3. Potassium and chloride in higher plants

3.1. Uptake and distribution of potassium and chloride

3.1.1. Removal of soil potassium by plants

Plant roots intercept K in the soil solution at the root surface. If plant roots take up K only from surface of particles within 2 mm of the roots, then only about 14% of available soil K would be taken up by plants when the root density was 4 cm cm^{-3} soil (Barber, 1985). Various studies have shown, however, that only 7% or less of the total K requirement of crops is available from the immediate vicinity of the roots (Nemeth, 1978; Drew and Nye, 1969). The concentration of K in the solution of most soils is insufficient to meet the total K requirement of the crop if calculated on the basis of mass flow (Barber, 1962).

The uptake of K by crops shifts the equilibrium between exchangeable and solution K to release exchangeable and non-exchangeable K into the K depleted solution (see above Fig. 2.1). Diffusion of K is the dominant factor controlling the availability of K even in paddy rice soils (Teo *et al*., 1994). It is apparent that the ability of the soil to supply K to the root is more important in determining K uptake than the ability of the root to absorb K (Barber, 1985). Using diffusion and mass-flow theory, some simulation models for nutrient transport in soil and uptake by plant roots, such as Baldwin-Nye-Tinker model, Claasen-Barber model, Barber-Cushman model, have been suggested and used to evaluate the soil K availability (Van Rees *et al*., 1990; Seiffert *et al*., 1995; Brouder, 1999).

3.1.2. Uptake of potassium

Potassium is the most abundant cation in the cells of nonhalophytic higher plants (Maathuis *et al*., 1997). Plant roots take up K from a wide range of soil concentrations, utilizing at least two distinct plasma membrane uptake systems with high and low affinities for K, respectively (Epstein *et al*., 1963). Simple diffusion and mass flow move K primarily from the soil solution to the cell wall space of the root cortex (Clarkson and Hanson, 1980). Once in the cell wall space, K is absorbed by the epidermal and outer layers of cortical cells. The suberized Casparian strip in the endosperm blocks the movement of K in the cell wall space from the root cortex to the xylem elements of the vascular tissue. Therefore, K must enter the plant mainly through the plasma membrane. Presumably, the cells of stems, leaves, flowers, and fruits have K uptake mechanisms that are fundamentally similar to those in cortical cells of roots (Leonard, 1985).

The rate of K absorption is influenced by the K content of the tissue (Siddiqi and Glass, 1982) and the concentration of K or competing ions (e.g. Mg^{2+}) in

the external solution (Fecenko, 1982). The availability of metabolic energy in the form of ATP (Petraglia and Poole, 1980) and the magnitude of the membrane electrical potential difference across the plasma membrane (Mercier and Poole, 1980) affect K uptake rates.

There is ample physiological information to suggest that the plasma membranes of root cells have transport proteins that distinguish K from chemically similar ions. These transporters facilitate the movement of K through the relatively impermeable lipid bilayer of the plasma membrane. The accumulation of K in root cells is ultimately at the expense of metabolic energy. ATP produced by aerobic respiration appears to be the energy source for K absorption. The K-stimulated plasma membrane associated ATPase may also be a K transporter. K uptake into root cells occurs by ATPase mediated electrogenic H^+/K^+ exchange (Leonard, 1985). Electrogenic K^+/H^+ exchange in excised wheat roots demonstrated that K influx was coupled with acidification of the external medium (Pakhomova, 1996). There is no evidence for the coupling between high affinity K^+ uptake and H^+ efflux in maize root cells (Kochian *et al*., 1989).

The introduction of electrophysiological and molecular techniques has enabled the study of K absorption mechanisms at the molecular level. Such approaches have confirmed the existence of discrete high and low affinity uptake systems at the plasma membrane of root cells, as well as at various other types of plant cells (Kochian and Lucas, 1988). High affinity K uptake from micromolar external K concentrations in the range of 5-40 μM is coupled to H^+ transport as demonstrated in *Arabidopsis* roots (Maathuis and Sanders, 1994).

3.1.3. Potassium channels in higher plants

There are three categories of transmembrane protein pumps, as well as carriers and channels that are used to describe ion transport mechanism across membranes, largely on the basis of kinetic criteria (Marschner, 1995; Poole, 1998; Raven *et al*., 1999). The three categories of transmembrane proteins move solutes at different speeds. Ion channels catalyze transport through membranes at rates between 10^6 and 10^8 ions per second per channel protein (Maathuis *et al*., 1997), while the numbers of ions transported by carriers and by pumps are only about 500-10,000 and fewer than 500 per second, respectively (Raven *et al*., 1999).

The old "moving-ferryboat" view of a carrier is now no longer considered valid. The numerous "carriers" that have been found in membranes are large proteins – too large to diffuse or spin around at the rate needed to account for the fluxes they catalyze. Only a part of the carrier protein undergoes a change of conformation when an ion is translocated across the membrane (Raven *et al*., 1999).

Ion channels are integral membrane proteins which transport solutes across the cell membranes (Poole, 1998). Potassium channels play diverse but defined essential roles in plant physiology. Specific K channels have been identified in a wide variety of plant cell types including guard cells of both *V. faba* and maize (Schroeder *et al*., 1987; Fairley-Grenot and Assmann, 1993) and in motor cells from leaf-moving organs of a leguminous plant (Moran *et al*., 1988; Ward and Schroeder, 1994), as well as in aleurone cells, stem tissue, mesophyll cells, cortical and stelar cells, root hairs (Maathuis *et al*., 1997).

There are two universal properties of ion channels: "gating" behavior and selectivity. The channel gate has two conformational states: "open" or "close", which permit or prevent ion permeation. This conformational switching can occur in response to ligands (e.g. Ca^{2+}) or to a change in membrane voltage after which the channel activates (open) or deactivates (close) (Maathuis *et al*., 1997).

The selectivity of ion channels is determined either by the conductance of different ions through the channel or by reversal potentials of different ions (Maathuis *et al*., 1997). Cation selectivity of the different channels varies widely from highly selective for K to a virtual absence of cation discrimination (Moran, 1990; Ward and Schroeder, 1994). Potassium channels derived from motor cells transfer several monovalent cations with a selectivity sequence $K^+ > Rb^+ > Na^+ \approx Cs^+ \approx Li^+$ (Moran *et al*., 1988). A type of K channel found in *V. faba* guard cells shows a cation selectivity sequence $K^+ >> Rb^+ > NH_4^+ >> Na^+ \approx Li^+ \approx Cs^+$ (Ward and Schroeder, 1994). It should be noted that the selectivity measured by conductance ratios is not always identical to that measured by reversal voltage analysis (Uozumi *et al*., 1995).

There are two main types of voltage dependent K channels defined as inward and outward rectifying K channel (K^+_{in} and K^+_{out}), respectively. K^+_{in} opens at more hyperpolarizing (very negative membrane voltages) to facilitate K uptake and K^+_{out} opens at depolarizing (less negative) voltages to carry an outward K current (Schroeder *et al*., 1994; Tester, 1990).

The direction of K flow through open channels depends on the difference between the prevailing membrane potential and the membrane voltage E_k. E_k can be assessed by calculation from the Nernst equation: E_k (mV) = 59 $\log[K^+]_{ext}/[K^+]_{cyt}$, in which $[K^+]_{ext}$ and $[K^+]_{cyt}$ are the K activities of the extracytosolic and cytosolic compartments, respectively. Typically, in higher plants $[K^+]_{cyt}$ is regulated roughly in the range of 80-200 mM (Walker *et al*., 1996), while K in soil solution and leaf apoplast ranges from around 0.5 mM to 10 mM, E_k would be –60 to –150 mV for a typical plant cell. If the difference in membrane potential is lower than the reversal value (zero current) in response to the negative membrane potential generated by the H^+-pump, the passive channel transporter may also mediate what has previously

been termed "high-affinity" uptake. AKT_1 channels mediate K uptake from solutions that contain as little as 10 µM K (Hirsch *et al*., 1998).
Molecular details for plant K channels are beginning to emerge. Several K transporter genes have been isolated from higher plants. The first identification of K channel cDNAs from plants was achieved by functional complementation of yeast cells that were defective in K uptake. Two distinct *Arabidopsis thaliana* cDNAs, termed AKT1 and KAT1, were independently cloned (Anderson, *et al*., 1992; Sentenac *et al*., 1992). Recently, two *Arabidopsis thaliana* K^+_{out} channels, KCO1 and SKOR, were also expressed in insect cells (Czempinski *et al*., 1997) and in *Xenopus oocytes* (Gaymard *et al*., 1998), respectively.
Nutrients taken up by roots are transported in the xylem to the aerial parts of the plant. Extensive studies in the recent years suggest diverse long-term transport functions of plant K channels (De Boer and Wegner, 1997). In roots, the K^+_{out} channels are probably involved in the release of K into the xylem. Most plant K channels may remain activated for long periods of time, which is critical for this proposed long-term transport function of K channels in plants.
Two major overlapping functions have been proposed for K^+_{in} channels: (a) they provide a pathway for low-affinity K uptake, which is driven by the H^+-pump established membrane voltage, and (b) they contribute to membrane voltage control by modulating the membrane conductance and by sensing the soil-to-cell K gradient, similar to a K electrode (Maathuis *et al*., 1997). The later function in particular could influence nutrient uptake by other transporters (Schroeder *et al*., 1994). Fu and Luan (1998) suggest that conformational changes in the transporter protein might be responsible for switching between the high and the low affinity mode for a single protein. Changes in external K concentration or membrane potential might trigger such a conformational switch.

3.1.4. Removal of soil chloride by plants

Crops can take up substantial amounts of Cl especially when available soil Cl is high because of the total amount of Cl in the soil or the amount of Cl applied in fertilizers or irrigation water. Application of 355 kg Cl ha^{-1} to winter wheat increased Cl concentration of flag leaves from 1.8 g kg^{-1} in the control to over 10 g kg^{-1} (Christensen *et al*., 1981). At peak accumulation, the Cl content in spring wheat was 18 and 61 kg ha^{-1} on sites testing low and high in Cl, respectively (Schumacher, 1988, cited by Fixen, 1993). By the time of crop maturity, Cl in the above ground portion of the plant had dropped to 50% and 43% of these peak values, respectively. Similar behavior was reported for K in wheat (Kafkafi *et al*., 1978). The amount of Cl removal in grain is very small. The concentration of Cl in the dry matter of wheat

grain is only 0.5 g kg^{-1} (Knowles and Watkin, 1931). In spring wheat, soybean and rice, the amount of Cl distributed in the grains was only 2.15%, 1.34% and 1.62%, respectively, of the crop's total Cl uptake (Pan *et al.*, 1991b).
Varying soil Cl at five sites caused the Cl content of sugar beet tops at harvest to vary from 28 to 148 kg ha^{-1} (Moraghan, 1987). The removal of Cl in soybean seed amounts to 0.45 kg ha^{-1}, which is less than the amount deposited annually in rainfall (Parker *et al.*, 1986).

