher nitrate incorporation into plant metabolism and, consequently, higher NUE by plants (Colmenero-Flores et al., 2019). The results of Rosales et al. (2012; 2020) confirm the previously known role of chlorine as an osmoregulator in plants (Flowers, 1988; Franco-Navarro et al., 2016; Colmenero-Flores et al., 2019). The authors discuss the interaction between chlorine and nitrate, noting that when chlorine is compartmentalized in vacuoles, nitrate is actively absorbed and included in nitrogen metabolism, which is impossible when nitrate is sequestered in the vacuole. In the absence of sufficient chlorine in the soil or in the presence of high nitrate concentrations, the latter can be compartmentalized (Siddiqi et al., 1990; Radcliffe et al., 2005). Thus, accumulation of the macronutrient Cl^- decreases compartmentalization of NO_3^- in the vacuole and facilitates its assimilation, thereby increasing NUE and plant biomass. According to the same premise, Cl should also play an adaptive role by improving plant growth under conditions of low N availability, which can also be explained by the anion-selective transport, and compartmentalization mechanism (Wen et al., 2017). The relationship between chloride and nitrate in higher plants is not limited to antagonistic interactions. Cubero-Font et al. (2016) described a molecular mechanism that determines the rate of NO_3^- /Cl^- accumulation rate in aboveground organs of *Arabidopsis thaliana* based on the Cl conductance of the AtSLAH3 channel, which in turn is regulated by environmental signals.

At the same time, Peinado-Torrubia et al. (2023) showed that chlorine nutrition in the range of macronutrient concentrations did not affect the efficiency of nitrate uptake, and the same NO_3-content was observed in root and xylem sap. Treatment with 6 mM Cl caused a decrease in leaf nitrate content with a simultaneous increase in nitrate assimilation and NUE. Chloride uptake increased the accumulation of transcripts and the activity of most of the enzymes involved in the assimilation of NO_3^- into amino acids, respectively increased the accumulation of organic nitrogen, mainly proteins. A decrease in the glycine/serine ratio and an increase in ammonium accumulation indicate a higher activity of the photorespiratory pathway in plant leaves under the influence of chlorine. Chloride increased the level of transcripts of genes encoding enzymes of the photorespiratory pathway. The authors conclude that higher nitrate use under the influence of chloride is associated with increased nitrate assimilation and photorespiration, which may contribute to the formation of ammonia, reducing agents and intermediates of C-N synthesis, and the corresponding plant growth. Chloride as a trace element increases water use efficiency through anatomically determined reductions in stomatal conductance and increased mesophyll diffusion to CO_2.

Chloride has traditionally been regarded as detrimental to agriculture because of its toxic effects in saline soils and its antagonistic interaction with nitrate (NO_3^-), which interferes with NO_3^- uptake. Cl^- is widely thought to antagonize NO_3^- uptake and accumulation in higher plants, thereby decreasing crop yields. The annual canarygrass (*Phalaris canariensis* L.) has a greater response to the application of chloride fertilizer than other cereal crops. The annual canarygrass sensitivity to Cl^- suggests that it could be used to study the role of Cl^- in cereal development and yield (Earl May & MacGregor, 2022).

Effect of chlorine on nitrogen use efficiency (NUE)

Chlorine can affect plant NUE through several mechanisms. NUE is the ability of plants to use available nitrogen efficiently. Chloride helps maintain osmotic balance and turgor pressure in plant cells, which promotes efficient water uptake and transport. Adequate water is critical for the transport of nutrients, including nitrogen, within the plant. By maintaining cell turgor, chloride ensures that root cells are in optimal condition for nutrient uptake, including nitrate uptake from the soil. Chloride ions are involved in the regulation of stomatal opening and closing. Efficient stomatal operation improves gas exchange and provides sufficient carbon dioxide for photosynthesis. Increased photosynthetic efficiency due to proper stomatal function supports overall plant growth and metabolism, resulting in better utilization of nitrogen for the synthesis of essential compounds.

Chloride can act as a cofactor for certain enzymes involved in photosynthesis and other metabolic processes. Increased enzyme activity contributes to better assimilation of nitrogen into amino acids and proteins.

Interaction of Cl^-/NO_3^-, and NUE

Chlorine (as chloride ion Cl^-) and nitrate (as nitrate ion NO_3^-) are important nutrients for plants, but their interaction can affect plant growth, nutrient uptake, and overall plant health. Understanding how these ions interact can help manage soil fertility and optimize plant nutrition. Chloride and nitrate ions can compete for uptake by root transporters because they are both anions. High concentrations of one ion can inhibit the uptake of the other due to competition at root uptake sites. Plant roots have specific transport proteins that may have different affinities for different anions. The presence of a high concentration of one anion can reduce the efficiency of uptake of other anions due to competitive inhibition. High levels of chloride in the soil can contribute to soil salinity, which can negatively affect the uptake of nitrate and other essential nutrients, reduce water availability, and alter the osmotic balance of the soil. Excessive chloride can lead to nutrient imbalances in the soil, affecting the overall availability of nutrients, including nitrate, for plant uptake.

Both chloride and nitrate play a role in maintaining osmotic balance in plant cells. Chloride helps regulate turgor pressure and stomatal function, while nitrate, as the primary source of nitrogen, supports growth and metabolic functions. The balance between these ions is essential for optimal plant health. Both chloride and nitrate help maintain the anion-cation balance in plant cells. Proper balance is essential for electrical neutrality, ionic balance, and overall cellular function.

Chloride is involved in the process of photosynthesis as part of the oxygen generating complex in PSII. Nitrates provide the nitrogen pool necessary for the synthesis of chlorophyll and other important compounds required for photosynthesis. Nitrate is the primary source of nitrogen and is reduced to ammonium (NH_4^+) for incorporation into amino acids and proteins. Chloride is not directly involved in nitrogen metabolism, but its presence can affect the efficiency of nitrate uptake and assimilation.

Chloride has been shown to increase the level of transcripts of genes encoding photorespiration enzymes. Accordingly, the interaction between different cellular organelles involved in photorespiration was revealed. Thus, it has been demonstrated that the increase in nitrate assimilation efficiency and NUE induced by chlorine nutrition is mainly due to the stimulation of photorespiration, which may contribute to the formation of ammonia, reducing agents and intermediates that optimize C-N recycling and plant growth. The role of chlorine as a potential tool to regulate NUE in plants has been confirmed (Peinado-Torrubia et al., 2023).

Cl^- accumulates in the leaves of tomato (*S. lycopersicum*) at the macronutrient level and promotes growth through osmotic, physiological, metabolic, anatomical and cellular changes that improve plant productivity under optimal nitrate nutrition. Cl^- application has been shown to reduce nitrogen deficiency symptoms and improve plant growth under low nitrate conditions. Cl^- dependent stress tolerance under low nitrogen conditions is the result of: more efficient use of available NO_3^-; improved regulation of osmotic and water status of plants; improved stomatal conductance and photosynthetic rate; and improved antioxidant response. The authors believe that favorable Cl^- levels increase the ability of plants to grow better with lower NO_3^- requirements and to withstand nitrogen deficiencies, contributing to more sustainable and resilient agriculture (Lucas et al., 2024).

Ensuring a balanced supply of both chloride and nitrate is critical. Excess chloride can reduce nitrate uptake, while insufficient chloride can impair stomatal function and osmoregulation.

In areas with high soil salinity, the use of salt-tolerant crop varieties can help prevent the negative effects of high chloride levels on nitrate uptake and overall plant health. Proper irrigation practices can help flush excess chloride out of the soil, reducing its negative impact on nitrate availability and uptake.

The interaction between chloride and nitrate is complex, involving competition for uptake, effects on soil salinity, and the need for a balanced supply of nutrients for optimal plant growth. Managing nitrate levels through balanced fertilizer application, soil testing and appropriate irrigation practices can help optimize plant nutrition and increase yields. Understanding these relationships helps develop better nutrient systems to ensure healthy plant growth and sustainable soil management.

Potential negative impact of chlorine on NUE

Excessive chloride in the soil can contribute to salinity, which can increase the likelihood of negative effects on plant water uptake and lead to osmotic stress. Osmotic stress impairs root function and reduces the efficiency of nitrogen uptake. High chloride levels can compete with nitrate for uptake by root transporters, potentially reducing nitrate uptake and availability for plant metabolism.

High chloride levels can have antagonistic effects on other nutrients, affecting the uptake of other essential nutrients, including nitrate. This imbalance leads to a decrease in NUE because it is more difficult for plants to absorb and use available nitrogen efficiently. Thus, chlorine affects the efficiency of nitrogen use by plants through its role in osmoregulation, stomatal function and enzyme activation. While optimal chloride levels can increase NUE by supporting overall plant health and nutrient uptake, excessive chloride levels can lead to soil salinity and nutrient imbalances that negatively affect NUE. Managing chloride levels through balanced fertilizer application, soil testing and field irrigation is essential to maximize NUE and ensure plant growth and development.

