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# Notes on Plant Nutrition

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# Plant Nutrition

# Chapter 1 Introduction

*Plant nutrition* is the study of absorption, translocation, and function of essential elements or nutrients in plants. Essential elements are chemical elements that meet criteria that determine that the elements are required for plant growth and development and therefore are called *plant nutrients*. *Soil fertility* is the study of delivery of essential elements from the soil to the plant. Soil fertility involves chemical, physical, and biological properties of soils. Some chemical properties of soil fertility are supply of plant nutrients and soil acidity. Some physical properties that affect soil fertility are texture, structure, depth, drainage, aeration, water, and temperature. Biological properties refer effects of organisms on soil fertility and may include harmful organisms such as diseases, insect pests, and weeds or beneficial organisms such as bacteria that conduct processes of mineralization and nitrification. It is difficult to sort properties of soil fertility into chemical, physical, and biological factors because of the interrelations and similarities of these factors.

### Reasons for Studying Plant nutrition

***Increases in crop yields***. Increased use of fertilizers has been a principal factor in increasing yields of crops in the World. Corn yields have increased sharply since about 1950 when fertilization of crops became a widely used practice in farming (Figures 1.1 and 1.2). Yields of wheat, rice, potatoes, and other crops have shown similar increases associated with the use of fertilizers. It is noted that fertilizer applications of nitrogen, phosphorus, and potassium have not increased since about 1990 although yields continue to increase. Perhaps, improvement of crop varieties and increased efficiency of fertilization have brought about the continued increases in yields without a matching increase in use of fertilization. Use of new varieties and improved efficiencies of nutrient use involve applications of plant nutrition.

***Crop species and varieties***. Crop species and varieties differ in their requirements for fertilization. Different species have different growth rates and responses to supplies of nutrients. Highly productive crops that produce large biomass will require more nutrition that crops that not as productive. The amount of removal of biomass by grain, shoots, or roots affects the nutritional requirements of crops. Some crops absorb nutrients throughout their growth cycle, whereas some crops may cease nutrient absorption at flowering and others may increase nutrient absorption at flowering. Different cultivars may accumulate different amounts of nutrients depending on the productivity, growth rate, and capacity of the crop to absorb, transport, and accumulate nutrients. The breeding of new varieties usually does not assess nutritional

requirements of crops. Varieties under development usually are grown at high levels of soil fertility in which plant nutrients are not limiting factors. The nutritional needs of new varieties must be assessed since these requirements may differ from those of traditional varieties. The quality of ornamental crops depends on the supply of plant nutrients to the crops. Failures to recognize the nutritional needs of different crop species and varieties may lead to limited productivity or crop failure. The supply of nutrients to crops of different growth rates and productivity, varying harvested parts, and genetic differences in nutrient accumulation demonstrate the need for studies of plant nutrition of crops. Figure 1.3 shows different patterns of crop response to fertilization. Plot A shows a yield response in which maximum productivity is reached below the maximum rate of fertilization, and a suppression in yield occurs. Plot B shows a crop response in which yields continue to increase as the rate of fertilization increases, indicating that productivity can be improved by increased fertilization. Plot C shows a response in which a maximum yield is reached below the maximum rate of fertilization, but yield suppression does not occur. Accumulation of nutrients with this type of response is called *luxury consumption*, as nutrient accumulation increases with increased amount of fertilization but no yield increase occurs.

***Increased nutrient-use efficiency***. Increasing efficiency of nutrient use requires knowledge of nutrient acquisition, translocation, and metabolism. Improvements of nutrient-use efficiency involve investigations of practices of application of fertilizers to soil and assess factors such as sidedressing of fertilizers in bands beside crops, broadcasting fertilizers across a field, and multiple applications of fertilizers in a growing season.

***Chemical forms of nutrients***. Much research addresses the effects of chemical forms of fertilizers on crop nutrition. Extensive investigations have been conducted to assess the relative effectiveness of ammonium and nitrate forms of nitrogen on crop production. Use of ammonium-containing fertilizers may increase nitrogen recovery by crops as the ammonium form of nitrogen is held to cation-exchange sites in soils, thereby giving some protection of nitrogen from losses by leaching. Urea that is applied to the surface of land is subject to losses of nitrogen by ammonia volatilization. Inhibitors of urease activity (conversion of urea to ammonium) and nitrification (conversion of ammonium to nitrate) are used agriculturally to restrict losses of nitrogen by volatilization or leaching. Nitrate on the other hand is not held to soils and may leach readily and be removed from the root zone. Ammonium, however, is toxic to plants. Extensive studies have been made and continue to investigate how the phytotoxicity of ammonium can be restricted. Phosphorus and potassium fertilizers vary in solubility and thus in the availability of these nutrients to crops. Nutritional studies

are conducted on these fertilizers to assess nutrient acquisition under various environmental conditions.

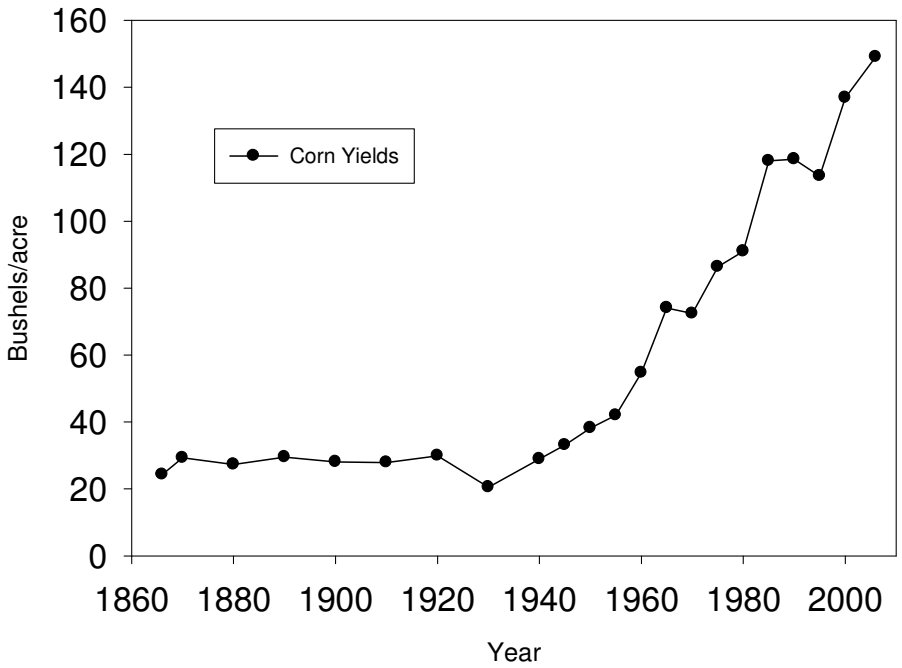



Figure 1.1. Yields of corn in the United States from 1864 to 2010. A bushel of shelled corn is 56 lb.

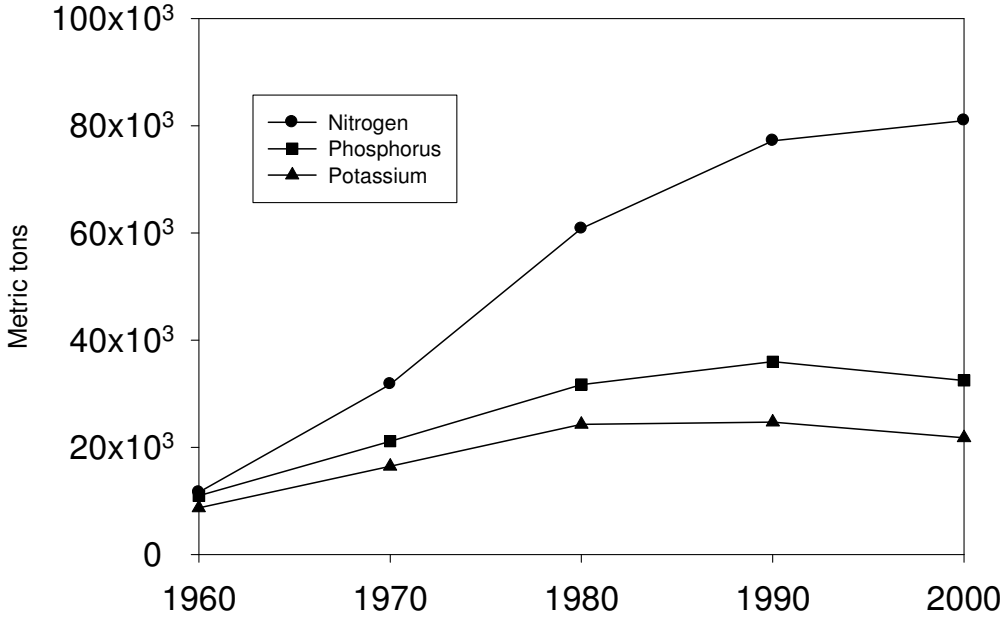



Figure 1.2. Worldwide consumption of nitrogen, phosphorus, and potassium supplied in fertilizers from 1960 to 2000.