3.1.5. Uptake of chloride

The uptake of Cl by plant roots is generally an active process that requires energy. Earlier studies suggested that Cl transport through the cell membrane involves a $2H^+/Cl^-$ symporter (Sanders, 1984) or occurs via an anti-port with hydroxyl ions energized by ATP (Jacoby and Rudich, 1980). The formation of a transmembrane pH gradient during H^+-ATPase functioning in sugar beet was found to require the presence of Cl in the incubation medium, though the anion had no effect on the ATPase activity in the plasma membrane (Gaivoronskaya and Molotkovskii, 1991). Therefore, the activation of ATP-dependent pH gradient generation by Cl is due to dissipation by the anions of the membrane potential produced by transmembrane transport of protons. Recently the H^+/Cl^- symporter was studied using electrophysiological methods (Felle, 1994) that demonstrated that nH^+/Cl^- would better describe the ratio between proton and Cl uptake. He concluded that the kinetics of Cl transport depended on the pH gradient across the plasma membrane rather than on the membrane potential.
Specific protein channels energized by ATP for Cl transport were suggested both for the plasmalemma (Lin, 1981) and the tonoplast (Martinoia *et al.*, 1986). Plant Cl channels were revealed in the membrane of *Arabidopsis thaliana* by the patch clamp technique (Lew, 1991) and cloned from *Arabidopsis thaliana* by Hechenberger *et al.* (1996) and from tobacco by Lurin *et al.* (1996). The Cl channel in plants is also voltage dependent (Lurin *et al.*, 1996) and its conductance (ranging from 5-40 pS) is independent of KCl on the cytoplasmic side until a threshold concentration of about 300 mM is reached (Lew, 1991). It was suggested that the potential-sensitive Cl channels were on the plasmalemma and their activity provided electric neutrality of H^+-ATPase action (Gaivoronskaya and Molotkovskii, 1991). The identification of a Cl channel provides a molecular probe for the study of voltage-dependent anion channels in plants.
Chloride ions always keep their negative charge, whereas SO_4^{2-} and NO_3^- are partly or completely reduced during metabolism in the plant. It was suggested that the physiological mechanisms for the control of Cl accumulation in plant cells operate at the cell or organ level (Cram, 1988). Changes in root

temperature and external Cl concentration affect Cl influx and accumulation (Cram, 1983; 1988).
It is difficult to determine the balance of individual ions across the plasmalemma and the tonoplast (Glass and Siddiqi, 1985). The situation is further complicated by interaction between the shoot and the root. Ion influx is regulated by the flux to the xylem and involves recycling in the phloem (Marschner, 1995). The fluxes of Cl in intact plants are very different from those measured in plants with excised roots (Collins and Abbas, 1985). Glass and Siddiqi (1985) proposed a homeostatic mechanism that senses vacuolar NO_3 plus Cl or total anion concentration. Deane-Drummond (1986) discussed a variety of other schemes. All of the suggested mechanisms must be balanced with plant growth, and yet there is no generally accepted view of the control of Cl uptake and transport in plants (Flowers, 1988).
The composition of the root cell membrane not only affects ion selectivity, but is also of particular importance in preventing Cl from entering the root. Salinity tolerance in grapes was positively correlated with the solubility of Cl in the lipids that constitute the root membranes (Kuiper, 1968). Enrichment of root cell membranes with phospholipids relative to their monogalactose diglyceride content limits Cl uptake (Kuiper, 1968). There were no apparent differences in the chemical composition of root microsomal membrane lipids between varieties of corn with low and high Cl uptake, and the composition of these membrane lipids was not affected by Cl salt (Hajibagheri *et al*., 1989).

3.1.6. Translocation and distribution of potassium and chloride

3.1.6.1. Potassium

Movement of K from the leaves to a developing sink by phloem transport is found in all plants and organs (Mengel and Kirkby, 1987) and has been demonstrated in potatoes (Dijkshoorn, 1972), apples (Hansen, 1980), plum (Lindner and Benson, 1954) and peaches (Popenoe and Scott, 1956). In potatoes, a flow of carbohydrates containing 2 g kg^{-1} K (dry matter basis) is constantly moving to the developing tuber (Dijkshoorn, 1972). Increasing the K content in potato leaves from 23 to 30 g kg^{-1} has been shown to increase tuber yields and lead to an increase in the resistance of the foliage to frost damage (Grewal and Singh, 1980).
Many crops, including soybean (Hanway and Johnson, 1985), cotton (Halevy, 1976), corn (Karlen *et al*., 1988), wheat (Kafkafi *et al*., 1978) and rice (Bao and Xu, 1993), stop taking up K from the soil after flowering or from the grain filling stage. The K in the ears comes only by retranslocation from the other plant organs (Hanway and Johnson, 1985; Karlen *et al*., 1988; Bao, 1989). Flowers, developing fruits and tubers serve as sinks for K. The K

accumulation rate and its distribution among various organs are well documented for some annual crops as shown in Fig. 3.1, Fig. 3.2, and Fig. 3.3. Data on the distribution of K in perennial crops is much more limited. Conradie (1981) has reported the total quantity and distribution of K in grapevines during one growing cycle (Fig. 3.4). The plant continuously takes up K from just after bud burst until one month after harvest. Up to bud burst, K is supplied from that stored in the roots and trunk. Potassium uptake by the roots then satisfies K demand, with most of the K going to the developing fruit until one month before harvest when the rate of K uptake from the soil cannot satisfy the demand of the fruit. Fruit K requirement is then satisfied by K from the leaves and shoots. This phenomenon of K retranslocation is very similar to that at the last stage of annual grain crops and cotton (Fig. 3.1, 3.2, and 3.3.), potato (Perrenoud, 1983) and sugar beet (Draycott, 1996). Sugar flow to the developing fruits may cause a shortage of carbohydrates in the roots that can inhibit K uptake so that the demand for K by the developing fruits cannot then be satisfied by K uptake from the soil. Then K is removed mainly from the leaves and the shoots. This is supported by the data of Conradie (1981) who shown that the amount of K in the leaves of grapevines at harvest was 35% lower than one month earlier. Then as soon as the fruit was harvested, K increased in all plant parts. A significant portion of the post-harvest K uptake was retained in the permanent parts of the vine.

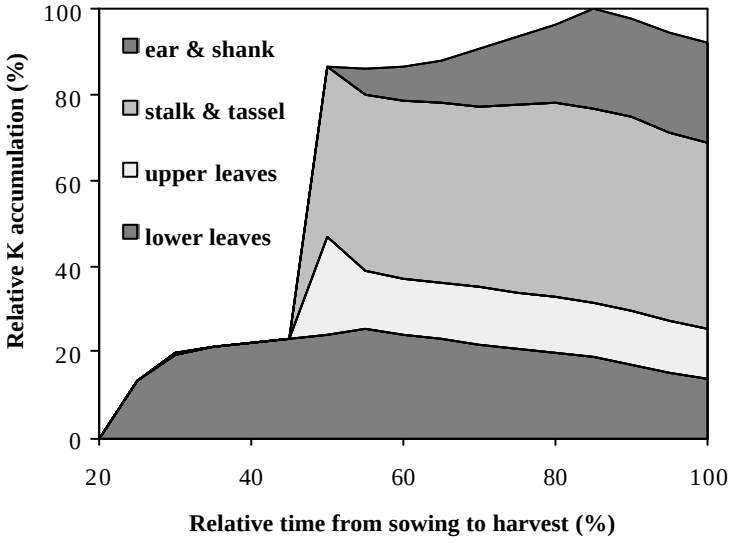

Fig. 3.1. Total relative quantity and distribution of potassium during the growing season of corn (Source: Karlen *et al*., 1988).

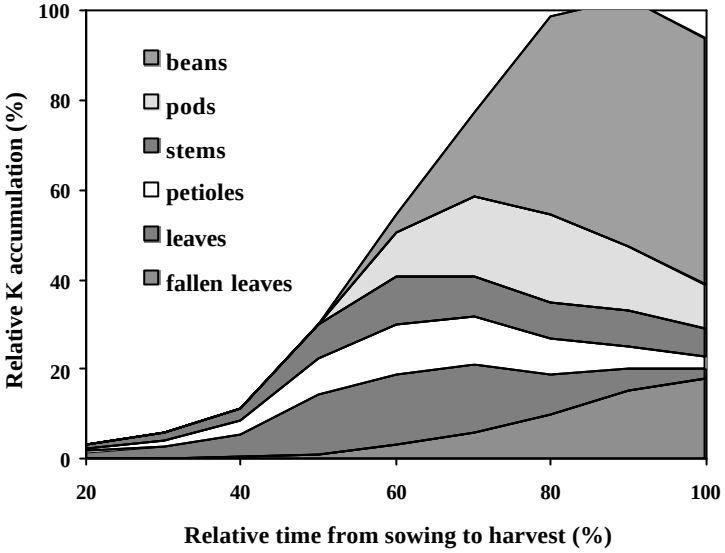

Fig. 3.2. Total relative quantity and distribution of potassium during the growing season of soybean (Source: Hanway and Johnson, 1985).

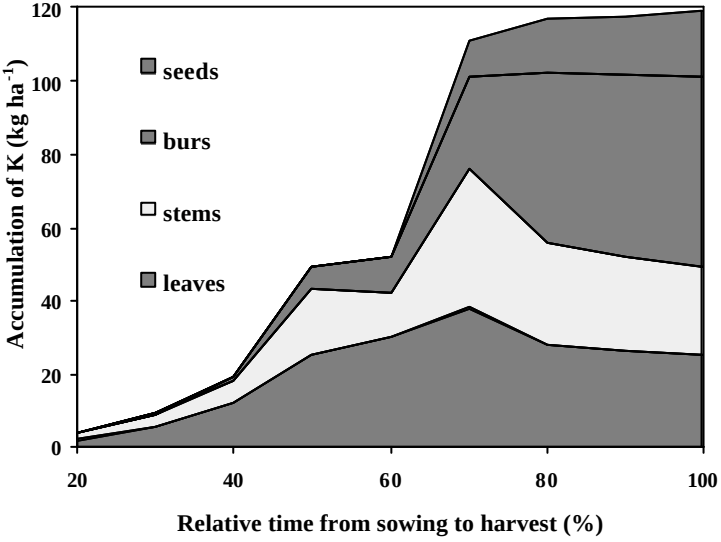

Fig. 3.3. Total quantity and distribution of potassium during the growing season of cotton (Source: Kerby and Adams, 1985).

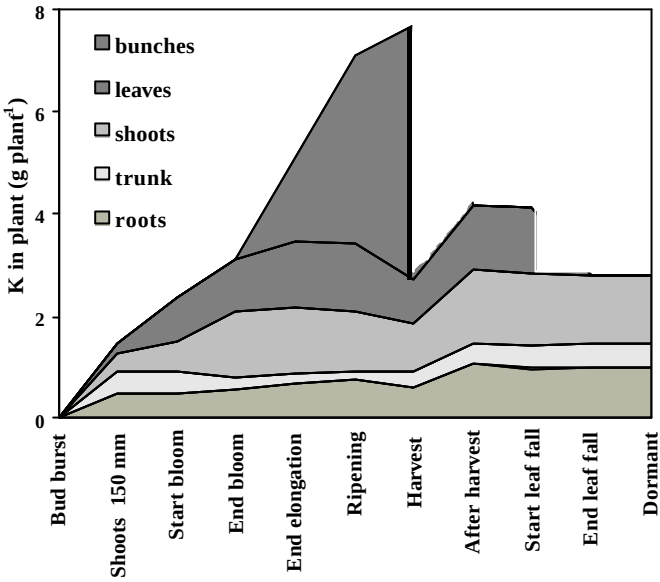

Fig. 3.4. Total quantity and distribution of potassium during one growing cycle of grapes (Source: Conradie, 1981).