NUE is an important agricultural trait defined as biomass or seed yield per gram of applied nitrogen fertilizer, determines plant yield per unit of applied N. It is an important agricultural trait for reducing the overuse of chemical fertilizers, which brings significant benefits to farmers and the environment (Han et al., 2016; Fan et al., 2017). Nitrate uptake and release are the key factors regulating NUE (Wang et al., 2012). Given the close interaction between Cl^- and NO_3^-, it is likely that Cl^- can significantly affect NUE. High concentrations of Cl^- can reduce NUE, possibly by limiting translocation of NO_3^- to the shoot and by Cl^- occupying space for storage of NO_3^- in shoot vacuoles (Teakle & Tyerman, 2010). Cl^- reduces NUE not only by limiting root uptake of NO_3^-, but also by limiting translocation from root to shoot or vacuolar compartmentalization. Several studies have reported a negative effect of Cl^- on root NO_3^- uptake (Siddiqi et al., 1990; Cerezo et al., 1997), which worsens NUE. Nevertheless, the net uptake of NO_3^- is the result of the difference between the influx of NO_3^- mediated by active transport and its passive leakage through anion channels. The efflux of root anions into the rhizosphere may be important for regulating H^+-ATPase activity, maintaining H^+ charge balance (Hawkesford et al., 2012), or regulating plant cell growth (Planes et al., 2015).

The release of chlorine from root cells can inhibit the release of nitrate and thus prevent the loss of this form of nitrogen. Interactions at the level of differential release of chlorine while retaining nitrate may underlie the increase in NUE (Wege et al., 2017). Vacuolar compartmentalization of NO_3^- to some extent determines the level of accumulation of this anion in plants. Previously, a relationship between the levels of nitrate accumulation in plant tissues and NUE levels has been demonstrated. Thus, in the *A. thaliana* aha2/aha3 line, a reduced ability to compartmentalize nitrate was observed in the absence of this function of the vacuolar ATPase, leading to an 80% reduction in NO_3^- accumulation and a corresponding inhibition of growth processes (Krebs et al., 2010).

In *Brassica napus*, an increase in NUE correlates with higher accumulation of NO_3^- in shoots and roots (Han et al., 2016). Thus, plants utilize Cl^- as a preferred osmoregulatory molecule, while NO_3^-, an important source of nitrogen for terrestrial plants, should be preferentially taken up and utilized as an osmolyte when Cl^- is deficient in the soil (Franco-Navarro et al., 2016) or due to high levels of NO_3^- in the environment (Glass & Siddiqi, 1985; Siddiqi et al., 1991; Radcliffe et al., 2005).

The addition of a salt supplement containing 5 mM Cl^- to the nutrient solution reduced the NO_3^- content in tobacco (*N. tabacum*) leaves by 6.5 times, while the addition of salts containing equivalent amounts of phosphate + sulfate salts reduced the NO_3^- content in leaves by 1.7 times (Franco-Navarro et al., 2016). Although plants treated with Cl^- had 3.6 times lower NO_3^- concentration than plants with phosphate-sulfate treatment, they had higher biomass. This indicates that Cl^- improves NUE despite a significant decrease in foliar NO_3^- storage. And Cl^- was transformed from an antagonist of NO_3^- to a nutrient that promotes more efficient N use. When NO_3^- is available, active transport mechanisms (often more selective for NO_3^- than for Cl^-) favor NO_3^- influx by inhibiting Cl^- uptake (Glass & Siddiqi, 1985; Wen & Kaiser, 2018). When NO_3^- availability is low, Cl^- influx is less inhibited, increasing root uptake and intracellular Cl^- concentration, which can substitute for NO_3^- in the osmotic function, making more efficient use of available N.

Cl^- nutrition, provided at macronutrient level concentrations, improves NUE through the simultaneous induction of both NO_3 assimilation and photorespiration pathways. Changes in the forms of N in shoots are well explained by the effect of Cl^- on the synthesis and activity of enzymes involved in these pathways of N metabolism. The increase in NH_4^+ and a significant decrease in the Gly/Ser ratio indicate the participation of photorespiration in the metabolic reactions to Cl^- nutrition. And the higher content of organic N in leaf tissues in combination with higher soluble proteins and lower free amino acids in Cl^- treated plants indicates that Cl^- promotes synchronized assimilation of N and C, producing more biomass in plants. It is suggested that optimal application of Cl^- in crop fields may be useful to improve biomass production even when N availability is scarce. Thus, Cl^- should be considered as a good candidate to reduce both N use on agricultural lands and the harmful NO_3^- content of vegetables (Peinado-Torrubia et al., 2023).

It has been shown (Abou Seeda et al., 2021) that Cl^-, in contrast to impairing NO_3^- nutrition, promotes NO_3^- utilization and improves NUE in plants. This is largely due to the improvement of N-NO_3^- utilization efficiency (NUTE) by Cl^-, which has little to moderate effect on N-NO_3^- uptake efficiency (NUPE) when NO_3^- is used as the sole N source. Clear positive correlations were found between leaf Cl^- and NUE/NUTE or plant growth at both intra- and interspecific levels. The optimal ratio of NO_3^- to Cl^- will be a useful tool to increase the yield and quality of crops, the sustainability of agricultural land, and to reduce the negative environmental and human health impacts of NO_3^-.

Interaction of Cl with other biologically important ions in higher plants

Chloride can be toxic to crops through antagonistic interaction with nitrates, especially on saline soils. Thus, high doses of chloride can reduce crop productivity. Annual canarygrass (*P. canariensis*) responds better to chloride fertilizers than other cereals. To find out how chloride affects this crop, long-term field experiments were conducted to determine the effects of macro- and microelements with chlorine. Field trials were conducted at six locations in Saskatchewan for 4 years using a randomized complete block design. *P. canariensis* yield was directly related to chloride nutrition in 7 of the 21 locations of the study, with a 70% increase in yield over 4 years. Both methods of application – side/belt or surface application of chlorine were effective. It should be noted that chloride affected the canary grass during the period of generative development. This peculiarity of the anion's action on cereals has been observed during the last 2 decades on the plants of various varieties of winter wheat in the Experimental Agricultural Production of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine. This peculiarity of chlorine action, its preference in the second half of the growing season, on light soils of Polissya of Ukraine may be primarily due to rapid leaching of the element at the beginning of spring vegetation of winter crops. As for the experiments in Canada, nitrogen was the main nutrient with which Cl^- interacted in this study. Canary grass was not more sensitive than other cereals to phosphorus, potassium, sulphur, zinc, copper, manganese and boron. Consequently, in Cl-sensitive areas, nutrient applications are ineffective in the absence of Cl fertilizers. In addition, the sensitivity of annual canarygrass to Cl suggests that it can be used to study the role of Cl in cereal crop development and grain yield (Earl May & MacGregor, 2022).

Note that during the period of generative development, the response to chloride was maximal. A similar response of cereals to chlorine was observed in PLS studies (see below).

Chlorine also affected the uptake of other macro- and microelements in dicotyledonous plants. Chen et al. (2010) summarized that Cl slightly affected the uptake and utilization of nitrogen (N), phosphorus (P), K, calcium (Ca), manganese (Mn), silicon (Si), sulfur (S), zinc (Zn), magnesium (Mg), iron (Fe), and copper (Cu) in potato (*S. tuberosum*) (Ma et al., 1993; Zhong & Ma, 1993). Studies on rice (*O. sativa*), soybean (*G. max*), cabbage (*B. oleracea*), strawberry (*Fragaria × ananassa* (Weston) Duchesne ex Rozier), peanut (*Arachis hypogaea* L.), and spring wheat (*T. aestivum*) showed that Cl has the effect of enhancing the action or antagonizing the action of other ions such as nitrate (NO_3^-), orthophosphate ($H_2PO_4^-$), and K^+ in plants. It has been found that the levels of nitrate (NO_3^-) and orthophosphate ($H_2PO_4^-$) depend on the concentration of Cl^-, and the element has an extremely competitive effect on the absorption of NO_3^- (Huber & Watson, 1974; Huber et al., 2012; Li et al., 2017). Studies have shown that the effect of Cl^- nutrition on phosphorus metabolism remains controversial (Ma et al., 1993): the use of Cl^- fertilizers did not reduce the P content of potato (*S. tuberosum*) grown on phosphorus-rich soil, and the concentration of phosphorus in old leaves and stems even increased. In the soybean study, however, the opposite was true. It was shown that relatively higher concentrations of Cl in plants affect P transport to seeds. It is likely that Cl can only affect P uptake when the Cl concentration exceeds 400 mg/kg. The effect of Cl on K also depends on the level of external Cl supply. Even at low levels of external Cl supply, plants take up more K^+ to balance the negatively charged Cl^- anions, but when Cl^- levels are high enough to be toxic, K^+ uptake in plants decreases due to impaired cellular metabolism.