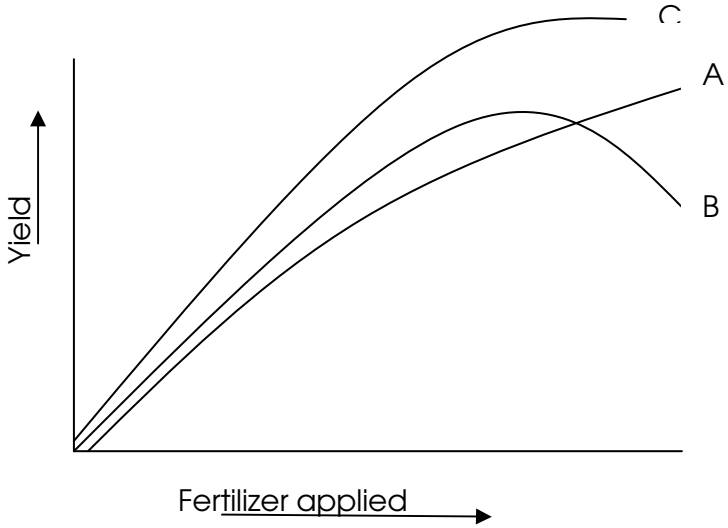



Figure 1.3. Yield of crops in response to fertilizer applied showing: A. a response in which crop yield increases with increases in fertilization; B. a response in which a maximum yield is reached at an optimum amount of fertilization and a restriction in yield occurs with increased fertilization; and C. a response in which a maximum yield plateau is reached at optimum fertilization.

***Environmental concerns***. Environmental concerns related to losses of nutrients into the environment are addressed in studies of plant nutrition. Nitrate leaching from soils has potential to reach surface and ground waters and lead to pollution of these bodies of water. Phosphorus due to its fixation in soils does leach, except in sandy soils low in iron and aluminum, but it can be lost from runoff across surface of land. Transport of soil particles moves phosphorus into bodies of surface water. Potassium is held in soils by fixation and cation exchange so that leaching is small except in sandy soils, where a third of potassium fertilization may be lost by leaching. Erosion of soils leads to substantial losses of potassium from soils. Nutrient management involves studies of plant nutrition to maximize nutrient-use efficiency and accumulation of nutrients in crops.

***Nutritional value of crops for humans and livestock***. Humans and livestock depend on plant-derived foods and feeds to supply mineral nutrients for animal diets. The accumulation of nutrients in plant-based diets is a subject of study in plant nutrition. Investigators address methods of increasing the mineral contents of fruits, vegetables, grains, and forages through management of plant nutrition and genetics. Plants are selective in nutrient absorption and accumulation, but this selectivity can be overridden through plant nutrition. Accumulation of nutrients generally increases with supply of nutrients within limits. Nutrients interact in synergistic and antagonistic relations with one another with some nutrients enhancing the accumulation of others and with some nutrients suppressing the accumulation of others. Biofortification of plant-based foods is

an area of study in plant nutrition in which attempts are made to increase the foods with iron, zinc, iodine, and other micronutrients of humans through plant nutrition.

***Research and teaching***. Training of future scientists with interests in plant nutrition is a major purpose of studies in this field. These scientists will lead investigations in matters that are discussed above—increasing crop yields through plant nutrition, conducting studies of nutrient accumulation among different crop species and varieties, increasing nutrient-use efficiency, addressing effects of chemical forms of nutrients on plant nutrition, addressing environmental concerns, and increasing the nutritive value of plant-based foods and feeds with improved nutrition of crops.
In this course, the lectures and laboratories help to train students for research and teaching in plant nutrition.

**Conducting Research**

***Setting up experiments***. Investigators must consider plants that can be used for the research to be conducted. Any plant may be suitable for studies of plant nutrition, but the selected plants must be suitable for use in the growing conditions that exist. For short-term experiments, as is the case in this course (all experiments must be finished in a semester), the plants must be fast growing. Researchers must consider what part of the plant is to be harvested. Duration and growing conditions will vary with sampling of vegetative parts and reproductive parts of plants.

The medium in which plants are grown must be considered. Many studies of plant nutrition are conducted in soil-less media or by hydroponics. Except for studies that are conducted in soils, it is expected that the medium will supply no nutrients and that nutrients will be supplied by solutions. Hydroponics is chosen for employment in many studies because this system allows researchers to control the nutrition of crops by adjusting the nutrient solutions that feed the plants.

***Collecting data***. In almost all studies of plant nutrition, investigators are interested in the effects of treatments of plant growth and crop yield. Fresh weights and dry weights of plant components are measurements of mass. Fresh weights are used in determining yields of produce or in cases where dry mass considerations are not important in assessing effects. Determining dry weights of crops often doubles the amount of work involved.

Dry weights are important for studies of plant composition. In studies of plant composition, concentrations of nutrients on a dry-weight basis usually are

evaluated. Investigators must consider whether to assess accumulation in the whole plants or in plant parts. Concentrations give an indication of the accumulation of nutrients in whole plants or plant parts. Plant parts vary widely in composition. Notable differences are evident between nutrient concentrations in vegetative and reproductive plant parts. Total nutrient accumulation is a product of concentration and plant dry mass. Total accumulation is important in evaluating nutrient removal from a soil or other media. Plant increases in mass result from the accumulation of carbon by photosynthesis, and carbon accumulation usually exceeds the rate of nutrient accumulation from soil or media. Large plants may have a lower concentration of nutrients than small plants that have restricted growth. This effect is referred to as *the dilution effect*, as the increased carbon accumulation dilutes the concentrations of other elements. Small plants or produce from these plants, especially if their growth is inhibited by some factor, may have high concentrations of nutrients. In this case, slow growth and restricted carbon accumulation lead to an enhancement in the concentration of nutrients in the plants. This response is called *the concentration effect*. Assessments of plant nutrient status can be assisted by considerations of the concentration or dilution of nutrients by crop growth. Since different organs of plants accumulate different concentrations of nutrients, evaluations of plant nutrition involve studies of nutrient distribution in plants.

*Absorption* or *uptake* is the process by which nutrients enter into plants. Most absorption of nutrients occurs by roots. Foliar absorption also occurs, and foliar feeding may be a way of supplying nutrients to severely deficient plants, although this practice has limited value in the total nutrition of plants. *Accumulation* should be used as the term to describe the amassment of nutrients in plant parts. Some people erroneously refer to the accumulation of plant nutrients in shoots as uptake.

*Translocation* is the process by which nutrients are distributed in plants. Translocation from roots to shoots is through the xylem and is driven principally by physical processes. Distribution of nutrients from leaves to other organs of the plant is through the phloem and is driven by energy-requiring processes that depend on plant metabolism. All nutrients are transported in the xylem, but some elements are not transported in the phloem. Nutrients that are transported in the phloem as called *mobile* elements. Elements that are not transported in the phloem are called *immobile* elements. Mobility of elements is important in considerations of plant nutrition and composition.

***Interpretation and application***. Researchers must interpret the results of experiments and develop conclusions about processes and factors that affect plant nutrition. Application of the research is developed from the interpretation

of the results. Studies of plant composition allow investigators to assess the nutritional status of crop. These interpretations can lead to development of recommendations to improve crop productivity. Researchers may not make recommendations based on research directly. However, it is important that researchers publish results so that other individuals can make recommendations concerning plant nutrition and agricultural practices.

# Chapter 2 Media for Plant Nutrition

A medium for studies of plant nutrition must supply plant nutrients, air, and water and provide support for the plants.