Generally the distribution of K in grains, as a percent of the total K in the aboveground parts of cereals is low, about 16-34% for corn (Welch and Flannery, 1985), 18-24% for most small grain crops (Beaton and Sekkon, 1985). Grain % K is relatively uniform and less influenced by K fertilizer than straw % K. Hybrid rice grain K as a proportion of the total plant K increased from 20% to 25% (Pong and Lao, 1993). A large proportion of whole plant K is found in reproductive organs in many other crops. Of the total K in the plant, 60-68% is found in the bolls of cotton (Bao, 1989; Kerby and Adams, 1985; Weir *et al*., 1986), 35-56% in the ears of corn (Chu, 1989; Karlen, *et al*., 1988), 60-70% in the fruits of tomato (Tanaka *et al*., 1974), 51-67% in fruit flesh of sweet pepper (Xu *et al*., 2001), 60-70% in the tubers of potato (Perrenoud, 1983), and 25-35% in roots of sugarbeet (Draycott, 1996).

3.1.6.2. Chloride

Chloride is partially stored in the vacuoles of leaf cells and there its negative charge is neutralized by Ca^{2+} and Mg^{2+} (Beringer *et al*., 1990). Differences in salinity tolerance between species of citrus depended on the compartmentation of the Cl under saline conditions (Lloyd *et al*., 1989). In leaf cells of NaCl-treated halophytes, Na tended to concentrate in the

cytoplasm whereas Cl was evenly distributed in the cytoplasm and the vacuole (Eshel and Waisel, 1979). The substantially greater sensitivity of NO_3 reductase activity to NaCl in bean leaves than in cotton leaves seems to be due to a decrease in ion compartmentation rather than a difference in salt tolerance of the enzyme itself (Gouia *et al*., 1994). Anderson and Steveninck (1987) suggested that the salt tolerance of alfalfa is due to restricted entry of Na and Cl into the roots as a result of retention of these ions in the vacuoles of the epidermis and upper cortex. Chloride compartmentation appears to be highly regulated. In the chloroplast, the Cl concentration remains relatively constant regardless of whether the plant growth medium is characterized by deficient or excessive levels of Cl (Maas, 1986).

Chloride has high mobility in both short-distance and long-distance transport. Leaf Cl content is controlled not only by root uptake but also by restricted translocation of Cl from the roots to the leaves. The type of rootstock controls leaf Cl accumulation in many crops (Downton, 1985; Velagaleti *et al*., 1990; Lahav *et al*., 1992; Stevens and Harvey, 1995). Tomato seedlings were able to prevent Na and Cl accumulation in the root and shoot as compared with the Cl-sensitive cucumber grown in the same solution (Al-Harbi, 1995).

Plants can use different mechanism to ensure Cl tolerance. They can restrict Cl transport to the shoots by a mechanism that resides in the roots (Grattan and Maas, 1985). They can withstand high Cl concentrations by restricting Cl uptake and transport to the leaves and/or they increase the ability of their leaf tissue to tolerate high Cl concentrations (Bar *et al*., 1997). Boursier *et al*. (1987) observed that salt-sensitive plants generally accumulate Na and Cl in the sensitive leaf tissue instead of retaining these ions in the root and stem. Shoot Cl concentration gives a more accurate evaluation than root Cl concentration of the extent of the injurious effect of Cl in citrus rootstocks (Zekri, 1993; Banuls *et al*., 1990). In the tolerant wild accessions and F_1 (*Le* x *Lpen*) of tomato, Cl concentrations in the leaves and the ratio of leaf Cl to stem Cl were lower than in the sensitive *Le* cultivar (Saranga *et al*., 1993). Leaf concentrations of Cl, Ca, Mg and K in salt tolerant soybean cultivars were low, while Na levels in the leaves of both sensitive and tolerant cultivars remained low under salt stress (Velagaleti *et al*., 1990). Reduced Na or Cl accumulation in the shoots was used as a physiological index of salt tolerance in rice, but there was no direct relationship between salt tolerance at the cellular level and at the whole plant level (Yan *et al*., 1992).

3.1.7. Restriction of chloride uptake by scion and rootstock selection

In the selection and breeding of salt-tolerant plant cultivars, attention should be given to the control of Cl uptake. Rootstocks that exclude salt are often deficient with regard to other desirable characteristics, and their use is therefore limited (Sykes, 1993). The ability of a particular species to exclude

Cl ions is independent of its ability to exclude Na ions; a good Cl ion excluder is not necessarily a good Na excluder, and vice versa (Sykes, 1993). Downton (1985) found that irrigation with water containing 0-50 mM Cl salt resulted in lower leaf Cl concentrations in grafted grapevines (except 'Dogridge') than in self-rooted vines. Under saline conditions, the use of a Cl-excluding rootstock of grapevine reduced leaf Cl concentration by 60% in grapevines grown in soil with free-draining root zones but only by 18% in vines with water-logged root zones (Stevens and Harvey, 1995).
Chloride concentration of avocado leaves from trees on West Indian salt-resistant rootstocks was almost one-third of that in leaves from trees on the Mexican rootstock as shown in Fig. 3.5 (Lahav *et al*., 1992).

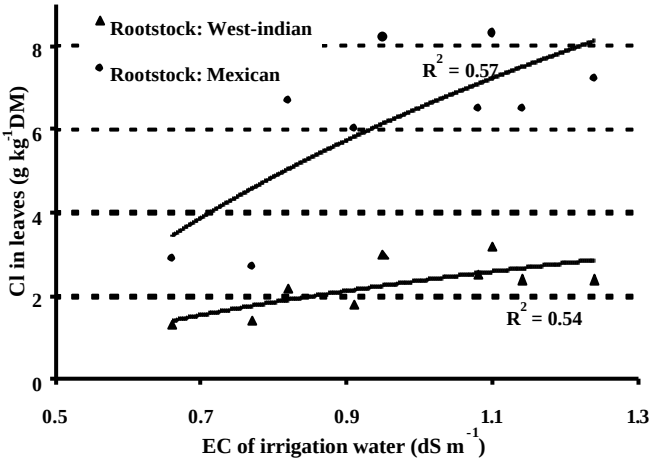

Fig. 3.5. Effects of salinity of the irrigation water and rootstocks on chloride concentration in the leaves of avocado (Source: Lahav *et al*., 1992).

In general, the sensitivity of citrus to Cl was found to be determined by its scion (Banuls *et al*., 1997), whereas Na levels in the leaves were greatly affected by both scion and rootstock (Lloyd *et al*., 1989). Harmful effects of high leaf Cl concentrations can be avoided if salt-excluding varieties are used as rootstocks. Turgor maintenance in leaves of the scion variety appears to be an important factor influencing the response to salinity of selected root-scion combinations (Walker *et al*., 1982).
The mean Cl concentrations were 24.3 g kg^{-1} and 7 g kg^{-1} in the early leaves of soybean Cl accumulators and excluders, respectively (Yang and Blanchar, 1993). The occurrence of leaf scorch, a symptom of Cl toxicity, in Cl accumulator cultivars was significantly higher than in Cl excluder cultivars.

The average grain yields of Cl accumulator cultivars were significantly decreased (by 16%) when Cl was added, while grain yields of excluder cultivars were not significantly reduced. Soybean scions of sensitive cultivars grafted onto the rootstocks of tolerant cultivars showed typical tolerant responses (Velagaleti *et al*., 1990).

3.2. Biochemical functions of potassium and chloride

3.2.1. Potassium

3.2.1.1. Potassium and the activation of enzymes

Boyer *et al*. (1943) reported that K was required for pyruvate kinase catalytic activity. Since then more than 60 enzymes have been shown to require K (Suelter, 1985). In K-deficient plants some gross chemical changes are presumably related to the high K requirement of certain regulatory enzymes. Potassium concentration in the cytosol and in the stroma of plants well supplied with K is in agreement with the monovalent ion concentration (100-150 mM) required for optimal protein hydration (Leigh and Wyn Jones, 1984). Potassium and other monovalent cations do not form complexes with the substrates prior to interacting with enzymes (Suelter, 1985), they activate enzymes by inducing conformational changes in the enzyme protein. In general, the enzyme conformational change induced by K increases the rate of catalytic reactions (Evans and Wildes, 1971).

Monovalent cation-activated enzymes fall into two broad classes: those catalyzing phosphoryl transfer reactions and those catalyzing elimination and hydrolytic reactions (Suelter, 1985). One of the typical K-influenced enzymes is starch synthase, which catalyzes the transfer of glucose to starch molecules. Potassium similarly activates starch synthase isolated from a variety of plant species and organs (e.g., leaves, seeds, and tubers). The range of K concentration for the maximum activity of this enzyme is 50-100 mM (Nitsos and Evans, 1969). Therefore, K deficiency causes the accumulation of soluble carbohydrates and a decrease in the starch content in plants. Higher concentration of K, however, may have inhibitory effects on this enzyme (Preusser *et al*., 1981).

Pyruvate kinase accelerates the conversion of 3-P-glyceraldehyde to pyruvate with concomitant energy production in the glycolytic pathway. Schuller *et al*. (1993) confirmed that K or NH_4, not Na, stimulated the pyruvate dehydrogenase kinase by lowering the K_m (Michaelis constant) at subsaturating ATP concentrations. The activation constants for NH_4 and K were 0.1 mM and 0.7 mM, respectively. Sodek *et al*. (1980) found that asparaginase, a very important enzyme in the N metabolism in plants, is dependent on K not only for activity but also for its stability. There may be reduced activities of certain enzymes in the Calvin cycle in leaf chloroplast of K-deficient leaves,

and their activity in situ may be indirectly limited by available K (Huber, 1985). The plasma membrane bound H-ATPase, which plays a key role in both the regulation of cytoplasmic pH and the driving force for cation and anion uptake, is also stimulated by K and is relatively insensitive to anions (O'Neill and Spanswick, 1984). The plasma membrane ATPase activity is influenced by pH and K concentration (Lindberg and Yahya, 1994). Relative low concentrations of mineral nutrients and low pH (5.3) in culture solution enhanced the specific K-activation of ATPase in roots of sugar beets.
Predicting whether or not an enzyme reaction will be activated by monovalent cations is complicated by the fact that two or more enzymes with different mechanisms have evolved to catalyze the same reaction. For example, *E. coli*, depending on growth conditions, synthesizes two isoforms of pyruvate kinase, but only one isoform requires K for its activity (Suelter, 1985).

3.2.1.2. Potassium and photosynthesis and respiration

Potassium deficiency results in reduced rates of net photosynthesis and translocation of assimilates and increased rates of dark respiration (Peoples and Koch, 1979). Potassium nutrition affects photosynthesis in two ways:

(1) Potassium affects the photosynthetic capacity possibly because of the dependence of protein synthesis and development processes on K. Thus, carbon exchange rates in an expanding leaf are rapidly restricted following the decrease in K supply. In addition, although maximum leaf expansion may be reduced by K deficiency, leaf initiation is not effected.