Studies on kiwifruit (*A. deliciosa*) showed (Chen et al., 2010) that increasing the concentration of Cl^- in the nutrient solution did not have a corresponding effect on the concentration of potassium in the leaves. On the contrary, the concentration of chloride in the leaves was partially dependent on the potassium status of the plant. Increasing the potassium concentration led to a decrease in the chloride concentration in the leaves at each yield level. It was also suggested that this effect was due to the simple accumulation of chloride in the tissues of severely potassium deficient plants rather than a specific physiological effect (Smith et al., 1987). As reported by Schnabl & Raschke (1980) there is a closer relationship between K and Cl, for example in the defense cells of some plants. It was reported that the Cl- status of the plants should have increased, which is also confirmed by the data (Marschner, 1995). Compared to the urea treatment (with the same amount of N), the content of Ca, Mg, K, Mn, Zn and Cu increased in radish (*Raphanus sativus* L.) and Chinese cabbage (*Brassica rapa* L. var. *pekinensis* (Lour.) Kitam. = *Brassica rapa* subsp. *pekinensis*) under ammonium chloride (NH_4Cl) treatment). These studies have shown that different plant species differ in the peculiarities of chlorine interaction with other ions.

Interactions between halogens

CIMMYT research also mentions interactions between halogens when chloride uptake by wheat (*T. aestivum*) plants is inhibited in the presence of bromide (Snowball & Robson, 1991). The uptake of chlorine ions by barley roots from NaCl solution under the influence of ions similar in chemical properties to bromine has been studied. It was found that chlorine and bromine ions are transported into plant cells by the same vectors, i.e. the effect of bromine ions is antagonistic to the uptake of chlorine (Abdyev & Kasumov, 2011).

The interaction between halogens may be essential in limiting the availability of chlorine to plants at high doses of fluoride in phosphate fertilizers, thus initiating the development of PLS. The source of the problem lies in the fact that raw phosphate ore contains high concentrations of fluoride, typically between 20,000 and 40,000 ppm (equivalent to 2% to 4% of the ore) (Wang et al., 2023; https://fluoridealert.org).

Effect of chlorine on plant diseases

The researchers Carl Sprengel (1787–1859), Justus von Liebig (1803–1873) and Jean-Baptiste Boussingault (1802–1887) are regarded as the founders of agricultural chemistry in respect to the use of chemical fertilizers and chemical plant protection means. Since publication in 1989 of "Soilborne Plant Pathogens: Management of Disease with Micro- and Microelements" edited by Art Engelhard the knowledge on that topic has greatly advanced and the reviewed book summarizes the progress made in this area and points the new goals for research (Lipa, 2008).

It is now clear that nutrients can influence plant disease susceptibility (Barker, 2007; Gupta et al., 2017; Tripathi et al., 2022; Jomova et al., 2022; Bera et al., 2024). Much of the literature on the biological activity of Cl^- is devoted to its role as a major component of salinity stress, which can reduce plant resistance to pathogens and diseases. The classic work in this area is Wade H. Elmer's chapter Chlorine and Plant Disease (Elmer, 2023); and the monograph Mineral Nutrition and Plant Disease itself has been reprinted nine times, from 2007 to 2021. Chloride increases plant resistance to diseases that require relatively large amounts of Cl^- (Huber et al., 2012; Weinmann et al., 2023). These doses are much higher than those required for its use as a micronutrient, but much lower than those required to induce salinity tolerance effects (Colmenero-Flores et al., 2019).

The vast majority of studies on chlorine nutrition are devoted to the effect of the element on the incidence of spiked grain crops. The chlorine content in wheat (*T. aestivum*) is 0.9 g/kg, in wheat straw 6 g/kg; in barley (*H. vulgare*) 1.2 g/kg (www.feedtables.com/content/chlorine). Higher levels of chlorine accumulation in barley (*H. vulgare*) coincide with certain requirements of the crop for chlorine supply during the growing season. In barley (*H. vulgare*) crops, in almost all varieties and hybrids, the formation of PLS during the period of grain filling is associated with chlorine deficiency.

Many diseases of various crops are suppressed by Cl at the macronutrient level. These include maize stem rot, *Gibberella zeae* (Warren et al., 1975) or *Gibberella fujikuroi* (Younts & Musgrave, 1958), wheat stripe rust (*Puccinia striiformis*) (Taylor et al., 1983), and pearl millet (*Pennissetum typhoids* L.) downy mildew (*Sclerophthora macrospora*) (Hegde & Karande, 1978). Yellow rust (*P. striiformis*) of winter wheat (*T. aestivum*) was one of the first leaf diseases to be inhibited by Cl^- (Russell, 1978). Chloride may also inhibit wheat septoria and the development of root rot caused by *Gaeumannomyces graminis* var. *tritici* (*T. aestivum* L.) directly or indirectly through its effect on overall productivity. The susceptibility of winter wheat (*T. aestivum*) plants to colonization by *G. graminis tritici* can be reduced by regulating the water potential in the plant. Since the osmotic potential of wheat (*T. aestivum*) plants is readily altered in the presence of Cl, fertilization with Cl salts allows the components of the plant water potential to be actively manipulated to suppress root rot (Christensen et al., 1981). The use of both KCl and $CaCl_2$ reduced the severity of spots caused by *Cochliobolus sativus*, a pathogen of a severe disease of wheat (*T. aestivum*), but the first compound reduced the level of disease damage by 11% more than the second (Sharma et al., 2006).

Huber & Wilhelm (1988) reported an inhibitory effect of chloride on blackleg and leaf rust in combination with ammonium fertilization. This relationship between high chloride and ammonium fertilization suggested a link with chloride-inhibited nitrification and the effect of acidification in the rhizosphere (Golden et al., 1981) as a prerequisite for direct inhibition of *Gäumannomyces graminis* hyphae or indirectly through increased manganese availability. A number of these studies have distinguished the effect of Cl^- from other related cations in the control of numerous plant diseases. In the conditions of experimental agricultural production of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (Kyiv region), the manifestations of PLS of winter wheat (*T. aestivum*) variety Novosmuglyanka were studied with simultaneous damage to plants from the basal part to the ear inclusive by powdery mildew (*Blumeria graminis* (DC.) (*Erysiphe graminis* DC. f. *tritici* Em. Marchal) (Fig. 2). At the same time, the disease was not controlled by the composition of fungicides propiconazole + ciproconazole, unlike the variants with chlorine in the nutrition systems, where PLS on the leaves was not observed, and powdery mildew was well controlled by the introduction of propiconazole with ciproconazole from the beginning of the restoration of spring vegetation, from the BBCH26 phase and during the period of generative development. The importance of component technologies in crop rotation for disease and weed control is mentioned in the review (Elmer, 2023). It has been shown that the replacement of sodium chloride (rock salt) with 2.4-D herbicides, which occurred in the 1940s for weed control in asparagus (*A. officinalis*), led to the emergence of *Fusarium* and root rot in asparagus plants.

Fig. 2. Under chlorine deficiency on the leaves of winter wheat in the period BBCH51-BBCH71, the appearance of physiological leaf spots (PLS) was observed. At the same time, the ear of plants was affected by powdery mildew (*Blumeria graminis* (DC.) Speer.), which is uncharacteristic of variants with the fall application of potassium chloride

Cl has a positive effect on the control of diseases of wheat like root rot, take-all, powdery mildew, glume blotch, tan spots, stripe rust, and leaf rust. It also helps reduce the severity of diseases like stalk rot (*Gibberella zeae*) and leaf blight (*Helminthosporium* spp.) in corn, sudden death syndrome in soya bean (*F. solani*), and physiological disorders of potato like hollow heart and brown centre in potato (Dordas, 2008).

Cl is reported to help in the control of a number of diseases such as stalk rot of corn, Northern corn leaf blight, and downy mildew of millet (Graham &Webb, 1991; Mann et al., 2004; Singh et al., 2015).

Several studies have considered possible mechanisms of manganese uptake growth in the presence of chlorine. For example, research (Ghiorse, 1988; Nealson, 2006) suggests that at neutral soil pH, an increase in glucose and organic acids may promote microbial oxidation of manganese by using these substrates and a corresponding decrease in pH. Alternatively, the presence of chloride may induce manganese-reducing microflora. It has also been suggested that manganese may enhance the formation of lignin protective barriers of the cell wall by activating secondary metabolism via shikimate (Huber & Wilhelm, 1988). Lignin barriers have been implicated in reducing damage caused by *Fusarium* pathogens. Increased activation of manganese-dependent peroxidase has also been implicated in the control of plant diseases (Kumar & Arora, 2022).