## Plant Nutrients

A plant nutrient is an essential chemical element. For a nutrient to be considered essential, it must meet the following criteria.

a. *The plant will not complete its life cycle in the absence of the element*. This criterion implies that the element has a vital metabolic function in plants or that the element is a part of a molecule of an essential plant constituent or metabolite. Growth will be abnormal or limited with an insufficient supply of the element, and symptoms of deficiency may develop. The essential element has a direct effect on growth or metabolism.

b. *The requirement for the element must be universal among plants*. The element is essential for all plants. If the element is required by some plants and not by all plants, it is called a *beneficial* element. Beneficial elements may enhance growth but are not required for plant growth.

c. *No other element substitutes fully for the essential element*. The role of an element in a plant is absolute in some nature. Partial substitution may occur, such as the substitution in some metabolic reactions fully or partially.

## List of essential elements

Seventeen chemical elements are recognized as essential elements or plant nutrients. Three of these elements are derived from the air (Table 2.1). The other 14 elements are derived from the soil or another medium. The elements that are derived from the air constitute the major portion of the dry matter accumulation in plants. Soil organic matter or organic molecules in nutrient solutions do not contribute to the carbon nutrition of plants. Hydrogen in plants is derived from water. Oxygen is required as elemental oxygen for respiration and enters into organic molecules carbon fixation in photosynthesis or perhaps by hydration of molecules. The concentrations listed in Table 2.1 may vary by 10% above or below the mean values presented.

The elements that are derived from soils are the common subjects of plant nutrition (Table 2.2). These elements often are called mineral nutrients and may compose in total about 10% of the dry weight of plants. All soil derived- nutrients are absorbed from solution in water in the soil or from nutrient solutions provided

to plants in soil-less culture. The concentrations of the individual nutrients vary widely among plants species and varieties and among plant parts. The concentrations of nutrients vary with their availabilities in soil, media, or nutrient solutions. Concentrations in various plant parts may be half or double or more those listed in Table 2.2.

Table 2.1 Plant nutrients derived from the air

| Element | Approximate concentration in plants, % dry mass | Source from air |
|---|---|---|
| Carbon | 50 | Carbon dioxide |
| Hydrogen | 5 | Water |
| Oxygen | 40 | Elemental oxygen, water, carbon dioxide |

Table 2.1 Plant nutrients derived from soil or medium

| Element | Approximate concentration in leaves | Source from soil or medium |
|---|---|---|
| ***Macronutrients*** | % dry mass | |
| Nitrogen | 3.5 | Nitrate ion, $NO_3^-$; Ammonium ion, $NH_4^+$ |
| Phosphorus | 0.4 | Phosphate ion, $H_2PO_4^-$ |
| Potassium | 2.0 | Potassium ion, $K^+$ |
| Calcium | 1.0 | Calcium ion, $Ca^{++}$ |
| Magnesium | 0.5 | Magnesium ion, $Mg^{++}$ |
| Sulfur | 0.2 | Sulfate ion, $SO_4^=$ |
| ***Micronutrients*** | mg/kg dry mass | |
| Iron | 100 | Ferrous ion, $Fe^{++}$; ferric ion, $Fe^{+++}$ |
| Manganese | 30 | Manganous ion, $Mn^{++}$ |
| Zinc | 20 | Zinc ion, $Zn^{++}$ |
| Copper | 5 | Cupric ion, $Cu^{++}$ |
| Molybdenum | 0.1 | Molybdate ion, $MoO4^=$ |
| Boron | 30 | Boric acid, $H_3BO_3$ |
| Chlorine | 100 | Chloride ion, $Cl^-$ |
| Nickel | 1 | Nickel ion, $Ni^{++}$ |

Elements that accumulate at considerable concentrations in plant dry mass (expressed in percent) are called *macronutrients*. Elements that accumulate in small concentrations (expressed in parts per million or mg/kg; one percent is 10,000 ppm.) in plants are called *micronutrients* or minor elements or trace elements. Micronutrient is the preferred term. Micronutrients are as important in plant nutrition as macronutrients, as each nutrient is required absolutely for plant growth. Concentrations of mineral elements in plant parts are commonly used guides for assessing the nutritional status of plants.

Elements that may be required by some plants or that improve the growth of some plants are called *beneficial* elements. Cobalt is required for nitrogen fixation, but nitrogen fixation is not a vital process in plants since these plants will grow well on mineral sources of nitrogen. Plants that do not fix nitrogen do not require cobalt for any metabolic process. Sodium improves the growth of some plants. Silicon, selenium, and vanadium have been studied for their beneficial effects on plants. Rare earths (lanthanides--atomic number 57 to 71--and yttrium and scandium) have been suggested as being beneficial elements and are used in fertilizers in some parts of the world.

**Types of Media**

Media for studies of plant nutrition include soils and various soil-less substrates including nutrient solutions. Each of these media has certain properties that make its use advantageous in studies of plant nutrition.

**Soil**

Most of our crops are terrestrial and depend on soil for nutrient supplies; therefore, soil is the natural medium for the growth of crops. Soils are convenient to use in investigations of plant nutrition, since no special apparatus is needed to use soil as the medium. Soils provide unique combinations of chemical and physical properties that are difficult to simulate. Plants roots grow normally in soil with development of root hairs and microfloral associations. Greenhouse studies made in soils may be easier to translate to field conditions that studies made in soil-less media.

On the other hand, soils present many difficulties in studies of plant nutrition. Soils are complex with nutrients being provided from mineral matter, organic matter, and soil water. Attempts to purify soil, such as by acid washing or removal of organic matter, change the soil into an artificial system. It is difficult to identify the soil factors that are affecting plants. Soil particles have heterogeneous sizes and mixtures of sands, silts, and clays and sesquioxides, thereby making it difficult to associate or separate physical properties with plant nutrition. Fixation of potassium, phosphorus, and other nutrients occurs in soils and may change quickly the availability of nutrients added. The organic matter of soil has different

states of stability, with certain fractions being labile and quick to release nutrients and other components being stable with slow release properties or even imposing immobilization of nutrients. The nutrients in the soil solution can be depleted quickly by plant growth, and renewal of the soil solution is at variable rates. Separation of factors of microfloral associations (nodules, mycorrhizae) from root factors is difficult. Microbial transformations of plant nutrients occur in soils. For example, ammonium is converted to nitrate, and urea is converted to ammonium and subsequently to nitrate, thereby destroying the original form of nitrogen added. These conversions occur at variable rates in different soils. Studies of absorption of ammonium or urea from soils would require considerable attention or would not be possible. Amino acids added to soils would be transformed rapidly, thereby eliminating the opportunity to study the use of organic molecules as sources of nitrogen for plants. Soils may have certain toxic factors that can affect plant growth or change the chemistry among nutrients and soil-borne elements. The chemistry of soil, such as soil acidity, can affect toxic factors. The effects of acidity on nutrition would be difficult to study in soils because of the interaction of soil acidity with availability or solubility of nutrients or toxic elements. For example, high soil acidity may enhance phosphorus fixation and increase the solubility of aluminum, manganese, and other toxins in soils. Kinetic studies of uptake of plant nutrients could not be studied in soils because of the transformations that occur in soils.

*Types of studies in soils*. Yield responses to fertilizers are appropriate studies for soils. Investigators may choose to study plant responses to total nutrients or forms of nutrients applied. Nutrient accumulation in plants can be studied with the understanding that soils may limit accumulation through chemical and physical processes. Soil acidity may interact with fertilization to affect crop nutrition. The effects of acidity on nutrient accumulation from soils and from nutrient solutions differ widely due to the complexity of soil chemistry. One should not try to transfer the effects of acidity in soils to the effects of acidity in soil-less culture in studies of plant nutrition.

**Solution culture**

Solution culture has been used since about 1856 to 1865 by investigators such as Wilhelm Knop, Julius von Sachs, Wilhelm Salm-Horstmar, and Nicholas-Theodore de Saussure. These scientists used hydroponics as a research tool to demonstrate the essentiality of elements. Solutions were formulated with an element missing from the preparation, and plant growth responses were studied. The essentiality of virtually every plant nutrient has been shown through hydroponics. The techniques used back in 1860 or before are basically the same as those used today. These scientists demonstrated the essentiality of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and iron. Demonstrations of the essentiality of micronutrients elements required improvements in purification

of nutrient solutions to ensure that micronutrients were deficient. Dates of discovery of essentiality of plant nutrients are listed (Table 2.3).