(2) Potassium appears to affect the activity of the photosynthetic system. This becomes evident when a mature leaf becomes K deficient (Huber, 1985). The reduction in the rate of net photosynthesis caused by K deficiency (Bednarz *et al*., 1998) is attributed to many factors, such as an increase in leaf mesophyll and stomatal resistance (O'Toole *et al*., 1980), an increase of dark respiration (Okamoto, 1969), and a decrease of leaf transpiration rates (Huber, 1984). The extent to which these factors affect photosynthesis depends on the severity of K deficiency and plant type. Bednarz *et al*. (1998) suggested that the initial decrease in net photosynthesis in cotton resulted from an increase in stomatal resistance with slight K deficiency. Then K deficiency becomes more acute, biochemical factors, i.e. decreasing carboxylation efficiency and increasing CO_2 compensation, also contribute to the decrease in net photosynthesis.

The role of K in photosynthesis is related to the establishment of the pH gradient across the chloroplast envelope. Potassium is the dominant counter ion to the light-induced H flux across the thylakoid membranes (Tester and

Blatt, 1989). Also, K is needed for the establishment of the transmembrane pH gradient necessary for the synthesis of ATP (photophosphorylation). The maintenance of high pH in the stroma when illuminated, needs an additional influx of K from the cytosol which is mediated by a H/K counterflow through the chloroplast envelope (Wu *et al*., 1991). Chloroplast electron transport activity and photophosphorylation are reduced in plastids from K-deficient leaves (Huber, 1985).
It is assumed that reduced cytosolic pH in K deficient leaves (Ward, 1960) may contribute to the typical increase in respiration rate that results in increase in malate and NADPH oxidation. The phloem loading stimulated by K results from K uptake into the mesophyll cells, which is linked to the release of sugars to the free space (Peel and Rogers, 1982). A low concentration of K in the free space may stimulate the proton pump, and thus, affect phloem loading with sugar. ATPase stimulated by a large K concentration in the phloem sap may provide a positive feedback and increase the uptake of sucrose into the phloem (Ho and Baker, 1982).

3.2.2. Chloride

More than 120 chlorinated organic compounds have been identified in higher plants (Engvild, 1986). The importance of these compounds in terms of the need for Cl in higher plants is not known. Besides wilting symptoms, the principal effects of Cl deficiency in most plants are manifested by reduction in leaf surface area relative to the rate of leaf cell division (Terry, 1977). A specific effect of Cl on cell extension may be assumed in some legume species, such as peas and faba bean, which contain substantial amounts of chlorinated indole-3-acetic acid (IAA) in their seeds. This chlorinated compound enhances hypocotyl elongation at 10 times the rate of IAA itself, probably because of the resistance of the former compound to degradation by peroxides (Hofinger and Bottger, 1979).

3.2.2.1. Chloride and activation of enzymes

At least three plant enzymes appear to require Cl for optimal activity: asparagine synthethase (Rognes, 1980), amylase (Metzler, 1979), and ATPase (Churchill and Sze, 1984). The fact that Cl plays a role in N metabolism is indicated by its stimulatory effect on asparagine synthethase, which uses glutamate as a substrate. Chloride increases the affinity of this enzyme for its substrate (Rognes, 1980). In plant species in which asparagine is the major compound in the long-distance transport of soluble N, Cl might also play a role in N metabolism (Marschner, 1995). The amount of certain amino acids and amides is exceptionally large in Cl deficient cabbage and cauliflower plants (Freney *et al*., 1959) as a result of either inhibition of protein synthesis or enhanced protein degradation.

The proton-pumping ATPase at the tonoplast is not affected by monovalent cations but is stimulated directly by Cl (Churchill and Sze, 1984). ATPase activity increased asymptotically with increasing Cl concentration, approaching a maximum at 50 mM. Chloride stimulates the vacuolar-type H^+-ATPase from guard cell protoplasts of *Commelina communis* (Willmer *et al*., 1995). The close relationship between KCl supply and ADP-glucose starch synthethase activity in roots of maize (Nitsos and Evans, 1969) is probably a reflection of two different stimulatory functions of K and Cl on ATPase activity located at the plasma membrane and the tonoplast, respectively (Fig. 3.6.). There are also striking similarities between the Cl-stimulated H^+-ATPase and the mechanisms regulating elongation of coleoptiles (Hager and Helmle, 1981).

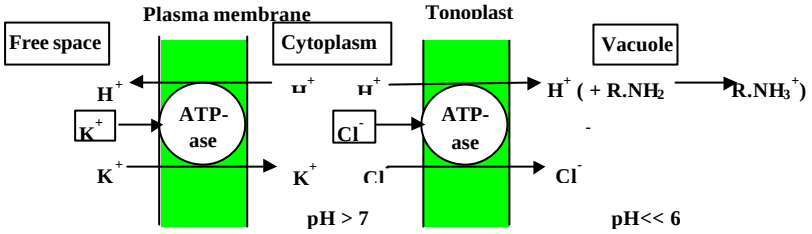

Fig. 3.6. Scheme of stimulating functions of potassium and chloride on ATPase activity in maize root cell (Source: Marschner, 1995).

3.2.2.2. Chloride and photosynthesis

Chloride is an essential cofactor in photosynthetic O_2 evolution in the water-splitting system of Photosystem II (PSII), initially reported by Arnon and Whatley (1949) and demonstrated by Izawa *et al*. (1969). The binding of Cl to membranes is needed for the activation of the O_2-evolving enzyme (Baianu *et al*., 1984). The preference for Cl over Br, NO_3 or I with regard to its ability to promote oxygen evolution is due to its specific ionic volume (Critchley, 1985). A number of proteins are involved in modulating the requirement for Cl in the evolution of oxygen (Andersson *et al*., 1984). It was suggested that Cl might act as a bridging ligand between manganese atoms during the transfer of electrons from water to PSII (Critchley, 1985), or as a structural component of the associated (extrinsic) polypeptides (Coleman *et al*., 1987). The various polypeptides (33, 24 and 18 kD) attached to PSII provide the charges for binding, while Cl protects the polypeptides from dissociation (Homann, 1988).

The mechanism of Cl action in PSII still remains controversial. Studies utilizing electron paramagnetic resonance of the manganese (Mn) involved in

PSII suggest that Cl is not bound to Mn (Yachandra *et al*. 1986). The site of action in the thylakoid membrane appears to be positively charged and to attract the ions by electrostatic force (Itoh and Uwano, 1986). As shown in sugar beet (Terry, 1977) and spinach (Robinson and Downton, 1984), even in plants with mild to severe decreases in growth, Cl levels in the chloroplasts remained large enough to maintain photosynthesis. The chloroplast Cl concentration in the leaf water was highly stable at 88-99 mM in spinach plants, regardless of the total plant Cl status (Robinson and Downton, 1984). Even in *Suaeda maritima*, a halophytic member of the Chenopodiaceae in which the Cl concentration in leaf cells can exceed 500 mM, the Cl concentration of the chloroplast remained at around 100 mM (Robinson and Downton, 1985). In Cl deficient leaves, however, it appears that nearly all of the Cl accumulates in the chloroplast. Therefore, the amount of Cl required by the plant for photosynthesis is very small.

3.3. Physiological functions of potassium and chloride

3.3.1. Osmotic and ionic balance

3.3.1.1. Potassium

Potassium is the main cation associated with either inorganic anions or organic acid anions in the vacuoles. In most instances cell extension is the consequence of the accumulation in the cells of K, which is required for both stabilizing the pH in the cytoplasm and increasing the osmotic potential in the vacuoles (Marschner, 1995). An inverse relationship exists between tissue concentration of K and reducing sugars in the cell (Pitman *et al*., 1971). Because K is taken up by plants together with an anion (Ben Zioni *et al*., 1971), particularly Cl^-, or NO_3^-, a substantial proportion of the effects of K on plant growth and sugar concentration are presumably the combined effects of both K and its accompanying anion. The inverse relationship between the K status of plants and cell sizes holds true both for leaves (Mengel and Arneke, 1982) and storage tissues (Pfeiffenschneider and Beringer, 1989).

Potassium is the dominant cation for counterbalancing immobile anions in the cytoplasm, the chloroplasts, and quite often also for mobile anions in the vacuoles, the xylem and phloem. Potassium is often the dominant counter ion for NO_3^- in long-distance transport in the xylem as well as for storage in vacuoles. After NO_3 reduction in the shoot, charge balance and pH homeostasis has to be maintained by a corresponding net increase in organic acid anions. The organic acid anions (mainly malate) and K as an accompanying cation are retranslocated in the phloem to the roots (Ben Zioni *et al*., 1971). The xylem transport of amino acids in nodulated legumes also use K as a charge balance and for recirculation (Jeschke *et al*., 1985).

Itoh *et al*. (1997) found that in K deficient soybean leaf growth was restricted by both decreased mesophyll cell numbers and cell enlargement but the water content and the water and osmotic potentials of the expanding leaves were not affected. Some cations, anions, sugars and amino acids can accumulate in the expanding leaf to compensate for the decrease in K concentration by as much as 92%. These findings suggest that the depression in the leaf growth occurs in K deficient plants even when the water relations of the leaves are normal.

3.3.1.2. Chloride

The ability of Cl to move rapidly across cell membranes against an electrochemical gradient and its relatively low biochemical activity are two important properties that make Cl particularly well suited to serve as a key osmotic solute in plants (Maas, 1986). The Cl concentration in wheat flag leaf tissue is closely related to its osmotic potential (Christensen *et al.,* 1981, Fig. 3.7), suggesting that an increased Cl concentration in the symplasm is probably the reason for the measured change in osmotic potential.

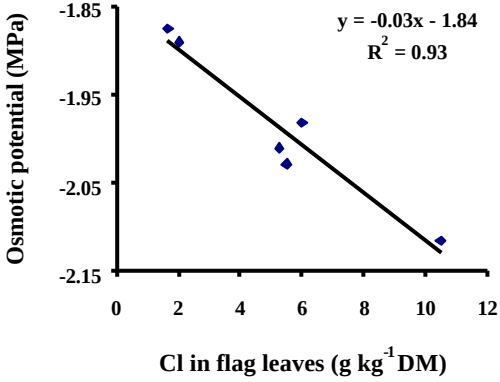

Fig. 3.7. Osmotic potential of youngest, mature winter wheat leaves (cv. Stephens) as related to chloride concentration in flag leaf (Source: Christensen *et al*., 1981).

The accumulation of Cl by plants contributes greatly to an increase in cell hydration and turgor pressure, both of which are essential for cell elongation (Maas, 1986). A similar osmotic function, as well as a close association between K and Cl, was reported for the guard cells of leaf stomata of *Vicia faba* (Talbott and Zieger, 1996). The stigma of grasses such as *Pennisetum americum* L. often extend within minutes at anthesis by cell elongation and

this is mainly mediated by the rapid transfer of K and Cl from the surrounding tissue into stigma primordium (Heslop-Harrison and Reger, 1986).

In the leaves of spinach exposed to 300 mM NaCl, Na and Cl are mainly sequestered in the vacuoles, and the K concentration in the chloroplasts is still sufficiently large to maintain photosynthesis (Schroppel-Meier and Kaiser, 1988).