In addition to the use of chlorine-containing fertilisers, e.g. KCl, etc., chlorine dioxide (ClO_2) can be used in chlorination technologies, for example to control postharvest stem-end rot of citrus fruit caused by *Lasiodiplodia theobromae*. The growth of *L. theobromae* mycelium on potato dextrose agar (PDA) was completely inhibited by 24 h exposure to ClO_2, which was provided by 0.5 g of solid granular ClO_2-generating mixture in a 7.7 litre airtight container. The *in vivo* experiments were carried out on artificially inoculated Tango mandarins and naturally infected American Early Pride mandarins. When ClO_2 treatment was started 0–6 hours after inoculation, the development of decay was significantly reduced compared to the control, and higher doses of ClO_2 were more effective. ClO_2 treatment had no negative effect on fruit quality characteristics. In addition, no visible physiological defects such as browning and skin bleaching were observed on ClO_2-treated fruit. These results suggest that ClO_2 gas can be used as a component of an integrated citrus postharvest decay management system to minimise fruit losses (Zhong et al., 2021). Thus, chlorination of plants is an important component of disease control. In modern understanding, the regulation of osmotic potential and increased manganese uptake, acting in concert, form a disease control mechanism (Elmer, 2023). In addition, chlorine-mediated changes in root microcenosis can provide biological control of disease, contributing to productivity and systemic resistance to pathogens (Cayanan et al., 2009; Elmer, 2023).

Chlorine fertilizers

The global mineral fertilizer market does not distinguish and therefore does not position a special group of fertilizers to supply plants with chlorine. The widely used potassium chloride is sufficient for this purpose, providing 0.9 kg of chloride per kg of potassium. Chloride fertilizers include NaCl, $CaCl_2$, $MgCl_2$ and NH_4Cl (Table 1). These salts are highly soluble in water or soil and are readily available for chloride uptake by plants.

Organic agriculture, which discourages the use of KCl and most salt-based fertilizers, obtains chloride primarily from manure and other natural sources.

Table 1
Mineral fertilizers containing chlorine

Fertilizer	Formula	Chlorine content, %
Sodium chloride	$NaCl$	61
Potassium chloride	KCl	47
Calcium chloride	$CaCl_2$	64
Ammonium chloride	NH_4Cl	66
Magnesium chloride	$MgCl_2$	74

Warnings about the long-term use of chlorine-containing fertilizers

With the long-term use of Cl-containing fertilizers such as ammonium chloride and potassium chloride, a significant amount of Cl^- enters the agricultural ecosystem as satellite ions and is incorporated into the nutrient cycle in soil and plants. Recently, many studies have focused on the effects of Cl^- fertilizers on crop yield and quality (Xu et al., 1999; Mengel et al., 2009), while less attention has been paid to the effects of Cl^- fertilizers on soil physical and chemical properties. In areas with high rainfall, Cl^- is easily leached and does not accumulate in soils, but long-term use of chlorine-containing fertilizers can significantly increase soil Cl^- (Zou & Gao, 2004). Chloride is a strong acidic anion that can directly cause soil acidification, which can also lead to the removal of a large amount of basic ions, further exacerbating soil acidification. Long-term use of chlorine-containing chemical fertilizers, especially at high application rates, can reduce nutrient availability in some soils because Cl can cause leaching of nutrients, making them unavailable for uptake by rice plants (Zou & Gao, 2004). Long-term use of NH_4Cl and KCl fertilizers reduced soil pH to 5.81 (Zhou et al., 2014). Soil acidification is an important aspect of soil quality and can have many negative impacts on ecosystems. For example, it can reduce the number of root hairs in plant root systems (Nadelhoffer, 2000; Braun et al., 2005), decrease crop yields (Zhao et al., 2010), and reduce biodiversity (Bobbink et al., 2010; Schroder et al., 2011; Zhang et al., 2015).

Wang et al. (2023a) conducted a long-term study (35 years) on the long-term effects of chlorine-containing fertilizers on soil ions and soil acidification in a peanut-corn rotation on brown soils in northeastern China. It was found that the use of chlorinated fertilizers significantly increased the Cl^- content in the soil profile compared to the untreated control. The Cl^- content in the 0–20, 20–40 and 40–60 cm soil layers treated with lower Cl^- concentration increased by 11.08, 9.01, 15.21 mg/kg, respectively, and in the higher Cl^- concentration – by 38.71, 34.71, 32.05 mg/kg, respectively, compared to the control. The application of chlorine-containing fertilizers significantly decreased the soil pH: by 0.41–0.17, 0.25–1.25, 0.91–0.88, respectively, in soil layers 0–20, 20–40 and 40–60 cm, compared to the control. Prolonged application of chlorine-containing fertilizers resulted in a decrease of K^+, Ca^{2+}, Mg^{2+} and Na^+ content in both upper and deeper soil layers and an increase of exchangeable Al^{3+} content, which led to soil acidification. This was particularly evident when high chloride fertilizers were applied. Long-term application of high chloride fertilizer had a greater effect on the deeper soil layers than low chloride fertilizer. Therefore, repeated, or excessive application of chlorinated fertilizers should be avoided on brown soils to achieve an optimal balance between reducing crop production costs and delaying soil acidification (Wang et al., 2023a).

Salinity and chlorine

Chlorine, mainly in the form of chloride ions (Cl^-), plays a significant role in soil salinity. Worldwide, chlorine salinization limits global food security. Sources of chlorine in soil include mineral weathering, chlorine from marine species, and anthropogenic pollution. For example, the use of saline water for irrigation is the major source of soil chloride. This is particularly common in arid and semi-arid regions where freshwater resources are limited. Some fertilizers contain chloride compounds, such as potassium chloride (KCl), which contribute to increasing soil chloride levels. Frequent irrigation, especially with water containing high levels of chloride, can lead to the accumulation of chloride and other soluble salts in the soil. When the water evaporates or is absorbed by plants, the salts remain, gradually increasing soil salinity. Inadequate drainage can exacerbate the accumulation of chlorides and other salts because waterlogging prevents these salts from being flushed out of the root zone. In arid regions, water containing dissolved salts, including chlorides, can rise by capillary action. When the water evaporates from the soil surface, the salts remain, resulting in surface salinity.

High concentrations of chlorides and other salts in the soil cause osmotic stress, which inhibits water uptake by plants and can lead to stunted growth, wilting and, in severe cases, plant death. Chlorine ions can be toxic to plants in high concentrations, resulting in symptoms such as leaf burn, chlorosis, and necrosis. High levels of chlorides and other salts can cause dispersion of soil particles, reducing the stability of the soil aggregate state. This can lead to a decrease in soil permeability and aeration, further affecting plant growth. Saline soils often have reduced fertility due to nutrient imbalances and the negative effects of high salt concentrations on soil microbiological activity. Using fresh water for irrigation instead of saline water can help reduce chloride accumulation in the soil where possible. Methods such as drip irrigation can minimize water use and reduce salt accumulation by delivering water directly to the root zone. Adding gypsum (calcium sulfate) to the soil can help displace sodium ions and improve soil structure, making it easier for chloride and other salts to leach out. Adding organic matter to the soil can improve soil structure, increase water infiltration, and promote microbial activity that can help reduce soil salinity. Periodic applications of excess water to flush salts, including chlorides, from the root zone can help manage salinity. This is most effective when combined with good drainage to prevent waterlogging. Planting salt-tolerant crops can help maintain agricultural productivity on saline soils. These crops are better able to withstand osmotic stress and chloride toxicity (Geilfus, 2018a).

In international scientific databases, the number of references to studies on the effects of salinity on physiological processes in plants exceeds hundreds of thousands. Salinity affects photosynthesis by reducing the availability of CO_2 due to diffusion limitations (Flexas et al., 2007), and a decrease in the content of photosynthetic pigments (Delfine et al., 1999; Ashraf & Harris, 2013). Salt accumulation in spinach (*S. oleracea*) inhibits photosynthesis (Di Martino et al., 1999), mainly due to a decrease in stomatal and mesophyll conductance to CO_2 (Delfine et al., 1998), and a decrease in chlorophyll content, which may affect light absorption (Delfine et al., 1999; Alvino et al., 2000).

In radish (*R. sativus*), about 80% of the reduction in growth at high salinity can be explained by a decrease in leaf area expansion and thus a decrease in light interception. The remaining 20% of the salinity effect on growth is most likely due to a decrease in stomatal conductance (Marcelis & Van Hooijdonk, 1999). Salinity reduces the overall photosynthetic capacity of a plant by reducing leaf growth and inhibiting photosynthesis, thereby limiting its ability to grow (Yeo, 2007). Salt accumulation in the root zone causes the development of osmotic stress and disrupts the ionic homeostasis of the cell, inducing both inhibition of the absorption of basic elements such as K^+, Ca^{2+} and NO_3^- and accumulation of Na^+ and Cl^- (Paranychianakis & Chartzoulakis, 2005).