Table 2.3 Approximate date of discoveries of essentiality of plant nutrients and scientists making the discoveries

| Element | Date | Scientist |
|---|---|---|
| Nitrogen | 1804 | de Saussure |
| Phosphorus | 1839 | Liebig |
| Potassium | 1856-1865 | Salm-Horstmar, Sachs, Knop |
| Calcium | 1860 | Sachs |
| Magnesium | 1860 | Salm-Horstmar |
| Sulfur | 1860 | Sachs |
| Iron | 1860 | Sachs |
| Manganese | 1920 | McHargue |
| Boron | 1923 | Warington |
| Zinc | 1926 | Sommer and Lipman |
| Copper | 1930 | Sommer |
| Molybdenum | 1939 | Arnon and Stout |
| Chlorine | 1954 | Broyer |
| Nickel | 1987 | Brown, Welch, and Carey |

# Chapter 3 Guidelines for Formulation of Nutrient Solutions

## Chemical Composition of Nutrient Solutions

*The composition of the solution should be constant*. All major and minor elements should be present and held in constant supply—except for the one being studied. It is important that all nutrients be present in constant supply in studies of plant nutrition to avoid issues of changes in composition on nutrient uptake and functions. In some experiments in which plant responses to variable supplies of a nutrient are being studied, that individual nutrient may vary, but all other elements should be held constant within the limits permitted by formulation of the solution.

Nutrients are added to solutions as soluble salts. Hence, leaving one nutrient out of the formulation usually takes two nutrients from the solution. The nutrient to be held constant is added with another soluble salt. Table 3.1 shows the salts of a full-strength nutrient solution and of a nitrogen-deficient solution.

Table 3.1 Soluble salts for macronutrients comprising a full-strength solution and a nitrogen-deficient solution

| Full-strength solution | Nitrogen-deficient solution |
|---|---|
| Calcium nitrate | Calcium chloride |
| Potassium nitrate | Potassium chloride |
| Magnesium sulfate | Magnesium sulfate |
| Potassium dihydrogen phosphate | Potassium dihydrogen phosphate |

In the nitrogen-deficient solution, calcium, potassium, magnesium, sulfur, and phosphorus are held constant with the supply of the full-strength solution. Chlorine varies between the two solutions since chloride salts are substituted for the nitrate salts of the full-strength solution. Chloride has low phytotoxicity; hence, chlorides can be used to supply calcium and potassium. Limits apply to the amount of chloride used in nutrient solutions. This limit is set commonly at 0.01M $Cl^-$.

***The solution should be aerated***. Due to the limited solubility of oxygen in water, simple diffusion does not supply adequate oxygen to roots in water culture. Warm water holds less oxygen than cool water. Air, oxygen, or mixtures of oxygen and nitrogen gases can be bubbled into solutions to provide oxygen to roots. Exposure of solutions to air by flowing them with exposure to air supplies

oxygen. Solid media should have a good division of air-filled pores and water-filled pores to supply oxygen to roots.

***The solution should be fairly concentrated with nutrients***. Nutrient solutions generally are several times more concentrated with nutrients than the soil solution (dissolved solutes in water in soil) if the volume of the containers used for water culture are small. The concentrations in large vats in which plants are grown can be much smaller that the concentrations in small containers. A nutrient solution that is flowing by roots of plants can be diluted relative to that of solutions in fixed-volume containers, and generally the faster the flow, the less the concentration needs to be. Much research has been conducted to develop nutrients solutions suitable for hydroponics. Concentrations of nutrients in a commonly used nutrient solution and in a soil solution are given in Table 3.2

Table 3.2 Concentrations of nutrients in a nutrient solution and in a soil solution

| Element | Nutrient solution[z] | Soil solution[y] | |
|---|---|---|---|
| | | Common occurrence | Range |
| | mg/L | | |
| Nitrogen | 210 | 10 | 10 to >200 |
| Phosphorus | 31 | <1 | 0 to 0.15 |
| Potassium | 234 | 40 | 10 to >200 |
| Calcium | 200 | 50 | 0 to 100 |
| Magnesium | 48 | 50 | 25 to 200 |
| Sulfur | 64 | 25 | 0 to 30 |

[z] Hoagland, D. R., and Arnon, D. I. 1950. The water culture method for growing plants without soils. Calif. Agr. Expt. Sta. Cir. 347 (Revised).
[y] Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley, New York.

The concentration of a nutrient solution should not be so high as to interfere with the water relations of plants. Solute lowers the osmotic potential of water. Water moves from a position of high osmotic potential to a position of low osmotic potential.

**Plant cell** **External solution**

**Ci**=concentrations of salts in solution inside a cell

**Co**=Concentration of salts in solution outside cell
(concentration in nutrient solution)

If **Ci** is less than **Co**, water will flow from inside the cell to the solution, or the plant will have difficulty absorbing water from the solution. This occurrence will lead to wilting of the plants. Generally, the situation is reversed so that the concentration of the solution inside the cells exceeds the concentration of the solution outside the cell, and water can enter the plants.

A guideline is that the osmotic potential of a solution should not fall below -2 atm. Commonly, the absolute value of this potential is used in informal communications, that is, the potential should not exceed |2| atm.

If the molar concentration of ions in a nutrient solution is known, the osmotic potential of a solution can be calculated from the Gas Law.

The gas law is written:

PV=nRT

where P is the pressure in atmospheres (atm; gas pressure or osmotic pressure);
V is the volume of the solution;
n is the number moles of gas or the molarity of the total solute;
R is the gas constant (0.082 L.atm/deg.mol); and
T is the absolute temperature (degrees Kelvin).

Under standard conditions of 0° C (273° K) and 1 atm pressure, the volume of one mole of gas is 22.4 L. If this volume of gas were compressed to one liter, the pressure would be 22.4 atm. Application of the gas law to solution, therefore, notes that one mole of total solute in a liter of water would have a potential pressure of 22.4 atm. That is to say, one mole of solute (all ions summed) would have the same pressure as one mole of gas compressed to one liter.

Osmotic potential is the reduction of the chemical potential of water as the result of dissolving substances in water. The chemical potential (energy) of pure water is higher than that of water with solute. Water diffuses by the process of osmosis from a place of higher potential of water to a place of lower potential of water. To reverse this flow through a semipermeable membrane, a pressure ($\Psi$) equal to the reduction due to osmotic potential would need to be applied to the solution. This reversed flow is called *reverse osmosis*.

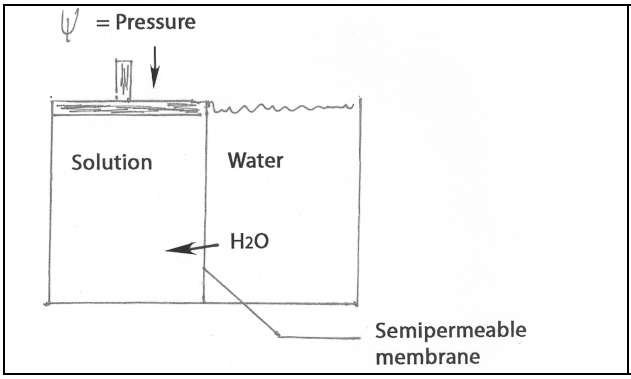



Figure 3.2 Illustration of movement of water from pure water to a solution. Water moves from a region of high potential energy of water to a region of lower energy. Ψ is the pressure that must be applied to a piston to reverse the flow of water.

***Use salts containing two or more essential elements***. If one salt can supply two or more elements, the osmotic potential of a solution will not be affected as much as if two salts are used to supply two nutrients. For example, potassium nitrate supplies two nutrients. Use of sodium nitrate and potassium chloride to supply the nitrogen and potassium would give a solution with twice the osmotic potential as the solution of potassium chloride as explained by the relationship below, which shows that only two ions are in solution with the use of potassium nitrate whereas four ions are in solution with use of potassium chloride and sodium nitrate (Sodium is not a nutrient, and chlorine is a micronutrient).

$KNO_3 = K^+ + NO_3^-$

$NaNO_3 = Na^+ + NO_3^-$
$KCl = K^+ + Cl^-$

A guideline in formulating a nutrient solution is that the total osmotic potential of a solution should not be below -2 atm. Application of this guideline gives considerable safety against osmotic damage to plants through impeded water relations.