Non-toxic or compatible organic solutes, such as glycine betaine, accumulate in the cytoplasm and its organelles for osmotic adjustment in salt-stressed plants (Schroppel-Meier and Kaiser, 1988), but such accumulation is much less obvious for the whole leaf tissue. An instructive example of the diversity of osmotic adjustment in various organs of the same plant is shown in Table 3.1. Sodium and Cl mediate osmotic adjustment in the leaves, whereas K, glycine betaine, and sugars mediate it in the flowers. Even within a given leaf the role of solutes may vary. In young leaves of sorghum, glycine betaine is important for osmotic adjustment only in the leaf blade, not in the leaf sheaths (Grieve and Maas, 1984).

Table 3.1. Chemical composition of *Aster tipolium* leaves and florets growing in saline substrate.

Component	Leaves	Florets
	mM[a]	
Na^+	360	56
Cl^-	320	51
K^+	72	133
Glycine, betaine	18	82
Total soluble sugars	53	493

[a] Plant water basis

Source: Gorham *et al*. (1980).

The osmoregulatory function of Cl in plants seems to operate at different levels. The Cl concentration range usually found in plants (50-150 mM of tissue water) exceeds its critical deficiency level by 1 to 2 orders of magnitude. Chloride serves as a main osmoticum in the vacuoles of plant tissue. Together with K, Cl has a role in maintaining xylem volume flow and root pressure (Marschner, 1995). In the phloem sap, Cl concentration might reach 120 mM and seems to play a role in phloem loading and unloading of sugars (Fromm and Eschrich, 1989). Because the Cl content of the whole plant may be 1 mM or less, the osmoregulatory functions of Cl are presumably confined to specialized compartments in tissues or cells, such as the extension zones of roots and shoots, pulvini, stigmata, and guard cells.

There the concentrations of Cl might be much higher than the average Cl concentration in the bulk tissue (Marschner, 1995).
Under saline conditions, the maintenance of low Cl concentrations in the leaves does not necessarily safeguard against a reduction in photosynthetic activity if the osmotic adjustment is not sufficient to offset the reduction in water potential. Conversely, osmotic adjustment and maintenance of turgor during salt treatment do not necessarily provide a safeguard against photosynthetic reduction in leaves that have accumulated high concentrations of Cl (Walker *et al*., 1982). The reduction of photosynthesis in the Cl sensitive leaves of the citrus variety Etrog citron was associated with high leaf Cl concentrations, while in leaves of the Cl tolerant variety Rangpur lime it was related to a loss of leaf turgor (Walker *et al*., 1982).
The observed benefits from Cl applied in field studies are most probably due to the osmoregulatory role of Cl in the plant (Flowers, 1988). The importance of this function for plant growth and grain yield is highly dependent on growing conditions such as water, temperature, and the presence of other ions that might potentially act as substitutes for Cl in its osmoregulatory role. An increase in Cl concentrations in the leaves of kiwifruit merely resulted in an equivalent decrease of NO_3 and did not change the concentrations of P, S or organic acids or the total anion concentration (Smith *et al*., 1987). Further research is needed to determine the specific functions that involve Cl in the response of plants to salt stress (Banuls and Primo-Millo, 1992).

3.3.2. Stomatal activity and regulation

3.3.2.1. Potassium

Stomatal opening occurs when K and corresponding anions, such as malate and Cl move into the guard cells, increasing cell turgor and, thus, opening the stomatal pore (Talbott and Zeiger, 1996). Fluxes of K are associated with stomatal movements in a wide range of plants, regardless of their location on the plant and evolutionary level (Willmer and Pallas, 1973). The influx of K into guard cells is presumed to be in response to the electronic H^+ extrusion (Outlaw, 1983). Part of the K^+ charge is balanced by Cl^- and part by $malate^{2-}$.
Sugars were suggested as an alternative osmotic solute for stomatal opening (Tallman and Zeiger, 1988), while Outlaw (1983) postulated that sucrose accumulation in the cytosol (synthesized internally or imported from surrounding cells) may be responsible. Talbott and Zeiger (1996) reported that guard cells of intact *Vicia faba* leaves during a daily light cycle of stomatal movements had two distinct osmoregulatory phases. In the morning phase, stomatal opening was correlated with K uptake and, to a lesser extent, sucrose accumulation. In the afternoon phase, in which the stomatal aperture was maximum, K content declined and sucrose became the dominant

osmoticum. Reopening of the stomata after CO_2-induced closure was accompanied by accumulation of either K or sucrose, depending on the time of day, indicating that a single environmental signal may use multiple osmoregulatory pathways. Malate accumulation, correlated with K uptake, was detected in the growth chamber with blue light enrichment but not in glasshouse conditions, whereas Cl was the main K counter ion in the glasshouse. These results indicate that guard-cell osmoregulation in the intact leaf depends on at least two different osmoregulatory pathways, K transport and sucrose metabolism.

Closure of the stomata is induced by darkness or ABA and is associated with a rapid efflux of K and accompanying anions from the guard cells and their steep increase in the apoplasm of guard cells (Bowling, 1987). ABA activates Ca channels in the plasma membrane and thereby activates voltage-dependent anion channels. This shifts the plasma membrane from a K conducting state to an anion conducting state, which further decreases membrane potential and enhances K efflux (Hedrich *et al*., 1990).

3.3.2.2. Chloride

Activation of a H^+ pump in the plasma membrane initiates K and Cl influx, accompanied by malate synthesis, resulting in osmotic water flow into the guard cells, a bowing apart of the guard cell pair, and consequent stomatal opening (Lee and Assmann, 1991). Using a Cl sensitive microelectrode, Penny *et al*. (1976) found a close correlation between stomatal activity and the movement of Cl between epidermal cells of *Commelina communis*. Chloride concentrations in the vacuoles of guard cells increased when the stomata opened and decreased when they closed.

The relative contributions of Cl and malate may vary among species, and may depend on the availability of external Cl (Raschke and Schnabl, 1978) and on the plant growth environment (Talbott and Zeiger, 1996). In plants grown in the glasshouse, a correlation between Cl and K in the guard cells was found during a single daily light cycle of stomatal movements (Fig. 3.8). The malate that accumulates during stomatal opening is synthesized in the guard cells (Du *et al*., 1997) and both its synthesis and its accumulation are affected by the Cl concentration in the growth medium (Raschke and Schnabl, 1978). The pH dependence of PEP carboxylase (Du *et al*., 1997) may provide an explanation for the coordination between Cl influx and malate synthesis. Chloride uptake will cause internal acidification, with consequent inhibition of PEP carboxylase. In the absence of external Cl, K^+/H^+ exchange will cause alkalization of the guard cells, thereby promoting malate synthesis.

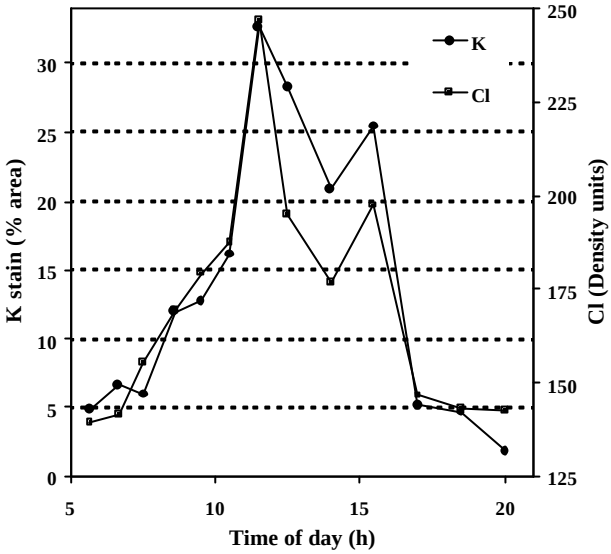

Fig. 3.8. Potassium and chloride content in guard cells of *Vicia faba* plant grown in the glasshouse during a single daily light cycle of stomatal movements (Source: Talbott and Zeiger, 1996).

In plant species such as onion (*Allium cepa* L.), which lack the functional chloroplasts for malate synthesis in the guard cells, Cl is essential for stomatal functioning (Schnabl and Raschke, 1980). Onion guard cells contain equivalent amounts of K and Cl. Stomatal opening is inhibited in the absence of Cl.
Palmaceae species, such as coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis Jacq*.), which might possess starch-containing chloroplasts in their guard cells (Braconnier and d'Auzac, 1990), also require Cl for stomatal functioning (von Uexküll and Sanders, 1986; von Uexküll, 1990). In Cl deficient coconut plants, stomatal opening is delayed by about 3 hours and this is thought to be a major factor responsible for growth depression and wilting symptoms in Cl deficient plants (von Uexküll and Sanders, 1986; Braconnier and d'Auzac, 1990).

3.4. Interaction of potassium and chloride with other nutrients

3.4.1. Potassium vs. Ammonium

High K levels are needed in the plant particularly when large amounts of N are supplied. Adequate K is needed to maintain N metabolism (Leigh and Wyn Jones, 1984). Ammonium has been shown to inhibit K absorption and increase K efflux (Ni and An, 1984; Bloom and Finazzo, 1986; Vale *et al*.,

1988; Beck and Feller, 1991; Adler and Wilcox, 1995) resulting in a decrease in net K influx. Potassium uptake through K inward transport channels is completely suppressed by NH_4 (Vale *et al*., 1988). As an N source, NH_4 may inhibit K absorption and impair K/Na selectivity or exchange mechanisms (Adler and Wilcox, 1995). This might explain the increase of salt sensitivity of plants given NH_4 (Speer *et al*., 1994). Chemically related ions compete with each other in uptake and may also interfere with their metabolic functions (Daliparthy *et al*., 1994). The inhibitory effect of NH_4 on net K uptake of maize seedlings resulted from an initial but temporary enhancement of K efflux and a constant inhibition of K influx (Topa and Jackson, 1988).

The competition between K and NH_4 is difficult to explain solely by competition for binding sites at the plasma membrane (Marschner, 1995). Whereas NH_4 is quite effective in competing with K, the inhibition of NH_4 uptake by K is not observed (Reese and Koo, 1975; Rufty *et al*., 1982; Topa and Jackson, 1988). Other results show that NH_4 and K do not compete for uptake (Daliparthy *et al*., 1994); the amount of K in the plants receiving NH_4 was large and exceeded the amount in plants receiving NO_3 (Dibb and Thompson, 1985; Silberbush and Lips, 1991).

Ajay *et al*. (1970) reported that K increased NH_4 assimilation in tomato and that K did not compete with NH_4 uptake, and Mengel *et al*. (1976) obtained similar results with rice. Potassium uptake by both *Ageratum houstonianum* (NH_4 tolerant) and *Salvia splendens* (NH_4 sensitive) plants was significantly enhanced in the NH_4 treatment (Jeong and Lee, 1996). The large absorption of NH_4 and K with the application of large amounts of K indicated a complementary effect on uptake between NH_4 and K (Dibb and Thompson, 1985). Beck and Feller (1991) found that the level and the time course for specific NH_4 stimulated, K release from *Lemna minor* L. into the external medium depended on the amount of NH_4 added (1-30 mM). K release was not detectable after the addition of large amount of NH_4 to duckweed grown on an NH_4 medium. The release of K was apparently associated with the transition from NO_3 to NH_4 utilization by the plant and might be linked directly or indirectly to the high initial absorption rate of NH_4. Zornoza *et al*. (1988) found that in pepper, K uptake inhibition by NH_4 only existed under conditions of high light intensity related to a pronounced yield decrease, but no effect was observed under low light intensity.