Specific ionic toxicity is caused by the accumulation of sodium, chloride or boron in the tissues of transplanted leaves to harmful levels. The accumulation of harmful ions can inhibit photosynthesis and protein synthesis, inactivate enzymes, and damage chloroplasts and other organelles (Lazar et al., 2003). These effects are more important for older leaves because they transpire longer and therefore accumulate more ions (Munns et al., 2002).

Deficiency of several nutrients in plants and nutrient imbalance can be caused by higher concentrations of Na^+ and Cl^- in the soil solution due to ion competition (i.e. Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+} and Cl^-/ NO_3^- in plant tissues) (Grattan & Grieve, 1998).

In romaine lettuce (*Lactuca sativa* var. *romana*), salinity increased carotenoid content (Kim et al., 2008). In two melon cultivars (Galia and Amarillo Oro), the application of salt stress from fruiting to harvest did not lead to a decrease in marketable yield and increased fruit quality (TSS) and maturity index in both cultivars (Botía et al., 2005).

Among the mechanisms of formation of plant response to salinity, changes in vacuolar chloride fluxes that affect the content and distribution of ions during early salinity stress are considered (Baetz et al., 2016).

Thus, chlorine in the form of chloride ions plays an important role in soil salinization, causing osmotic stress and toxicity to plants, as well as negatively affecting soil structure and fertility. Effective management practices, such as improved irrigation practices, soil fertilization, periodic leaching, and the use of salt-tolerant crops, are important to mitigate the negative effects of salinity and maintain soil health and agricultural productivity.

Sensitivity of crops to chlorine

Some crops can tolerate higher levels of chloride without adverse effects, while others are more sensitive and may show symptoms of toxicity or growth retardation when exposed to higher chloride concentrations. Understanding the sensitivity of specific crops to chloride is important in developing nutrient systems and irrigation practices. Below are some of the differences in how crops respond to chlorine.

Tolerant crops or crops that require the presence of chlorine in their nutrient systems. These crops can tolerate higher levels of chloride without significant negative effects. Barley (*H. vulgare*) is known for its high tolerance to salinity and chloride. Sugar beet (*B. vulgaris saccharifera*) and table beet (*B. vulgaris cruenta* L.) (collectively known as *B. vulgaris* L.) can tolerate relatively high levels of chloride in the soil. Spinach (*S. oleracea*), celery (*A. graveolens*), and asparagus (*Asparagus* spp., includes several species, the most important being *A. officinalis*) are generally more tolerant of chloride than other vegetable crops. Sorghum (*Sorghum bicolor* (L.), Moench,), millet (*Panicum miliaceum* L.), hemp (*Cannabis sativa* L.), cotton (*G. hirsutum*), etc. show good tolerance to chloride and Salinity. Tomatoes (*S. lycopersicum*) should also be included in this group. Although tomatoes are moderately sensitive to salinity, they tolerate chloride better than some other crops. We note the existing difference in the response of wheat and barley (*H. vulgare*) to the presence of chlorine in the nutrient systems. Unlike winter wheat, known varieties/hybrids of winter and spring barley (*H. vulgare*) require chlorine, in the absence of which numerous leaf spots are regularly observed during the generative period of vegetation.

Moderately sensitive crops have a moderate tolerance to chloride and can tolerate low to moderate levels without serious adverse effects. It is advisable to use KCl as the main application on these crops. Wheat (*T. aestivum*) and other cereals, except barley (*H. vulgare*), have a moderate tolerance to chloride, but excessive levels can cause problems. Corn (*Z. mays*) can tolerate moderate levels of chloride, but high concentrations can cause toxicity. Soybeans (*Glycine max* (L.) Merr.) are moderately sensitive to chloride; high levels can affect growth and yield. Cabbage (*B. oleracea* var. *capitata*) is moderately sensitive to chloride. This group also includes rapeseed (*B. napus* L.), cabbage (*B. oleracea* var. *capitata*), rice (*O. sativa*), soybean (*G. max*), meadow clover (*T. pratense*), peanut (*Arachis hypogaea* L.), and carrot (*Daucus carota* L.).

Carrots (*D. carota*) can tolerate moderate levels of chloride, but high concentrations can cause toxicity. However, carrots are only partially tolerant of chlorine. It is better to apply potassium chloride to carrot crops under the previous crop.

Sunflower (*H. annuus*) – selected hybrids (Li et al., 2020), grape (*Vitis* L.), radish (*R. raphanistrum* subsp. *sativus* L.), pea (*Pisum sativum* L.), kohlrabi (*Brassica oleracea* var. *gongylodes* L.), spinach (*S. oleracea*) (some hybrids and varieties) are also partially tolerant to chlorine.

Crops that are sensitive to chloride may suffer from symptoms of toxicity or stunted growth when exposed to chloride. In these crops, potassium should be applied mainly in the form of sulfate. Potatoes (*S. tuberosum*) are the first to be mentioned. The species is highly sensitive to chloride; excessive levels can cause leaf scorch and yield reduction. Beans (*P. vulgaris*) are very sensitive to chloride; high levels can lead to significant yield losses. Mustard (*Sinapis alba* L.) and many sunflower (*H. annuus*) hybrids are sensitive to chlorine (Céccoli et al., 2022). Lettuce (*L. sativa*) is sensitive to chloride, with high levels causing leaf burns and quality degradation. Strawberries (*F. ananassa*) are sensitive to chloride, with excessive levels causing leaf burns and reduced fruit quality. *Citrus* spp. are sensitive to chloride; high levels can cause leaf scorch and negatively affect yield and fruit quality.

In moderately sensitive and sensitive crops, when excess chlorine is absorbed, low starch content in tubers and root crops is observed: low sugar content in watermelons, grapes, etc.; damage to seedlings, negative impact on the combustion efficiency of tobacco, etc.

Chlorine ions are easily absorbed by the leaves when applied to the leaves with saline irrigation water. As a rule, the concentration of chlorine ions in irrigation water does not exceed 100–150 mg/L, and the specific electrical conductivity (EC) is in the range of 1–3 mS/m. As a rule, the permissible concentration of chlorine ions does not exceed 100 mg/L for highly sensitive vegetable crops and 300 mg/L for sensitive crops (Machado & Serralheiro, 2017).

Most vegetable crops have a low tolerance to continuous salinity, and are accordingly classified by sensitivity level/salt tolerance classes: sensitive, moderately sensitive, moderately resistant, tolerant, and unsuitable. Most vegetable crops are sensitive or moderately sensitive (Maas, 1986, 1990; Maas & Grattan, 1999).

One salt-tolerant crop is asparagus (*A. officinalis*). Sensitive crops include beans (*P. vulgaris*), carrots (*D. carota*), onions (*A. sepa*), spinach (*S. oleracea*) and strawberries (*F. ananassa*). Purslane (*Portulaca oleracea* L.) and beetroot (*B. vulgaris* var. *cruenta*) are moderately tolerant; broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*), celery (*A. graveolens*), eggplant (*Solanum melongena* L.), lettuce (*L. sativa*), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), peas (*P. sativum*), peppers (*Capsicum annuum* L.) and potatoes (*S. tuberosum*) are moderately tolerant. Tomato (*S. lycopersicum*) is moderately sensitive (cited in Maas, 1990; Grattan & Grieve, 1998; Grattan, 2002).

It can be noted that the level of chloride translocation to reproductive organs can determine the values of salt tolerance of a plant species. For example, in *Vicia faba* L., the level of chloride transportation to meristematic tissues was found to be the determinant of salt tolerance to sodium chloride (Franzisky et al., 2019).

Crop sensitivity to chlorine varies widely, with some crops being highly resistant and others highly sensitive to elevated chloride levels. Understanding the specific tolerance of each crop is essential for effective nutrient management and efficient irrigation. By controlling soil chloride levels, using appropriate fertilizers and water sources, and selecting appropriate crops, growers can mitigate the negative effects of chloride and optimize disease resistance and crop productivity (Johnson et al., 1957; Marschner, 1995, 2011; Zhang et al., 2015). Chlorine toxicity can occur because of chlorine fertilizers such as Muriate of Potash (KCl), rainfall in coastal areas, and even runoff from roads when applied for de-icing. However, most chloride toxicity results from unsafe levels in irrigation water and should be monitored.

Varietal specificity of the response of cereal crops to chlorine nutrition

Based on more than 30 years of field research, we can conclude that approximately more than 50% of the known varieties among those registered in Ukraine respond positively to chlorine fertilizer applications. In contrast to wheat (*T. aestivum*), almost all known barley (*H. vulgare*) varieties respond positively to chlorine fertilizers. It is expected that innovative barley (*H. vulgare*) hybrids, which have been introduced by a number of companies in recent years, will realize their high genetic potential under chlorine nutrition throughout the growing season. Because of the high salt index of potassium salts, which can cause damage to plants during foliar application, it is advisable to apply chlorine in early spring in the form of water-soluble potassium chloride in doses of 20–50 kg/ha in physical weight (tucks), or in later stages in the form of calcium chloride. Calcium chloride is significantly less toxic to cereal crops when applied foliarly. Doses of $CaCl_2$ can be 1–5 kg/ha.