Hoagland and Arnon Solution no. 1 is used commonly to grow plants. It has the following composition of salts to supply macronutrients:

0.005 M $KNO_3$
0.005 M $Ca(NO3)_2$
0.001 M $KH_2PO_4$
0.002 M $MgSO_4$

In the solution, these salts provide the following ionic concentrations:

0.005 M $KNO_3$ = 0.005 M $K^+$ + 0.005 M $NO_3^-$
0.005 M $Ca(NO3)_2$ = 0.005 M $Ca^{++}$ + 0.010 M $NO_3^-$
0.001 M $KH_2PO_4$ = 0.001 M $K^+$ + 0.001 M $H_2PO_4$
0.002 M $MgSO_4$ = 0.002 M $Mg^{++}$ + 0.002 M $SO_4^=$

The sum of the molarity of all ions in solution is 0.031 M. Applying the gas law, the osmotic potential of this solution is -0.694 atm:

PV = nRT
P(1 L) = 0.031 mole (RT) = 0.031 x 22.4 atm
P = 0.694 atm

This number has a negative value since it refers to the potential of water, which has been lowered by the salts. The absolute value is smaller than the guideline of -2 atm; hence, this solution will have little or no effect on the water relations of plants.
Another guideline is that the electrical conductivity of a nutrient solution should not exceed 2 dS/m (decisiemens/meter). This unit is the same as 2 mmho/cm, which is the common unit for expressing electrical conductivity. The electrical conductivity of a solution is due to the migration of ions in an electrical field:

CATHODE (negative charge) ← Cations (positive charge)
Anions (negative charge) **->** ANODE (positive charge)

Another guideline is that the total dissolved solids (TDS) should not exceed 2,000 to 4,000 mg/L (ppm). Total dissolved solids cannot be measured directly but can be calculated or estimated from electrical conductivity. People who use a TDS meter have an electrical conductivity (EC) meter with a scale that has been adjusted to read TDS.

A common conversion is that TDS = 1,000 x EC. Some conversions use a factor of 640 whereas others use 800.

Calculation of TDS requires knowledge of the composition of a nutrient solution. For example, with Hoagland and Arnon no. 1 solution:

0.005 M $KNO_3$ adds 0.505 g of solute
0.005 M $Ca(NO3)_2$ adds 0.820 g of solute
0.001 M $KH_2PO_4$ adds 0.136 g of solute
0.002 M $MgSO_4$ adds 0.240 g of solute
For a TDS of about 1.7 g of solute per liter or 1,700 mg/L

The TDS of 1,700 mg/L is below the guideline concentration of 2,000 to 4,000 mg/L.

The concentrations of micronutrients in a nutrient solution is so small so that the contributions of micronutrients to the salinity of a nutrient solution is not a

significant figure in consideration of any of the guidelines of osmotic potential, electrical conductivity, or total dissolved solids.

Use salts of macronutrients to supply macronutrients

The considerations of total solute in a nutrient solution address cautions to avoid osmotic desiccation of plants, that is, to avoid hindering water relations of plants. This guideline is implemented to avoid toxicities from micronutrients. For example, sulfur is supplied as magnesium sulfate. This salt gives two nutrients. Manganese sulfate would not be used even though it also supplies two nutrients but would be avoided because the manganese supply at a concentration to supply the sulfur would be toxic to plants.

***The acidity of the nutrient solution should be between pH 4 and 7.5***. Below pH 4, hydrogen ions are directly toxic to plants (Figure 3.1). Above pH 7.5, micronutrients (except molybdenum) and phosphate are precipitated, and the micronutrients may become deficient in the nutrient solution. Below pH 5, ammonium if present in the solution becomes toxic. The chemistry of soil acidity is quite different from the chemistry of acidity in a nutrient solution Aluminum and manganese increase to toxic levels in the soil solution as acidity rises (pH falls). In nutrient solutions, aluminum will not be added generally, and manganese is too low to cause problems in relation to acidity. In soils, phosphorus is precipitated by iron and aluminum as acidity rises.

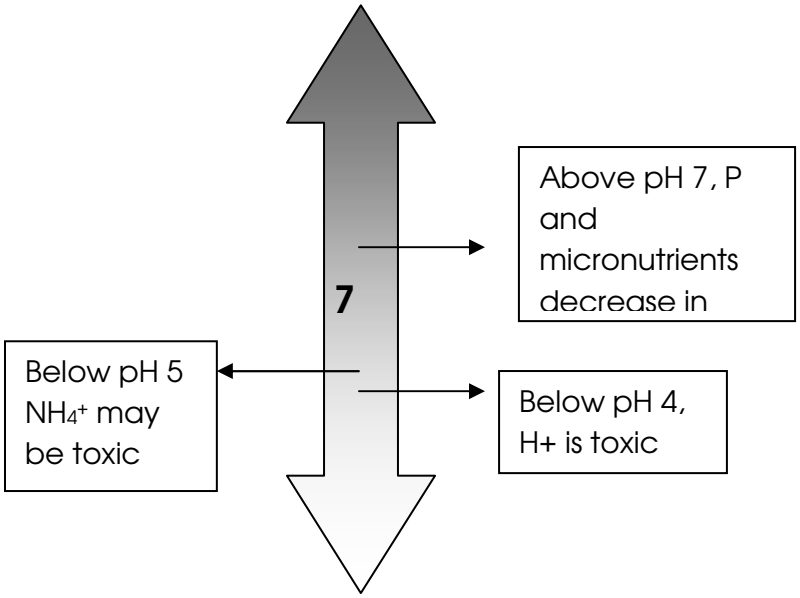



Figure 3.1 Illustration of effect of solution acidity on the availability and toxicities of some plant nutrients

In nutrient solutions, the solubility of phosphorus declines as acidity falls (pH rises). In solutions, the following relationship between orthophosphate (the most highly hydrated for of phosphate) occurs:

$H_3PO_4 = H_2PO_4^- + H^+ = HPO_4^{2-} + H^+ = PO_4^{3-} + H^+$

The solubility of some salts of the species of phosphate decreases as the ionization proceeds from left to right.

Phosphoric acid ($H_3PO_4$) dominates at pH below 2; and $H_2PO_4^-$ and $HPO_4^{2-}$ are equal at pH 7, whereas $PO_4^{3-}$ dominates above pH 12. In a nutrient solution, these ions can react with calcium or micronutrient cations to form precipitates. Therefore, as the pH rises in a nutrient solution, the solubility of phosphates and micronutrients declines.

Micronutrients in solutions are affected by solution acidity as they are precipitated as phosphates or hydroxides, for example with iron as $Fe(OH)_2$, $Fe(OH)_3$, $Fe_3(PO_4)_2$, $FeHPO_4$, and $FePO_4$. In nutrient solutions, P concentration is 30 times that of Fe so P precipitates Fe. In soil, Fe concentration is 50 times that of the P, so Fe precipitates P.

The solubilities of some common calcium phosphates are given below (Table 3.3).

Table 3.3 Solubilities of some common calcium phosphates in water at room temperature

| Calcium salt | Common compound | Solubility | |
|---|---|---|---|
| | | g/L | Molar |
| Monocalcium phosphate $Ca(H_2PO_4)_2$ | Triple superphosphate | 1.8 | 0.0077 |
| Dicalcium phosphate $CaHPO_4$ | Calcium dihydrogen phosphate | 0.003 | $2.20 \times 10^{-5}$ |
| Tricalcium phosphate $Ca_3(PO_4)_2$ | Bones | 0.002 | $6.45 \times 10^{-6}$ |

**Control of pH**

Most solutions will have a pH between 4.5 and 7 upon formulation but are not well buffered. So pH drifts as plants grow and absorb nutrients. The faster that plants grow, the faster the change in pH. If cation absorption dominates, pH of solution drifts downward. In ammonium-based solutions or nitrogen-deficient solutions, cation absorption exceeds anion absorption, and pH can fall to values below 4.

If anion absorption dominates, pH of solutions rises. In nitrate-based solutions such as Hoagland and Arnon no. 1, pH will rise, often exceeding pH 8 as plants grow (Figure 3.2). In ammonium-based solutions, the pH will fall as plants grown, falling to pH 3.0 or lower. With nitrate nutrition, anion absorption exceeds cation absorption, and $OH^-$ is excreted to maintain electrical balance in the solution, thereby raising the pH of the solution. Plants absorb nitrogen readily, and with nitrate nutrition, absorption of nitrate dominates so that anion absorption exceeds cation absorption.