In view of these inconsistent conclusions on interaction of K and NH_4, the inhibition of K uptake in some cases may result from the decline of plant growth rate when NH_4 is the major N source.

3.4.2. Potassium vs. Calcium and Potassium vs. Magnesium

Potassium applications increase K tissue levels in plants and have a fairly consistent effect on lowering tissue concentrations of Ca and Mg in most

plant species (Marschner, 1995). With excess K applications, antagonism between Mg and K may induce Mg deficiency symptoms in cereals, maize, citrus, potatoes, fruit trees and sugarbeet (Fecenko, 1982). Some of the reported decreases in cation content were due to the "dilution effect" caused by larger yields in response to K application (Dibb and Thompson, 1985). Evidence for the antagonistic effect of the three elements was related to charge balance among them (Mengel and Kirkby, 1987).

Of special interest in the relationship of K and Ca is the phenomenon known as the *Viets effect* (Viets, 1944). In experiments with excised barley roots, Viets (1944) found that the presence of Ca in the outer medium stimulated the uptake of K. Other multivalent cations such as Mg, Sr, Ba and even Al had similar but less pronounced effects. However, the internal Ca of the roots did not influence K uptake. In general, it is thought that higher Ca concentrations in the outer solution either enhance the uptake or reduce the loss of the K ion (Dibb and Thompson, 1985). Epstein (1972) stated that K absorption is decreased in the absence of Ca rather than increased by its presence. The true *Viets* effect would occur only when Ca at higher than normal levels stimulates the uptake of another nutrient. Mengel and Kirkby (1987) considered that a deficiency of Ca in the outer solution results in higher efflux rates of K, resulting in the *Viets* effect, namely a larger K concentration in plant tissue with the greater Ca availability.

A strong antagonism between K and Ca was well characterized by Garcia *et al*. (1999). In culture solution, Ca enrichment resulted in a 30% decrease of the K concentration in all the organs of grape compared to that in control plants. The Ca concentration in plants grown in the K enriched solution was lower than that for plants grown in the low K solution, although the later contained less Ca in the solution than the former. In vineyards established on acid soils, liming might depress K uptake and favor the production of more acidic wines (Garcia *et al*., 1999).

Gold specks in tomato fruit walls are deposits of calcium oxalate symptoms of Ca excess, high air humidity and high Ca:K ratios result in more gold specks (De Kreij *et al*., 1992). Increasing the K:Ca ratio in the culture solution suppressed Ca uptake and reduced *gold specks* on tomatoes (Sonneveld and Voogt, 1990).

In alfalfa, *one way* competition between K and Mg has been found (Fig. 3.9). Increasing K decreased Mg dramatically (Fig. 3.9-A), whereas added Mg depressed K only slightly (Fig. 3.9-B). A similar K/Mg antagonism was recently reported for grapevine (Garcia *et al*., 1999). Symptoms of Mg shortage tend to be more pronounced on sandy soils, which have a small cation exchange capacity and are poor in basic cations, than on clay soils (Fecenko, 1982). Salmon (1964) stated that the Mg deficiency did not result solely from a low soil exchangeable Mg but occurs from antagonism with K in very acid soils and in soils with much exchangeable K.

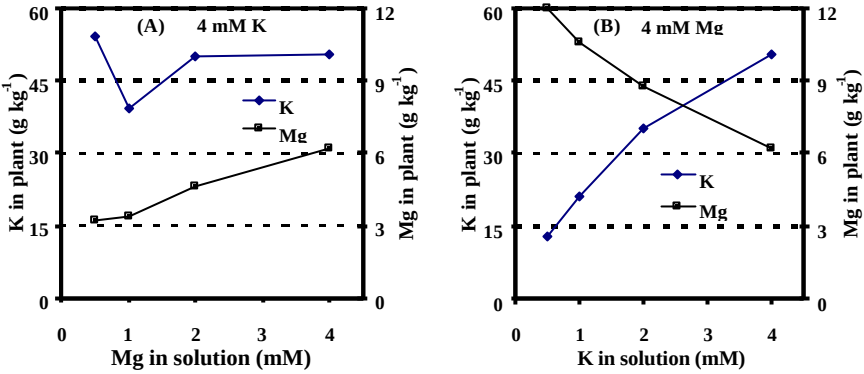

Fig. 3.9. Effect of potassium and magnesium in the nutrient solution on potassium and magnesium concentration of alfalfa (Source: Omar and El Kobbia, 1966).

In forages, high K levels can result in an induced deficiency of Mg and in a decreased Mg availability in ruminant animals that consume mainly forages. This may lead to a metabolic disorder in cattle usually referred to as grass tetany or hypomagnesemia (low levels of magnesium in the blood) (Grunes and Welch, 1989).
Grass tetany is most common in lactating cows grazing lush spring pastures. Fertilizing pastures with large amounts of K, especially in spring, is associated with increased incidence of grass tetany. Therefore, it is recommended to avoid large K applications during the grass tetany season (Robinson *et al*., 1985). Instead, the estimated K required should be divided into a number of smaller applications.

3.4.3. Potassium vs. Sodium

The adverse effect of Na on plant growth is attributed to its antagonistic relationship with Ca, K and Zn in plants and increased salinity and alkalinity hazards in soils (Shukla and Mukhi, 1979). The synergistic or antagonistic effect between K and Na depends on the amount of each element presented in the soil and on the plant type (Marschner, 1971). A number of reports have demonstrated the antagonistic effects of K and Na (Shukla and Mukhi, 1979; Rajarathinam *et al*., 1988; Cordovilla *et al*., 1995; Song and Fujiyama, 1996). The K/Na relationship is important for sugar beet, rhodesgrass, carrots and to the some extent, cotton. In general, the beneficial response of these crops to Na is greatest when K is in short supply, and the effects are less with an increase in soil K levels (Daliparthy *et al*., 1994). Sodium can partially substitute for K in

metabolic functions (Figdore *et al*., 1989). In salt tolerant *Atriplex haliumus* L., there are specific uptake sites for Na, and Na uptake is not inhibited by excessive K (Mozafar *et al*., 1970). Loué (1978) concluded that the interaction of K and Na is always significant and negative in plants that respond to Na. Nevertheless, even in such cases, both nutrients were required to maximize crop yields. Their interaction resulted in higher growth and increased tolerance to low external osmotic potential (Mozafar *et al*., 1970).

3.4.4. Potassium vs. Micronutrients

There are conflicting findings on the interaction between K and some micronutrients. Their interaction is not fully characterized and needs more study, especially under field conditions (Daliparthy *et al*., 1994).

Zinc: Several reports show the effect of K on the P - Zn interaction in corn. At soil K saturation, the "P-induced Zn deficiency" was relieved (Ward *et al*., 1963). Stukenholtz *et al*. (1966) indicated that with rising levels of soil or applied K, the intensity of P-induced Zn deficiency was diminished. The application of K increased the desorption and availability of Zn, which in turn prevented P-induced Zn deficiency (Adriano *et al*., 1971). However, K application did not affect the Zn concentration in the foliage of bluejoint (Laughlin, 1969) but reduced it in alfalfa (Smith, 1975).

Boron: Reeve and Shive (1944) observed that the severity of B toxicity symptoms in tomato and corn was increased when the K concentration in the growth substrate was increased. On the other hand increasing K concentration, increased B-deficiency symptoms at low levels of B. No definite effect of K on B uptake was observed in Brussels sprouts and cauliflower (Gupta, 1979).

Other micronutrients (Fe, Cu, Mn, Mo): Potassium deficiency decreased the Fe-excluding capacity of rice roots, and Fe toxicity in rice was decreased by increasing the K supply (Tanaka and Tadano, 1972). The application of large amounts of K had only a limited influence on the accumulation of Cu and Fe in corn (Stukenholtz *et al*., 1966). Synergetic effects have been reported for K and Mn (Leggett *et al*., 1977; Smith 1975) and K and Mo (Loué, 1978).

3.4.5. Chloride vs. Nitrate

The antagonism between NO_3 and Cl uptake was demonstrated in avocado (Wiesman, 1995; Bar *et al*., 1997), barley (Smith, 1973; Glass and Siddiqi, 1985), broccoli (Liu and Shelp, 1996), citrus (Chapman and Liebig, 1940; Banuls *et al*., 1990, 1997; Bar *et al*., 1997; Cerezo *et al*., 1997), corn (Imas, 1991), kiwifruit (Smith *et al*., 1987), melon and lettuce (Feigin, 1985; Wei *et al*., 1989), groundnut (Wang *et al*., 1989; Leidi *et al*., 1992), potato (James *et al*., 1970), strawberry (Wang *et al*., 1989), tobacco (Fuqua *et al*., 1976),

tomato (Kafkafi *et al*., 1982; Zabala, 1984; Feigin *et al*., 1987) and wheat (Wang *et al*., 1989; Silberbush and Lips, 1991).

Increasing concentrations of NO_3 linearly decrease Cl concentrations in plants (Table 3.2.). An increase of 1 mmol NO_3 g^{-1} DM prevented the accumulation of 2.38 mmol Cl g^{-1} DM in the tomato plant (Kafkafi *et al*., 1982). The inhibition of NO_3 uptake by Cl depends on the plant species and the concentrations of both NO_3 and Cl in the uptake medium (Cerezo *et al*., 1997). In root cells, the high-affinity, saturable system for NO_3 uptake that operates at small NO_3 concentrations (Siddiqi *et al*., 1990) is inhibited by high external Cl, whereas the low-affinity linear system that operates at high NO_3 concentrations seems to be inhibited by high internal Cl (Cerezo *et al*., 1997). The competition of Cl vs. NO_3 was found to be stronger in salt-sensitive plants, such as groundnut, than in salt-tolerant plants, such as cotton (Leidi *et al*., 1992).

The Cl content of citrus leaves was 27-39 g kg^{-1} DM in NO_3-deficient plants and only 5.3 g kg^{-1} DM in plants with an ample NO_3 supply (Adler and Wilcox, 1995). Higher rates of KCl application may be needed in systems where NO_3 levels are high (Fixen *et al*., 1986b). In kiwi fruit, the severity of leaf necrosis following KCl application was attributed not to Cl toxicity but rather to N deficiency enhanced by competition between Cl and NO_3 (Buwalda and Smith, 1991). On the other hand, chlorosis resulting from NO_3-induced Fe deficiency in avocado rootstocks could be prevented by increasing the Cl levels (Bar and Kafkafi, 1992).