Physiological leaf spot (PLS) in cereals under chlorine deficiency

PLS, especially during the generative development of crops, is an important factor limiting the productivity of crops, especially wheat (*T. aestivum*). Leaf spot in wheat (*T. aestivum*) can be caused by various factors, including fungal, bacterial and viral pathogens, as well as physiological stress. Chlorine can influence the development and severity of leaf spot by affecting plant physiology and soil conditions. One of the primary causes of leaf spot in wheat (*T. aestivum*) is fungal infection. The most common fungal diseases causing leaf spot in wheat (*T. aestivum*) are septoria spot (caused by *Zymoseptoria tritici*), brown spot (caused by *Pyrenophora tritici-repentis*), and leaf rust (mainly caused by *Puccinia triticina*). Less common are lesions caused by bacterial leaf streak (caused by *Xanthomonas translucens*) and wheat streak mosaic virus (WSMV – Wheat streak mosaic virus is a plant pathogenic virus of the Potyviridae family that infects plants of the Poaceae family, especially wheat; it is globally distributed and is transmitted by the wheat curl mite, especially in regions where wheat is widely grown), which can cause leaf discoloration and spotting.

Deficiencies or toxicity of essential nutrients can cause PLS. Factors such as drought, salinity and temperature extremes can cause stress-related symptoms of leaf spot.

It is generally accepted that chlorine plays a complex role in the development of leaf spot in wheat (*T. aestivum*). Adequate chloride levels in plants can increase resistance to certain fungal diseases. Chlorine ions can induce systemic acquired resistance (SAR) and activate plant defense mechanisms. Conversely, salinity stress caused by high chloride levels can weaken plants and make them more susceptible to pathogen attack, leading to an increase in the incidence and severity of leaf spot disease. Although it is an important micronutrient, excessive chloride levels can lead to salinity stress and toxicity, promoting PLS and increasing plant susceptibility to disease. Effective management of soil chloride levels through proper irrigation, fertilization and soil amendment practices is critical to mitigate the negative impact on wheat (*T. aestivum*) health and productivity.

PLS in wheat, also known as abiotic or non-pathogenic leaf spot, is not caused by pathogens but by other factors such as nutrient imbalances, environmental stresses, and chemical toxicity. Understanding the mechanisms of induction of these spots is important for effective management and mitigation. One of the major factors involved in the induction of PLS in wheat (*T. aestivum*) is nutrient imbalance. Nitrogen deficiency can cause chlorosis (yellowing) and necrotic spots on older leaves due to insufficient chlorophyll production and impaired photosynthesis. Potassium deficiency causes marginal chlorosis and necrosis, often starting at the leaf tips and margins and progressing to interveinal necrosis. Magnesium deficiency results in interveinal chlorosis and necrosis because magnesium is essential for chlorophyll and enzyme function. Boron toxicity results in chlorotic and necrotic spots, especially on leaf tips and margins, due to boron accumulation. Manganese toxicity causes brown necrotic spots due to excess manganese interfering with physiological processes.

Water deficiency also causes dehydration and oxidative stress, resulting in cell damage and the formation of necrotic spots. High temperatures accelerate the metabolic rate, leading to heat damage, protein denaturation and oxidative stress, causing necrotic spots on leaves. High levels of sodium and chloride ions create osmotic stress, reducing water uptake and causing ionic toxicity, leading to chlorosis and necrosis. Incorrect application of pesticides, incorrect application rates or transfer from non-target areas/crops can cause chemical burn, chlorosis and necrotic spots on leaves. In the conditions of the experimental farm of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (Kyiv region), we observed pronounced leaf lesions of wheat (*T. aestivum*) with PLS during the period of generative development of the crop when using 0.5 L/ha of the herbicide pivot, BASF (imazethapyr, 100 g/L) on the predecessor pea (*P. sativum*) (Table 2). These patches were insensitive to the use of fungicides from the azoxystrobin and triazole classes and were not observed in 2008 and 2009 when imidazolinones were not used on the preceding crop.

Table 2
Aftereffects of imidazolinone herbicides on winter wheat leaves that may cause physiological leaf spot (PLS); Kyiv region, 2005–2009

Growing season, year	Application of imidazolinone class herbicides* on the precursor – peas	Physiological leaf spot (PLS) appearing during the generative period of winter wheat development (BBCH51-BBCH71)
2005	Imidazolinones have been used on peas in 2004 – the previous crop	PLS are observed
2006	Imidazolinones have been used on peas in 2005 – the previous crop	PLS are observed
2007	Imidazolinones have been used on peas in 2006 – the previous crop	PLS are observed
2008	Imidazolinones have not been used on peas in 2007 – the previous crop	PLS are not observed
2009	Imidazolinones have not been used on peas in 2008 – the previous crop	PLS are not observed

Note: for using 0.5 L/ha pivot herbicide, BASF (imazethapyr, 100 g/L) on the preceding peas, previous crop in the crop rotation before winter wheat.

Necrotic spots of physiological character after imazethapyr application were also found on weedy rice plants. The increase in the dose of imazethapyr resulted in an increase in the level of leaf lesions with physiological spots (Zhang et al., 2024). It was found in many plant species that imazethapyr inhibited the ROS scavenging system and increased ROS formation (Traxler et al., 2023).

PLS in wheat (*T. aestivum*) is caused by a variety of factors, including nutrient imbalances, environmental stresses, chemical toxicity, and oxidative stress. Extreme soil pH can affect nutrient availability and uptake, leading to nutrient deficiencies or toxicity that cause PLS. Understanding these mechanisms is critical to developing effective management and mitigation strategies to maintain plant health and productivity. Regular monitoring, balanced fertilization, optimal irrigation, and judicious use of chemicals are key practices to prevent and manage PLS in wheat (*T. aestivum*).

Numerous studies have shown that ROS are the cause of plant stress and aging. The pathology of several biotic and abiotic diseases is also associated with the uncontrolled generation and insufficient scavenging of ROS. In recent years, leaf spot lesions of unknown etiology have become an increasingly problematic lesion in spring and winter barley (*H. vulgare*) and several winter wheat (*T. aestivum*) cultivars in Germany. The symptoms are like net leaf spot and ramular leaf spot, but the pathogens have not been identified (Fig. 3). The severity of symptoms varied greatly between cultivars. The most affected varieties of both spring and winter barley (*H. vulgare*) showed significantly higher levels of superoxide (O_2^-) and lipid peroxidation (malondialdehyde, MDA) production, but lower levels of antioxidant potential, expressed as superoxide dismutase (SOD) activity, catalase activity, and integrated water-soluble antioxidant capacity, compared to non-susceptible varieties (Wu & von Tiedemann, 2001, 2002, 2004).

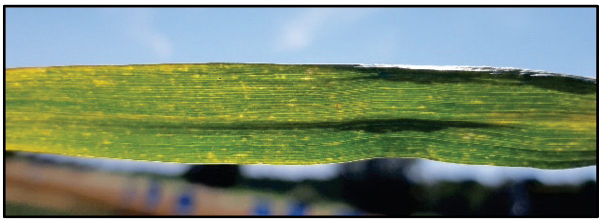

Fig. 3. Chlorophyll content "sinking" – sun-transparent PLS spots in winter wheat leaves during the generative period of development were observed under improved fungicide control, which may indicate the role of chlorine deficiency; near Helmstedt, Lower Saxony, Germany; 2018: fungicide control with BBCH31 – ciproconazole + azoxystrobin, BBCH39 – benzovindiflupyr (SDHI) + prothioconazole + tebuconazole + prothioconazole, BBCH61 – propiconazole + difenoconazole

Leaf H_2O_2 levels also increased with spot intensity. The histochemical localization of O_2 and H_2O_2 in tissues adjacent to leaf spots indicates that these two ROS are involved in the formation of leaf spots. Reductions in symptom severity with strobilurin and azole fungicides were consistently associated with increases in SOD activity and ROS levels and inhibition of O_2 production. However, peroxidase activity was significantly higher in sensitive cultivars and in more severely affected tissues and decreased with fungicide application. This suggests that a possible genetic mechanism based on imbalanced ROS metabolism contributes to the development of PLS. PLS in winter (*H. vulgare hiemalis*) and spring (*H. vulgare vernalis*) barley depends on the genotypic oxidative stress in the field.

The imbalance in ROS metabolism in chloroplasts may be the cause of PLS in susceptible varieties, which is consistent with the fact that light was important for the induction of PLS expression under both field and greenhouse conditions. Both biochemical and histochemical analyses showed that the expression of PLS symptoms was closely correlated with the accumulation of superoxide (O^{2-}). Together, these data indicate that PLS in barley (*H. vulgare*) is genotype-specific, but its expression is induced by certain environmental stressors, among which photosynthetically active light / actinic light plays an important role.