With ammonium nutrition, cation absorption exceeds anion absorption because absorption of ammonium exceeds absorption of anions (Figure 3.2). Hydroxide ions are released to maintain electrical neutrality in the nutrient solution.

In a nitrogen-deficient solution, absorption of $K^+$, $Ca^{++}$, and $Mg^{++}$ exceed absorption of $H_2PO_4^-$ and $SO_4^=$, and pH will fall as $H^+$ is released to maintain electrical neutrality in the solution.

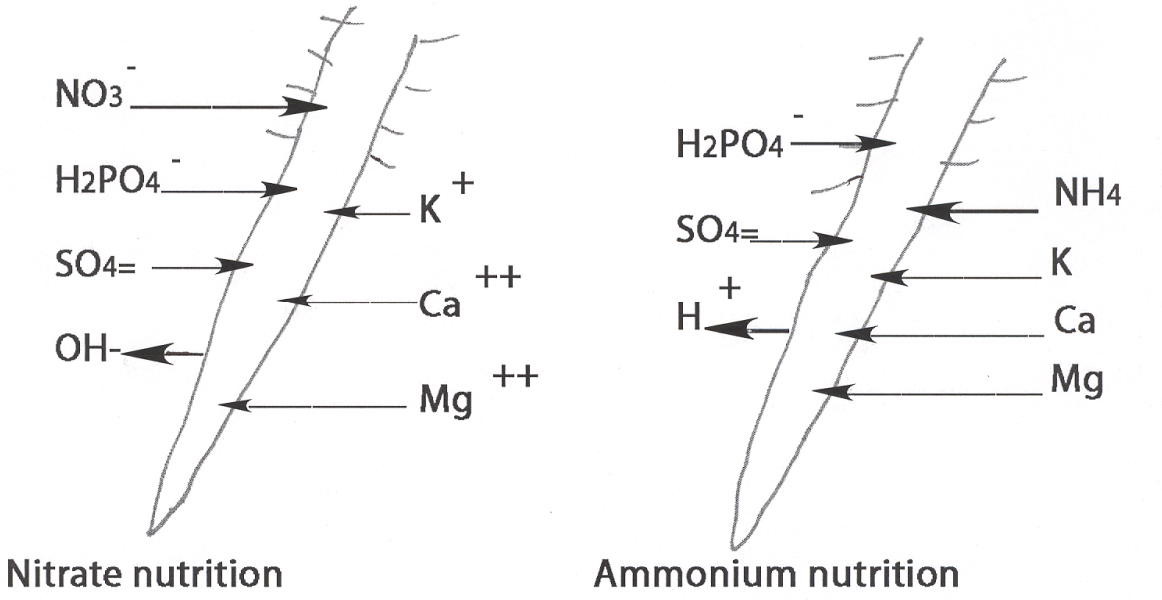


Figure 3.2 Differences in nutrient ion absorption with nitrate nutrition and ammonium nutrition. With nitrate nutrition, anion absorption exceeds cation absorption, and pH rises. With ammonium nutrition, cation absorption exceeds anion absorption, and pH falls.

Methods of Control

*Add acid or base to adjust to desired pH and readjust frequency*. Hydrochloric acid (HCl) or sodium hydroxide (NaOH) are used commonly to adjust pH. The effect of this adjustment is temporary, lasting for only days or for hours if plants are growing rapidly in solutions of small volumes. The acid and base also add unwanted ions ($Na^+$, $Cl^-$). Phosphoric acid or sulfuric acid can be used to lower pH, and KOH can be used to raise pH. These additions may change the nutrient content of solutions slightly.

*Use buffered solution*. Weak acids or bases or soluble salts of weak acids or bases can be added or used in formulation of nutrient solutions to regulate pH. Hoagland and Arnon Solution no. 1 is composed of salts of strong acids and is not buffered. Nitrate will be the principal ion absorbed from this solution, thus, the pH of the solution with growing plants will drift toward alkalinity. Hoagland and Arnon Solution no. 2 (sometimes called Johnson's solution) was modified with the addition of ammonium phosphate to buffer the solution (Table 3.4). The ammonium phosphate adds buffering to the solution. The ammonium ion is a weak acid and can donate hydrogen ions to the solute ion: $NH_4 = NH_3 + H^+$. Inclusion of the ammonium in the solution also ensures that some of the nitrogen will be absorbed in cationic form, thereby balancing some of the effects of nitrate absorption. The buffering of Solution no. 2 is weak since only about 6.7% of the nitrogen is supplied as ammonium. For strong buffering against a drift toward alkalinity, the ammonium should be about 12 to 25% of the total nitrogen supply, and nitrate should be about 75 to 88% of the total supply. These concentrations of ammonium and nitrate in a solution will hold the pH at 6.0 or so. If ammonium comprises more than 25% of the nitrogen supply the solution will be acidic, perhaps pH 5.5.

Table 3.4 Concentrations of salts in Hoagland and Arnon Solutions no. 1 and no. 2.

| Hoagland and Arnon Solution no. 1 | Hoagland and Arnon Solution no. 2 |
|---|---|
| 0.005M $KNO_3$ | 0.006M $KNO_3$ |
| 0.005M $Ca(NO_3)_2$ | 0.001M $NH_4H_2PO_4$ |
| 0.001m $KH_2PO_4$ | 0.004M $Ca(NO_3)_2$ |

0.002M $MgSO_4$ 0.002M $MgSO_4$

---

Other possible buffers include carbonates, phosphates, acetates, citrates, acetic acid and citric acid. Weak acids such as acetic acid or phosphates provide buffering by limited ionization: HAc =$H^+$ + $Ac^-$; $H_2PO_4$-= $H^+$ +$HPO_4^{2-}$; $HPO_4^=$ = H+ +$PO_4^{3-}$. Buffering with weak acids or bases may provide nutrients or toxins to solutions.

Salts of weak acids or bases provide buffering based on the hydrolysis reaction of the ions. A weak acid will hydrolyze to acidify the solutions, such as the reaction with the ammonium ion:

$NH_4^+ + H_2O = H^+ + NH_4OH$

A weak base will hydrolyze to produce a basic solution, such as the reaction with bicarbonate or acetate:

$HCO_3^- + H_2O = OH^- + H_2CO_3$

Acetate + $H_2O$ = $OH^-$ + Acetic Acid

Solid buffers are added to hydroponics containers. These buffers dissolve only slightly in the solution but provide buffering by the reaction of hydrogen ions with the buffers to prevent drifts toward acidity. As long as the solid phase is present in the medium, buffering will be provided. Calcium carbonate is used as a solid buffer to prevent drift toward acidity, especially in solutions where ammonium is a substantial supplier (25% or more) of the nitrogen. Calcium carbonate will provide buffering by the direct reaction of the compound and hydrogen ions:

$CaCO_3 + 2H^+ = Ca^{++} + CO_2 + H_20$

Hydrogen ions also will react with products of dissolution of calcium carbonate:

Dissolution: $CaCO_3 + H_2O = Ca^{++} + HCO_3^- + OH^-$

Reactions: $HCO_3^- + H^+ = H_2CO_3 \rightarrow CO_2 + H_20$
$H^+ + OH^- = H_2O$

Calcium dihydrogenphosphate (dicalcium phosphate) provides buffering through reaction with hydrogen ions and liberation of hydrogen ions:

$CaHPO_4 = Ca^{++} + HPO_4^=$

$HPO_4^{=} + H^+ = H_2PO_4^-$ corrects drift toward acidity

$HPO_4^{=} = H^+ + PO_4^{\equiv}$ corrects drift towards basicity

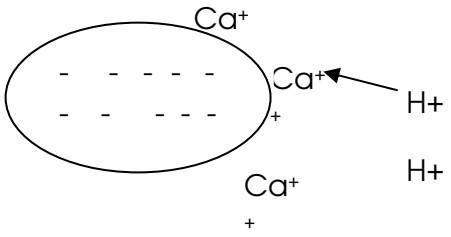
Dicalcium phosphate is sparingly soluble, so the concentration of phosphate is not elevated substantially in the solution.