Deane-Drummond (1986) suggested that there are two populations of Cl carriers and NO_3 carriers, each differing in their sensitivity to external NO_3 and Cl. The influx of ^{36}Cl from outside of the plasmalemma into the cytoplasm was initially insensitive to external NO_3, but became sensitive after a lag period of 3 to 6 h in barley plants previously grown in solutions lacking NO_3 (Glass and Siddiqi, 1985). The results of kinetic analysis suggested that the inhibition of ^{36}Cl influx by external NO_3 was complex. Chloride efflux, however, was found to be insensitive to external NO_3. A time-course study and other experiments led to a model (Glass and Siddiqi, 1985) for the regulation of Cl influx, involving both the negative feedback effects from vacuolar (NO_3+Cl) or total anion concentration, and the inhibition by external NO_3 of Cl influx at the plasmalemma. The combined effects suggest an explanation for the discrimination against Cl in favor of NO_3 accumulation, when the later ion is available.

Bar *et al*. (1997) suggested that while both Cl and NO_3 anions are taken up by the root against their electrochemical gradient, NO_3 is reduced after uptake whereas Cl maintains its negative charge. As a result, the active uptake of Cl is reduced as the Cl electrochemical potential gradient builds up during its accumulation. Chloride-stimulated ATPase activity is more sensitive than basal ATPase activity to NO_3 (Griffith *et al*., 1986).

Table 3.2. Antagonistic uptake effects between nitrate and chloride.

Crop	Plant part	Concentration range mmol g^{-1} DM		Equation		Source
		Cl (Y)	NO_3-N (X)			
Avocado	Leaves	0.12 - 0.74	1.29 - 1.86[b]	Y = 2.51 - 1.35X	r^2=0.70, n=11	Bar *et al*., 1997[a]
Barley	Root	0.008 - 0.052	0.003 -0.084	Y = 0.05 - 0.52X	r^2=0.96, n=13	Glass and Siddiqi, 1985
Barley	Shoot	0.021- 0.093	0.017 - 0.107	Y =0.098- 0.55X	r^2=0.89, n=13	Glass and Siddiqi, 1985
Broccoli	Shoot	0.05 - 1.00	0.10 - 0.65	Y = 1.50 - 2.97X	r^2=0.70, n=12	Liu and Shelp, 1996[c]
Citrus seedlings	Leaves	0.15 - 0.50	1.7 - 2.7[b]	Y = 0.82 - 0.34X	r^2=0.97, n=3	Chapman and Liebig, 1940[a]
Kiwifruit	Leaves	0.02 - 0.10	0.14 - 0.26	Y = 0.29 - 1.47X	r^2=0.99, n=3	Smith *et al*., 1987[a]
Melon	Shoot	0.08 - 1.00	0.60 - 0.93	Y = 2.87 - 3.22X	r^2=0.80, n=4	Feigin *et al*., 1987[a]
Tomato	Shoot	0.25 - 1.00	0.18 - 0.89	Y =1.25 - 1.25X	r^2=0.89, n=8	Feigin *et al*., 1987[a]
Tomato	Shoot	0.16 - 2.30	0.5 - 1.4	Y = 3.21 - 2.38X	r^2=0.79, n=36	Kafkafi *et al*., 1982
Groundnut	Shoot	0.094 - 0.180	0.003 - 0.035	Y = 0.18 - 2.86X	r^2=0.80, n=6	Wang *et al*., 1989[a]
Strawberry	Shoot	0.03 - 0.26	0.016 - 0.058	Y = 0.36 - 6.25X	r^2=0.93, n=6	Wang *et al*., 1989[a]
Spring wheat	Shoot	0.10 - 0.20	0.014 - 0.140	Y = 0.21 - 0.91X	r^2=0.84, n=7	Wang *et al*., 1989[a]

[a] Recalculated from the original data. [b] Total N concentration of plant grown with nitrate-N as sole N source.

[c] Field grown 'Emperor' broccoli

The fact that the total N content of plants did not decrease in response to Cl applications (Ourry *et al*., 1992; Liu and Shelp, 1996), led Liu and Shelp (1996) to suggest that Cl absorption does not compete directly with NO_3 absorption. The addition of moderate amounts of Cl to the growing medium of broccoli plants decreased the NO_3 content by increasing the extent of NO_3 reduction.
Chloride application may be used as a strategy to decrease the NO_3 content of vegetables (Liu and Shelp, 1996), particularly in plants such as spinach, lettuce and cabbage, which are classified as NO_3 accumulators (Maynard *et al*., 1976). The strategy of applying KCl instead of KNO_3 at the reproductive stages may also be suitable to other crops, such as tomatoes; this would prevent unnecessary vegetative growth caused by excess N (Hand and Fussell, 1995).
Bar *et al*. (1997) found that increasing NO_3 concentration from 2 to 16 mM in the irrigation solution that contained 16 mM of Cl, relieved Cl toxicity symptoms in avocado leaves (Fig. 3.10; Plate 3.1).
Increased Cl availability could increase the optimal application rate of N fertilizer for potatoes (James *et al*., 1970). Supply of KCl or $CaCl_2$ and maintaining suitable NO_3 : Cl ratio can enhance the production of better quality carrots via decreasing the NO_3 content of the carrot without causing reductions in the yield (Inal *et al*., 1998). These findings suggest that the interpretation of soil and plant tissue analyses for N-NO_3 would have to be modified to take into account the uptake competition between NO_3 and Cl.

3.4.6. Chloride vs. Ammonium

When nitrogen is taken up in the ammonium form, relatively more anions have to be taken up to maintain the electrical neutrality of the process (Teyker *et al*., 1992). As a result, when Cl is present in the external solution, NH_4 uptake increases the salt sensitivity of plants like pea (Speer *et al*., 1994), corn and wheat (Lewis *et al*., 1989), and potatoes (Harward *et al*., 1956).
Plants fertilized with NH_4, usually contain much more Cl in the tissue than plants fertilized with NO_3 or NH_4 + NO_3, irrespective of the Cl level in the nutrient solution. Experiments with marigold, petunia, and salvia (Jeong and Lee, 1992), potato (Cao and Tibbitts, 1993), and oilseed rape (Ali *et al*., 1998) all showed that the concentration of the anions of Cl and P in the shoots and roots were increased when NH_4 rather than NO_3 was present in the solutions. When the ratio of NH_4 to NO_3 was relatively high, both the vegetative and grain yields of wheat were more susceptible to NaCl than when plants were fertilized with NO_3 only (Silberbush and Lips, 1991).
Speer and Kaiser (1994) suggested that the intracellular compartmentation capacity of plants given NH_4 was considerably lower than that of plants given NO_3. Why this should be the case remains unclear.

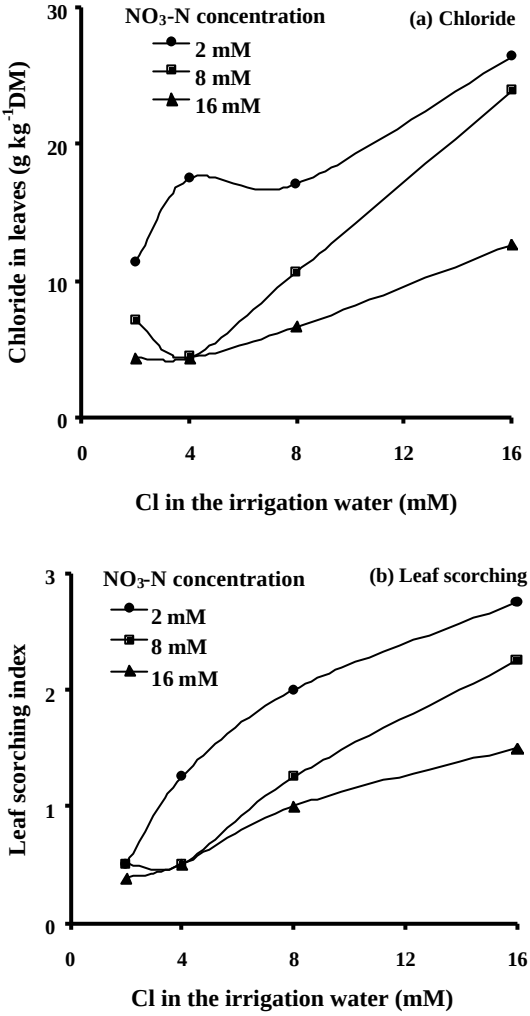

Fig. 3.10. Effects of chloride and nitrate in the irrigation water on leaf chloride concentration (a) and leaf scorching index (b) in avocado cv. *Degania* (Drawn on the basis of data from Bar *et al*., 1997).

3.4.7. Chloride vs. Phosphorus

The interaction of Cl with P appears to be complex. It seems unlikely that competition in uptake between H_2PO_4 and Cl ions is important, because of the great differences in their physical and physiological properties

(Champagnol, 1979). The Cl content in potato petioles was not affected or stimulated by phosphate fertilization when KCl was applied as the sources of both K and Cl (Fig. 3.11.). In hydroponic experiments, both Cl and SO_4 in the culture solution impaired the uptake of P by potato roots (Hang, 1993). In tart cherry leaves, an excess of applied KCl suppressed P and increased Mn (Callan and Westcott, 1996). The results of pot experiments with potatoes grown on Caribou loam soil using ^{32}P led Gausman *et al*. (1958b) to suggest the existence of an optimal or critical level of Cl for maximum P uptake. The optimum Cl level appeared to be 300-450 mg kg^{-1} soil. Phosphorus uptake was stimulated by small amounts and suppressed by higher amounts of Cl (Wang *et al*., 1989). The optimal soil concentration of Cl for maximum P uptake differs among crops; it is about 237-437 mg kg^{-1} for strawberry, 437 mg kg^{-1} for groundnut and 837 mg kg^{-1} for spring wheat (Fig. 3.12.). Chloride had very little effect on the P content of tomato plants grown in nutrient solution (Kafkafi *et al*., 1982), while many reports describe a decline in P uptake when plants were grown in soil under conditions of Cl salinity (Kafkafi, 1987).

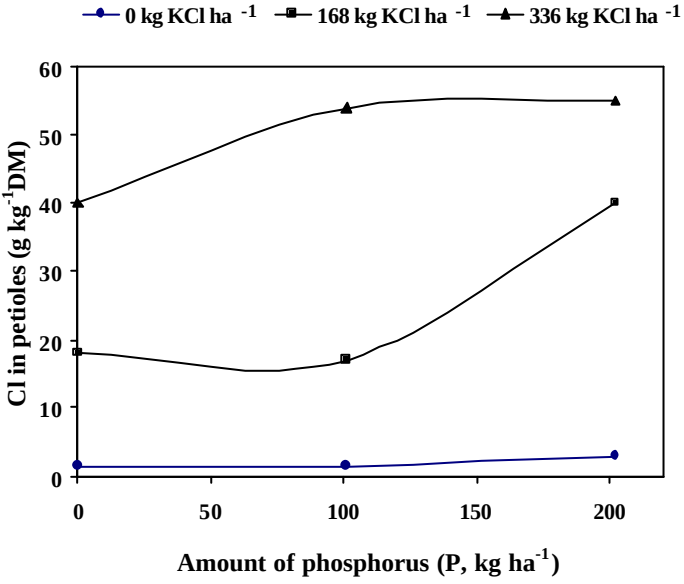

Fig. 3.11. Effect of phosphorus and KCl application rate on chloride concentration in potato petioles (125 days after planting) (Source: James *et al*., 1970).