In a classic study, it was confirmed that physiological leaf spots develop on cereal leaves when the nutrient system is deficient in chlorine (Schwenke et al., 2015). It has been suggested that the necrosis that develops in Cl-deficient plants is associated with the accumulation of H_2O_2 during the release of Cl from the Mn cluster of the EOC (Imaizumi et al., 2022). Binding and functions of two chloride ions in the oxygen center of PSII. The authors believe that the necrosis that develops in Cl-deficient plants is related to the accumulation of H_2O_2 upon Cl release from the Mn cluster of the OEC (Imaizumi et al., 2022; Imaizumi & Ifuku, 2022). This scheme of PLS formation is confirmed by the description of typical spot manifestations: numerous small to large, yellow, often barely visible, to brown and completely dark spots on barley (*H. vulgare*) leaves, which form even under correct fungicide protection. An example of this is the damage to wheat (*T. aestivum*) leaves in fields in Germany (Fig. 3).

Fig. 4. "Subsidence" of chlorophyll content – transparent through spots / PLS dots in leaves of winter wheat Novosmuglyanka variety during the generative period of development; Kyiv region, 2022

This scheme of formation of physiological spots on leaves in the absence of chlorine may not be so obvious in production conditions, including conditions of regular application of chlorine fertilizers in crop rotation. In the conditions of Kyiv and other regions of Ukraine, due to the lack of resources in the period 2019–2024, physiological spots were observed in the form of completely/throughly transparent dots/spots on the leaf, which may indicate inhibition of chlorophyll synthesis rather than chlorophyll degradation (Fig. 4). Considering that chlorine in micromolar concentrations affects transport processes on membranes, that the element is easily washed through the soil profile, its deficiency occurs in the second half of the growing season, during the period of generative development, which may be the initial mechanism of formation of PLS in the form of transparent/transparent leaf spots. The development of these spots in the generative period of development, during grain filling, can be significantly accelerated at high levels of actinic light and, accordingly, significantly limit the productivity of cereal crops and their quality.

In a review of sensitive genotypes of bread wheat (*T. aestivum*) and durum wheat (*T. durum*) (Schwenke et al., 2015), it was reported that the Cl requirements of crops are usually met by atmospheric wet deposition and the application of chlorine-containing fertilizers. Thus, Cl deficiency in soil-grown crops is extremely rare, and the resulting deficiency symptoms are not well described or understood. The classic literature on nutrient deficiency (Kopsell & Kopsell, 2015) describes how Cl deficiency leads to a number of distinct symptoms, such as hypertrophic leaf curling, twisting, and patchy necrosis, referred to as "Cl deficiency leaf spot syndrome" (Schwenke et al., 2015). A specific mutation, PsbP-D139N, was found to significantly increase oxygen evolution activity in the absence of PsbQ, but not significantly in its presence. The D139N mutation increased the Cl^- retention capacity of PsbP and induced a unique structural change in the OEC, as revealed by light-induced Fourier transform infrared (FTIR) difference spectroscopy and theoretical calculations. Our results provide insight into the functional significance of chlorine in the water-oxidizing reaction of PSII (Imaizumi et al., 2022). Therefore, a number of studies suggest that the necrosis that develops in Cl-deficient plants, namely physiological leaf spotting, is associated with the accumulation of H_2O_2 following the release of Cl from the Mn cluster of the OEC (Imaizumi et al., 2022).

Chloride deficiency results in conspicuous light brown spots with reddish dots in the center. In cereals, leaf tips become soft and wilted, symptoms similar to copper deficiency in plants. The link(s) between function and symptom are not fully understood, but Cl^- plays an important role in the photoconversion of the OEC. Incorrect assembly can lead to photoinhibition and consequently to the accumulation of ROS in the thylakoid lumen. The accumulation of H_2O_2 and other ROS during Mn deficiency is probably the main cause of the formation of the characteristic necrotic spots (Lilay et al., 2024).

Chloride and fluoride and PLS

Many publications on the mechanisms of PLS in winter wheat and other cereals consider the dominant cause to be redox homeostasis or chlorine transport processes across membranes as the main cause of leaf injury and do not consider one of the possible causes of leaf injury.

There are some references in the literature on the effect of chlorine on phosphorus nutrition. Thus, on *Stylosanthes hamata* L. Taub, "Verano" and *Trifolium repens* L., "Ladino", it was shown that the maximum dry matter yield and P uptake were recorded in the tops and roots of *T. repens* under the treatment of 8 μM P/100 μM Cl and in the tops of *S. hamata* under the treatment of 8 μM P/30 μM Cl. The maximum root yield in *S. hamata* was recorded at 30 μM Cl, with no significant response to P at this level of Cl. P uptake by *S. hamata* roots was maximal in the treatment with 8 μM P (Chisholm & Blair, 1981). These differences can be explained in accordance with the above-described peculiarities of the effect of chlorine on plant transport processes. At the same time, the effect of phosphorus fertilizers on chlorine uptake and metabolism remains poorly understood.

We mentioned above that phosphate fertilizers can have a high fluoride content. We also mentioned above the possible antagonistic interaction between halogens. Therefore, when phosphate fertilizers are applied, so high levels of fluoride contamination are possible with a corresponding inhibition of chlorine supply to plants. The manifestations of PLS under chlorine deficiency may also be related to the effect of fluoride in reducing chlorophyll synthesis, degrading chloroplasts, and disrupting photosynthesis. Therefore, to provide plants with chlorine while avoiding the interaction of fluoride and chlorine, the recommendation for phosphorus nutrition of plants may be to use phosphate fertilizers with low fluoride content, namely monopotassium phosphate produced in Belgium or Israel, as opposed to phosphate fertilizers produced from African phosphate rocks.

Chlorine and crop yield

Thus, chlorine is one of the major inorganic nutrients that are important for the productivity of cultivated plants. Chlorine, in the form of chloride ion, plays a vital role in osmoregulation, stomatal function, photosynthesis and nutrient balance, which contributes to plant productivity. Both a lack and excess of chloride can negatively affect plant growth and productivity. Proper management of chloride levels through balanced fertilization, soil testing and good irrigation practices is essential to optimize plant development and achieve high productivity.

According to many farmers and plant nutrition physiologists, only a small amount of chlorine is needed in nutrient systems to achieve optimal productivity at moderate nitrogen doses (Geilfus, 2018a). However, numerous studies have shown that increasing chlorine doses can result in a corresponding increase in productivity for many crops (Christensen et al., 1981; Timm et al., 1986; Inal et al., 1998; Xu et al., 1999). The research results discuss how plant yields were increased, whether it was due to the accompanying cations or whether other anions could replace Cl^- in stimulating growth.

Consistent with the functions of Cl^- as a beneficial macronutrient (Franco-Navarro et al., 2016; Colmenero-Flores et al., 2019), several physiological disorders affecting the growth and yield of durum wheat (*T. durum*) in the field have been shown to be due to soil Cl^- deficiency (Schwenke et al., 2015). Crops such as tomato (*S. lycopersicum*), lettuce (*L. sativa*), spinach (*S. oleracea*), and Swiss chard (*B. vulgaris* var. *cicla*) showed an increase in plant biomass in the range of useful macronutrients (40–110 mg/g DM) under 5 mM Cl^- treatment (Colmenero-Flores et al., 2019). Such Cl^- levels are an order of magnitude higher than the concentrations traditionally considered toxic in plants (Xu et al., 1999), negating the view of Cl^- as a harmful element for agriculture (Colmenero-Flores et al., 2019).

Mori et al. (2008) showed that chloride in the metabolism of the halophyte *Suaeda salsa* (L.) Pall. contributes to the reduction of nitrate and the incorporation of nitrogen into amino acids and protein. Christensen & Brett (1985) demonstrated the participation of chlorides and divalent cations in the regulation of nitrogen metabolism, resistance to take-all, and productivity. The need for chloride as a companion anion for potassium instead of carboxylates such as malate can also be considered a useful function in plant species such as palms (Arecaceae), kiwi (*Actinidia chinensis* var. *deliciosa* (A. Chev.) A. Chev.) or onions (*Allium* spp.), which have guard cells without chloroplasts. In palm plantations located far from the sea in Indonesia or Côte d'Ivoire, chloride fertilizers such as KCl or $CaCl_2$ have been used to increase yields and improve nut quality (Manciot et al., 1979, 1980; Lins et al., 2021). In onion (*Allium* spp.) cultivation in Georgia (USA), it was shown that this crop is very sensitive to chlorine and that plants receiving a certain amount of chloride from irrigation water when fertilized with $CaCl_2$ had healthier, darker green leaves (Randle, 2004). Chlorine nutrition in the presence of divalent cation pools is an important component of take-all disease control, and wheat grain yield (Thomason et al., 2001).