Other solid buffers include calcium oxide and other species of calcium phosphate. Calcium sulfate does not provide buffering as it is a salt of a strong base and a strong acid.

*Resins*. Polystyrene resins have active groups that exchange hydrogen ions (R $SO_3^-$ $H^+$) or hydroxyl ions ($R_4N^+$ OH). Resins can be used to buffer acidic or alkaline solution.

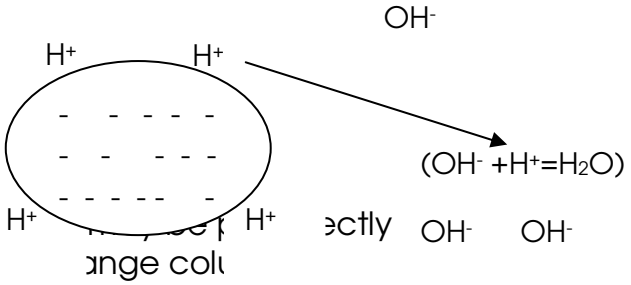
$Ca^{++}$ saturated resins buffer acid solutions.

**Calcium resin** **Acidic solution**




$H^+$ saturated resins are used to buffer alkaline solutions:

**Acidic resin** **Basic solution**




ectly e flowed through a resin

ange colu

**Add no toxic substances.** Toxic ions or molecules should be kept from the nutrient solution. No $Al^{+++}$, $Pb^+$, $F^-$, $CN^-$ , for example, should be added. Minor elements should be in nontoxic concentrations. Sodium ($Na^+$) should not exceed 0.01M. Chloride ($Cl^-$) should not exceed 0.01M. No more than half of N from $NH_4^+$ unless the solution is buffered. Nitrogen should not be supplied in any amount form nitrite ($NO_2^-$). No organic substance should be added in any quantity unless

sterility is maintained. Salts of weak organic acids will cause solution to be alkaline. Acetate salts will cause solution to become strongly basic due to the hydrolysis reaction:

$Acetate^- + H_2O = Acetic\ acid\ (HAc) + H^+$

One can calculate the resulting pH from the hydrolysis reaction of acetate:

$$K_h = \frac{Kw}{Kb} = \frac{10^{-14}}{1.8x\ 10^{-5}} = 5.56x\ 10^{-9}$$

$(HAc)(OH^-)/(Ac^-) = X^2/(Ac^-) = 5.56 \times 10^{-9}$

If $(Ac^-) = 0.001$ M, $X^2 = 5.56 \times 10^{-12}$

$X = (OH^-) = 2.35 \times 10^{-6} = 10^{-5.63}$

pOH = 5.63; pH = 8.37

# Chapter 4 Materials for Plant Nutrition

For selection of plant materials, consider the hypothesis to be tested. These investigations may address issues of the amounts of nutrients needed to supply deficient, optimum, or toxic concentrations of nutrients in a solution. Critical concentrations in plant parts may be considered. The critical concentration of a nutrient in plant parts is the concentration below which growth or yields will be limited by the supply of nutrients. Uptake or absorption of nutrients requires special consideration of plant species, parts of plants, or other organisms. Studies of transport of nutrients and accumulation in plants will require selection of the proper plants and plant parts.

Plant or organisms must respond to the treatments imposed and in the time allotted for the experiment. Some plants may grow slowly and may not develop fast enough to allow completion of an experiment in the limited time that is available.
Investigators should consider the facilities that are available to conduct the investigations. Special apparatus may need to be acquired or developed. Laboratories and instruments for chemical analysis may be needed.

**Examples of Materials**

***Intact plants***. Intact plants are useful to study gross effects of nutrition on quality, growth, yield, root to shoot transport, sites of accumulation, and accumulation with time. Whole plants would not be appropriate for ion uptake or kinetic studies, as translocation of nutrients would confound the studies.

In studies with intact plants, it is important that the concentration of element and total accumulation in plants or plant parts be considered. For example, consider the following two cases:

For one plant 10 g dry weight of leaves had a K of 2.5% for at total K accumulation of 0.25 g. Another plant had 12 g of dry weight of leaves at a K concentration of 2.1% and a total accumulation of 0.25 g. A scientist reported that the first plant had more K in it than the second one. The first plant had a higher concentration of K, but the two plants accumulated equal amounts of potassium. Sometimes, it is necessary to factor concentration times mass to determine total accumulation to get the full understanding of plant nutrition.

***Excised roots***. Excised roots are favored for studies of ion uptake. Fibrous roots are usually more desirable than tap roots. Wheat, barley, rye, corn, or other grass roots are commonly used. Fibrous root systems have numerous about equally sized roots extending in a complex network from the base of the plant.

In contrast, a taproot is a thick, fleshy primary root with some branch roots. Carrots, beets, radish, and dandelion have taproots. Intact taproots may not be useful for studies of ion uptake, but sections of these roots may be used.

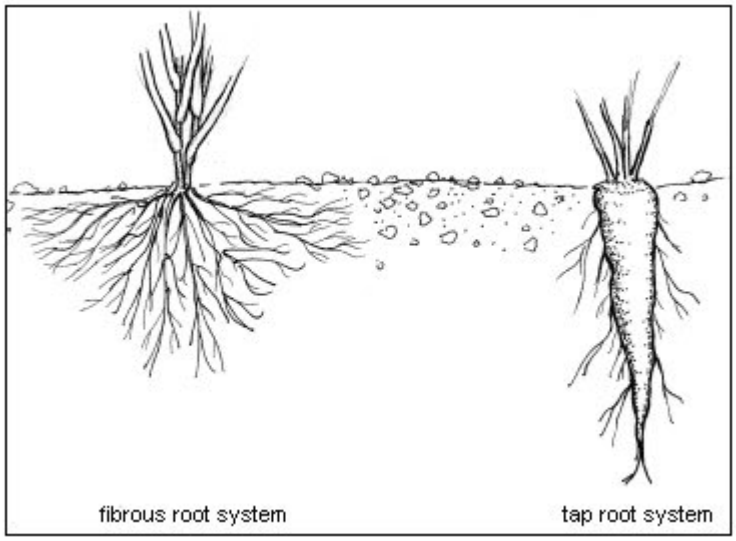



Figure 4.1 Drawings of fibrous and tap root systems

***Storage tissues***. Sections of storage tissues are used for investigations of ion uptake. Storage tissues are not analogous to roots but provide tissues with uniform parenchyma. Roots are not formed of uniform parenchyma and differ in structure or anatomy from tip to base. The heterogeneity of cellular structure can confound studies of uptake. Use of storage tissues allows for studies of ion uptake by tissues that are formed of cells that are essentially alike. White potato, Jerusalem artichoke, radish hypocotyls, carrot roots, and beet roots give tissues that are uniform and useful for studies of ion uptake. Disks are cut from the storage tissue and sliced into thin sections for use.

***Attached or excised whole leaves or leaf discs or strips***. Ion uptake by leaves is a useful study to elucidate foliar absorption or loss of nutrients by plants. Leaves may be left on the plants to assess plant responses to nutrients sprayed on leaves. However, studies of uptake may be confounded by transport from the leaves, so, excised leaves would have an advantage in this case. In some research, leaf cuticles are isolated by enzymatic action to yield a semipermeable membrane to study transport of ions across leaf cuticles.

***Isolated cells***. Leaves are macerated in a solution having about the same osmotic concentration as leaf interiors. Some cells are broken during the process, but many cells are left intact after the residue is separated. The osmotic

concentration should be the same inside and outside the cells so that the cells do not rupture. Cells also can be isolated enzymatically using cellulases, hemicellulases, pectinases, and polygalactonases to separate cells.

Isolated cells give direct contact of cells with solutions so that the pathway of diffusion is reduced relative to that which occurs in leaf disks, strips, or whole leaves.

***Algae***. Single-celled algae such a *Chlorella* spp. and *Euglena* spp. are free living and have chloroplasts. These organisms mimic individual cells of leaves. Since the organisms are free-living, the problems of osmotic rupturing of isolated leaf cells are not present. These organisms are easy to culture. Chlorella often populates nutrient solutions that are exposed to light and air.