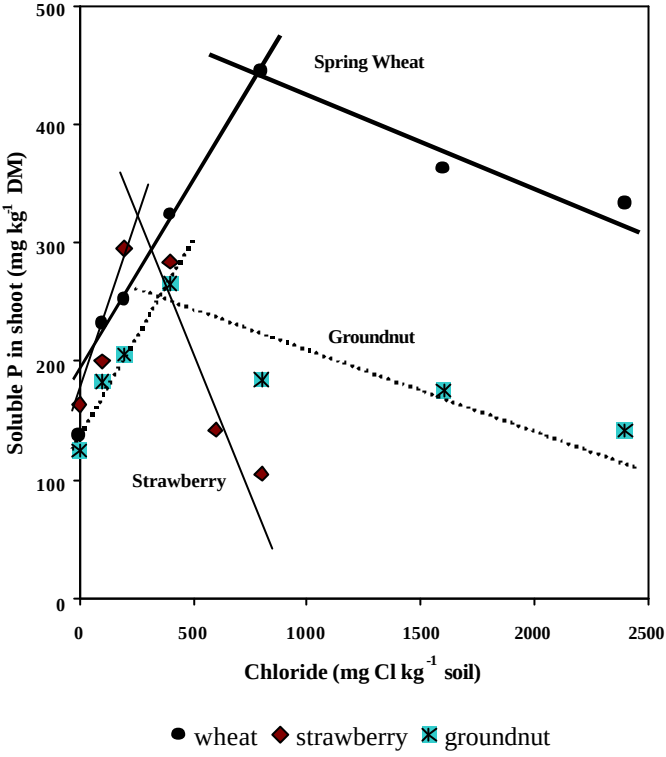

Fig. 3.12. Effect of chloride application in soil on phosphorus concentration in shoot of different crops (Based on data from Wang *et al*., 1989).

Several mechanisms that operate simultaneously are responsible for the apparently conflicting evidence. (1) An increase in NO_3 uptake results in an increase in pH near the roots (Marschner and Romheld, 1983). Then the pH in the rhizosphere may reach values at which P availability is reduced, thus decreasing P uptake. (2) On acid soils, an increase in the concentration of Cl reduces the rate of nitrification. Where NH_4 fertilizer is added, this leads to high NH_4 concentrations in the soil and of NH_4 availability to the roots (Christensen *et al*., 1986). (3) An increase in Cl concentration reduces NO_3 uptake, thereby attenuating the increase in the rhizospheric pH, which may result in an enhanced availability of P. (4) An increase in Cl concentration induces an increase in electrical conductivity (EC) near the root. As a result, the rate of root elongation is reduced and a decline in total plant growth commonly occurs under saline conditions. The uptake of P and Fe are also influenced by the reduction in root elongation (Kafkafi and Bernstein, 1996).

Since all of the above mechanisms operate simultaneously, and also depend on the concentration of other nutrients and soil pH, it is difficult to make a quantitative prediction with respect to the direction of the effects of Cl on P uptake under variable soil conditions.

3.4.8. Chloride vs. Potassium

In the leaf blade of muskmelon the accumulation of Cl did not reduce that of K (Adler and Wilcox, 1995). Increasing concentrations of Cl in the nutrient solution had no consistent effect on K concentrations in the leaves of kiwifruit (Smith *et al*., 1987). An increase in soil Cl of up to 600 mg kg^{-1} soil did not affect plant K in groundnut or wheat (Wang *et al*., 1989). Copra yields of oil palm increased with increasing leaf Cl content, while K concentrations remained relatively constant (Fig. 3.13, von Uexküll and Sanders, 1986). In citrus leaves K and Mg were not affected by the Cl content of the nutrient solution (Bar *et al.* 1996). The effect of Cl on plant K uptake does not follow the nearly synchronous pattern that exists between K and Cl observed in guard cells of intact attached leaves of *Vicia faba* (Talbott and Zeiger, 1996). The cytoplasmic K concentration (100-140 mM) is independent of the external K supply even in the presence of 100 mM of NaCl (Jeschke and Wolf, 1988).

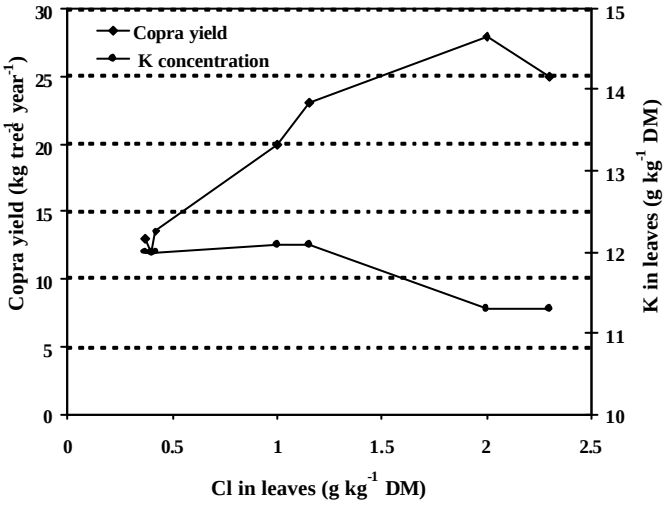

Fig. 3.13. Relation between chloride concentration in leaves and copra yield and potassium concentration in leaves of oil palm cv. *Frond 14* (Source: von Uexküll and Sanders, 1986).

In some plant varieties, Cl tended to inhibit K uptake at high Cl concentrations. Callan and Westcott (1996) found that K concentrations in tart cherry plants declined in the third year when KCl was applied as the sole K source at a rate of 0.58 kg per tree, indicating an inhibitory effect of accumulated Cl on K uptake (Fig. 3.14).

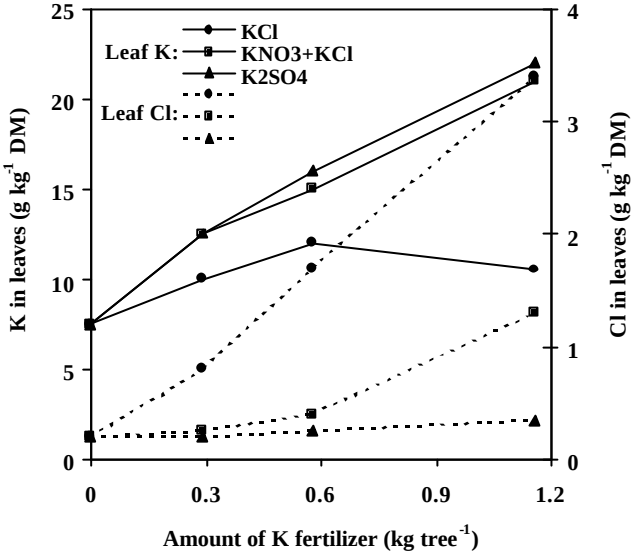

Fig. 3.14. Effect of different potassium fertilizers on potassium and chloride concentration in leaves of tart cherry tree (third year results) (Redrawn from Callan and Westcott, 1996).

Chloride applications tended to increase the K content and decrease the Ca content of citrus seedlings, while an increase in NO_3 concentration in the culture solution increased K:Ca ratios and reduced Cl uptake (Table 3.3). An increase in applied Cl from 90 to 250-400 mg L^{-1} caused a significant increase in the K content of avocado leaves (Lahav *et al*., 1992). Application of Cl at a rate of 100 mg kg^{-1} soil had a stimulatory effect on the K content of strawberry plants (Wang *et al*., 1989). When grown in nutrient solutions containing equivalent amounts of K, plants generally take up more K when KCl rather than K_2SO_4 is the K source. Jackson and McBride (1986) found that petiole K in potatoes given KCl was larger than when K_2SO_4 was applied. The K concentrations in the leaves of kiwifruit were significantly greater for vines receiving KCl than for vines receiving K_2SO_4 (Buwalda and Smith, 1991). The kiwifruit uses Cl rather than organic anions for charge balance and thus takes up much K (Buwalda and Smith, 1991).

Table 3.3. Influence of nitrate on the cation content (g kg^{-1}) in citrus leaves at two levels of chloride.

Nitrate	Chloride concentation in culture solution								
	----------0.28 mM----------				----------20.28 mM----------				
(mM)	K	Ca	K/Ca	Mg	Cl	K	Ca	K/Ca	Mg
0.05	30.6	29.9	1.0	2.3	27.8	40.2	29.0	1.4	2.8
0.10	26.5	30.3	0.9	2.4	17.9	31.6	26.8	1.2	2.5
0.50	25.6	26.7	1.0	2.7	8.7	29.0	22.3	1.3	2.3
5.00	27.6	24.8	1.1	2.4	5.3	32.1	20.9	1.5	2.2

Recalculated from Chapman and Liebig (1940).

3.4.9. Chloride vs. Calcium

Calcium ions are required for the maintenance of membrane integrity and ion transport regulation (Marschner, 1995). The ability of Ca to mitigate the adverse effects of NaCl by inhibiting Na uptake has been tested in many experiments (Banuls *et al*., 1991; Chien *et al*., 1991). The higher the concentration of NaCl in the medium, the more Ca is required up to a maximum of 10 mM to achieve the maximum gain in fresh weight (Kafkafi and Bernstein, 1996).

Accumulation of Cl in orange leaves grafted on either Cl-tolerant or Cl-sensitive rootstocks was reduced when external Ca concentrations were increased. At the same time, the Cl concentration in the roots remained constant or was slightly increased (Fig. 3.15). The distribution of Cl in the plants suggests that a high external Ca level increased Cl accumulation in the basal stem and roots, reduced the transport of Cl from the roots to the leaves, and increased photosynthesis and stomatal conductance (Banuls *et al*., 1991; Banuls and Primo-Millo, 1992).

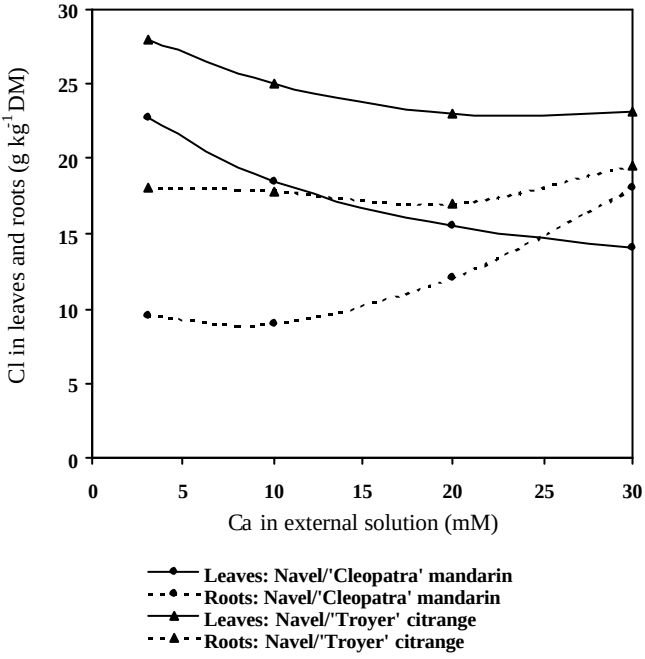

Fig. 3.15. Effects of calcium concentration in the external solution on chloride concentration in citrus leaves and roots of two scion/rootstock combinations (Recalculated and drawn from Banuls *et al*., 1991).