Numerous studies have demonstrated the importance of Cl fertilization for crop yields (Geilfus, 2018a). Studies have shown (Fixen, 1986a) that at least some of the observed yield increases were due to the Cl content of the fertilizer when applied to chloride-deficient soils. Several studies have shown that chlorine-containing fertilizers, mainly in the form of KCl, have improved the productivity of wheat (*T. aestivum*) and other crops in the Great Plains and Pacific Northwest of the United States. Diaz-Zorita et al. (2004) reported that 253 kg/ha of KCl in wheat (*T. aestivum*) resulted in a 7% increase in grain yield compared to the no-Cl control.

Wheat and barley (*T. aestivum* and *H. vulgare*) have been shown to be more sensitive to KCl fertilization than oats. It has also been found that chloride fertilizers can contribute to better vegetative growth conditions, resulting in more grains per unit area. Studies conducted in China on major crops and vegetables have shown that increased yields of rice (*Oriza sativa*), wheat (*T. aestivum*), rapeseed (*B. napus*), Chinese cabbage (*B. Rapa* subsp. *pekinensis*), and asparagus (*A. officinalis*) can be achieved by applying more chloride fertilizers (Li et al., 1991; Chen et al., 2010).

Smith et al. (1987) found that in Cl-deficient plants, in addition to cell division and cell expansion, leaves and roots are particularly affected (damaged). Higher Cl concentrations in plants and soil systems cause salt stress for plants. Cl deficiency can be compensated by applying sufficient fertilizer (KCl).

After conducting field trials at 14 sites over a 3-year period, it was suggested (Fixen et al., 1986a) that the Cl concentration in wheat (*T. aestivum*) plants is strongly correlated with the Cl content in the upper 60 cm of the soil profile, and that soil Cl levels > 43.35 kg/ha are sufficient to obtain almost maximum wheat yields. At the same time, no diseases were detected, indicating that Cl had a positive effect on pathogen control. In this work, cereal species differed in their response to KCl application (Fixen et al., 1986b). Spring wheat (*T. aestivum*) responded better to chlorine application in four of the six experimental plots. Barley (*H. vulgare*) responded well to chlorine in three of the six plots, while oats did not respond to KCl in any of the five plots studied. This study showed that wheat and barley (*T. aestivum* and *H. vulgare*) were more sensitive to the presence of KCl than oats (*Avena sativa*).

The yield response to chloride is consistent with the concept of crop response to mobile nutrients. That is, yield can be directly related to the amount of Cl if this element was deficient in the soil. Studies conducted in Canada in 1996–1998 on clay loam and fine sandy loam soils showed that soil chlorine levels were low in all years at the fine sandy loam site and at the clay loam site. It is also important to note that Cl is a highly mobile element and is easily washed through the soil profile, so crops grown on low-C soils, such as sandy and sandy loam soils, often produce greater yield increases than chlorine fertilizers (Freeman et al., 2006).

During drought, small morphological changes in leaves can significantly increase WUE, resulting in a competitive yield advantage (Flexas et al., 2016). Nutrient systems that provide an appropriate application of Cl^- based on the amount of available moisture can increase the WUE of plants in the crop and thus increase yield.

The most common Cl^- concentration in the leaves of 670 species belonging to 138 families of terrestrial plants collected from their natural habitats was ~5 mg/g DW (Watanabe et al., 2007), which is well above the critical requirement as a trace element, but below the beneficial Cl^- level required to induce defense in tobacco (*N. tabacum*) plants (20–50 mg/g DW). Thus, plants can often benefit from Cl^- fertilization in different environments. Franco-Navarro et al. (2021) observed Cl^- stimulation of plant growth during an extended period of water deficit (WD), corresponding to better water status and WUE. The adoption of agronomic practices that ensure favorable Cl^- levels and optimal Cl^-: NO_3^- ratios in the field/soil will improve WUE & NUE, and crop drought tolerance, as well as reduce the use of nitrogen fertilizers and nitrate pollution, contributing to more sustainable agriculture; creating profitable crop production in resource-scarce environments.

Conclusions

Chlorine is an essential nutrient, a deficiency of which reduces plant productivity. Chlorine-containing substances have been known and used for a long time. The most common chlorine compound, sodium chloride (table salt), has been known since ancient times. It was used as early as 3000 BC and brine as early as 6000 BC. Cl substances are mentioned in ancient texts from different cultures. Chlorine was discovered in 1774 by Carl Wilhelm Scheele. He obtained it by reacting pyrolusite (manganese dioxide, MnO_2) with hydrochloric acid (HCl, then known as muriatic acid). Scheele thought that the gas produced contained oxygen. It was Sir Humphry Davy's proposal and confirmation in 1810 that chlorine was an element, and he also named the element. Chlorine has been considered a biologically important element almost since its discovery. Research into the effects of chloride fertilisers was carried out in the second half of the last century. In 1948, Warburg & Lüttgens argued that chloride was an important trace element for plant growth and showed that it was necessary for the water distribution system at the site of PSII oxidation. In the 1950s, Broyer et al. (1954) finally demonstrated the biological importance of chlorine for plants.

Chloride is the most abundant inorganic anion in plant cells, an element available in most agrophytocenoses. Cl^- levels can reach toxic levels for many plant species, especially sensitive species. The average Cl^- content in plants ranges from 2.0–20.0 mg/g DM, but for Cl^- sensitive and Cl^- tolerant glycophyte species, the critical (often toxic) Cl^- content in tissues can be around 4–7 and 15–35 mg/g DM, respectively. Chlorine deficiency in plants has characteristic symptoms: wilting, numerous spots and reduced productivity.

Chloride performs a wide range of functions in plants, primarily forming turgor and osmoregulation, respectively, affecting transport processes on membranes (plasmalemma, tonoplast, etc.), water & nitrogen use efficiency (WUE & NUE), and affects the functioning of PSII, and is therefore an important part of agricultural plant productivity. Chloride stimulates the structural and functional role of the plasma membrane, sugar transport, as well as nitrogen fixation and assimilation in the plant. Recent studies have discussed the role of chlorine in nitrogen assimilation and photorespiration. It has been shown that Cl plays an important role in the OEC by adjusting the affinity of different amino acid residues for manganese (Mn) (Imaizumi & Ifuku, 2022). Nitrogen assimilation and photorespiration become more efficient when fed with chloride.

Chlorine acts as a counterion, balancing the positive charges of potassium (K^+) and other cations in plant cells, which is essential for maintaining electrical neutrality and proper ionic balance in cells.

Chlorine, in the form of chloride ions, plays a significant role in soil salinity. Sources of chlorine in soil include mineral weathering, chlorine from marine species and anthropogenic pollution. Fertilisers such as potassium chloride (KCl) help to increase the chloride content of the soil. Planting salt-tolerant crops can help maintain agricultural productivity on saline soils. The sensitivity of crops to chlorine varies according to the type of crop. Some crops can tolerate higher levels of chloride without adverse effects, while others are more sensitive and may show symptoms of toxicity or growth retardation when exposed to higher chloride concentrations. Understanding the response of specific crops to chloride is important for the development of nutrient systems and irrigation practices.

Chloride increases plant resistance to diseases that require relatively large amounts of Cl^-. These doses are much higher than those required for its use as a trace element, but much lower than those required to induce salinity control effects (Elmer, 2023). The vast majority of research on chlorine nutrition has been devoted to studying the effect of the element on the incidence of leaf spotting in cereals. PLS form on the leaves of cereal crops when there is a lack of chlorine in the nutritional systems (Schwenke et al., 2015). The necrosis that develops in Cl-deficient plants is thought to be associated with the accumulation of H_2O_2 during the release of Cl from the Mn cluster of the OEC. Physiological spotting in the form of completely/partially transparent dots/spots on the leaf was observed, which may indicate inhibition of chlorophyll synthesis rather than degradation. Given that chlorine at micromolar concentrations affects transport processes on membranes and that the element is easily leached through the soil profile, its deficiency occurs in the second half of the growing season, during the period of generative development, which may be the initial mechanism for the formation of physiological leaf spotting in the form of transparent/translucent leaf spots. The development of these spots in the generative period of development, during grain filling, can be significantly accelerated by high levels of actinic light and, accordingly, significantly limit the productivity of cereal crops and their quality.

A possible component of chlorine deficiency and leaf damage in wheat and other cereals by PLS may be the application of phosphate fertilizers with high fluoride content, such as phosphate rock, etc. Therefore, in high productivity technologies, it is advisable to use only potassium monophosphate produced in Belgium or Israel.

Therefore, the use of chlorine fertilisers, mainly potassium chloride in the basic (fall or spring) application, ammonium chloride, calcium chloride, etc. in the foliar application, is important to provide plants with chlorine during the growing season to increase WUE & NUE, increase plant resistance to pathogens, control physiological leaf spotting and increase productivity of cereals and other agricultural crops. Chlorine's role in increasing WUE & NUE is particularly important for the country's profitable crop production in the face of resource shortages.

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