*Nitella* spp. (fresh water) and *Valonia* spp. (sea water) are multicellular algae. They have structures of large, one-celled bladders or long-celled stems than can be isolated easily and used to study nutrient absorption.

| | |
|---|---|
|  |  |
| Stem cells of Nitella | One-celled bladders of Valonia |

Figure 4.2 Photographs of Nitella and Valonia

***Bacteria and fungi***. These organisms are easy to culture. Bacteria provide free-living, non-green, single-celled material for studies of plant nutrition. Hyphae of fungi are somewhat rootlike but with much simpler structures than roots.

***Cellular organelles***. Subcellular structures are used in studies of ion uptake and function in plant metabolism. Mitochondria and chloroplasts are isolated by

maceration of cells in an osmotically adjusted medium followed with centrifugation and suspension. Mitochondria and chloroplasts are membranous organelles. Studies of ionic accumulation in the interiors of these structures have led to understanding of the relationship between plant metabolism and plant nutrition. The c*hemiosmotic hypothesis of photosynthetic phosphorylation* is based on studies of ion accumulation in chloroplasts and formation of adenosine triphosphate (ATP).

# Chapter 5 Diagnostic Criteria for Assessing the Nutritional Status of Plants

Much attention has been given to diagnosis of the nutritional status of plants so that corrective action can be taken and so that an understanding of the function of nutrients in plants is gained. These diagnoses involve visual inspections, fertilization, plant and soil analyses, biochemical tests, and instrumental analysis.

## Visual Diagnoses

Involves assessments based on recognition of characteristic symptoms of deficiency followed by remedial action to correct the deficiency. Nutrient deficiencies disrupt metabolic functions in plants and induce maladies that appear as symptoms on plants. Leaves are the most commonly diagnosed plant parts for assessment of deficiencies, although fruits and other organs may show symptoms that are characteristic of certain nutrient deficiencies. Visual symptoms alert a grower of a problem with plant nutrition.

The fact that symptoms are observed and not the actual deficiencies causes problems in visual diagnosis. Other disorders may cause similar symptoms to develop. Deficiencies of several nutrients may cause similar symptoms, particularly at advanced stages of development. Generally, the more advanced the development of symptoms, the more similar the deficiencies of diverse nutrients appear. Pathogenic diseases often cause symptoms that resemble nutrient deficiencies. Symptoms resembling those of nutrient deficiencies may develop under abiotic stresses, such as poor light intensity, heat, drought, or waterlogging. Often, a grower is not aware of the characteristic symptoms of a particular nutrient, and this lack of knowledge can lead to misdiagnosis.

Symptoms may be missed or fail to develop. The failure of a symptom of a deficiency to develop is called *hidden hunger*.

## Application of Nutrients to Confirm Diagnosis

Visual diagnosis often is followed by application of fertilizers to confirm the diagnosis or to correct the deficiency. These applications may be foliar or soil applications of the nutrients suspected to be deficient. Observations are made for the recovery of the plants following fertilization. The time for recovery may be several days or weeks.

## Soil Analysis

A quick soil test is run by testing laboratories or with soil-testing kits. This test gives an indication of the availabilities of plant nutrients, usually the macronutrients. In some cases, a direct analysis for the nutrient suspected as being deficient is made. A quick test usually reports soil pH. Soil salinity, micronutrients, and nonessential or toxic trace elements may be included in the test. Some soil analyses involve growing of indicator plants in the soils or conducting bioassays with microorganisms. In each case, the growth of the organisms is observed and compared to expected standard observations.

**Plant Analysis**

This analysis often is referred to as plant-tissue testing. It involves a direct analysis for the element in question. Results of the tests are compared with those of plants having deficient, sufficient, or toxic concentrations of the element. Many textbooks and references on plant nutrition have tabulated data on sufficiency ranges of concentrations of nutrients in many plants.

For reliable information, material to be tested must be from specific plant parts from similar plants. Tissue concentrations vary greatly among plants and plant parts and with plant age.
The *Critical Concentration* of a nutrient in a plant is the concentration below which growth or yields will be limited. Determinations of critical concentrations require a lot of research and are available for only a limited number of crops. When assaying for critical concentration or applying critical concentration to diagnosis, investigators must know plant species, variety, age, plant part and position, environmental conditions (light, water, temperature), and sometime the time of day of sampling. A critical concentration curve is presented (Figure 5.1). The critical concentration is measured in what is called the transition zone between limited growth and adequate or full growth. Often, the amount of growth is taken as 90% or 95% of the full growth in the adequate zone. The 90% or 95% is not chosen because it is acceptable growth, but because it is in the transition zone. If the critical concentration is set in the adequate zone, no reading of critical concentration could be made from the flat portion of the curve. No special statistical treatment of the data is required to determine the critical concentration.

The accumulation of nutrients in the adequate zone in which nutrients rise in the plant but growth does not increase is called *luxury consumption*. Eventually nutrients may accumulate to toxic concentrations, and growth will fall below the maximum yield.

**Biochemical Diagnosis**

Biochemical analyses are enzymatic tests to assay for a deficiency. Symptoms of deficiencies of minor elements often are similar, occurring with identical appearances on the same plant parts. Enzymatic activities in quick tests may tell what nutrient is lacking or not lacking. For the assay to be useful, it must be run conveniently and quickly. Leaf disks or crude enzyme preparations are used in the assays. Biochemical diagnosis is applied often to assay woody plants, such as citrus. Iron is required for peroxidase activity. A quick assay of peroxidase activity can assess whether iron is adequate or not. Zinc is required for carbonic anhydrase. Copper is required for ascorbic acid oxidase, and molybdenum is required nitrate reductase. Quick assays of activities of these enzymes may inform investigators of specific deficiencies that cannot be distinguished fully by other observations.

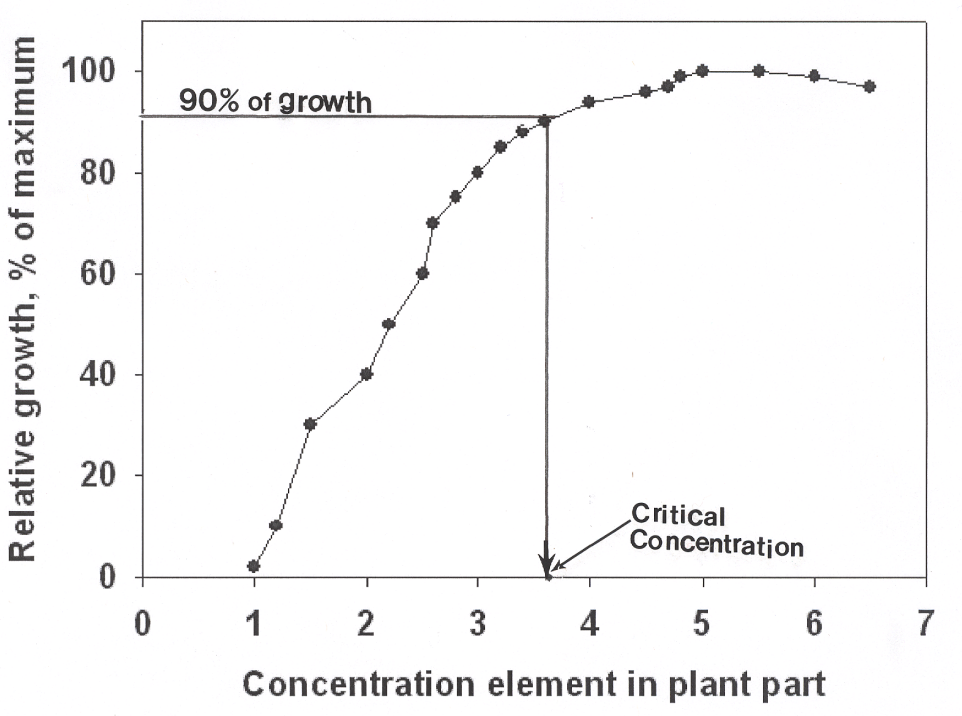



Figure 5.1 Critical concentration curve for plant response to concentration of nutrient in the tissue of a particular part of a plant. Concentration is in arbitrary units, and growth is relative growth with maximum growth set at 100%

**Electron microprobe**

The electron microprobe is a research tool that may be used to assist in diagnoses. The microprobe is similar to an electron microscope. Tissues are

bombarded with electrons, and electrons are emitted from elements in the tissues. Assays can be run to be specific for a nutrient. The analyses can determine accumulation and movement of nutrients. For example, movement or accumulation of calcium, magnesium, and aluminum across roots has been followed with an electron microprobe. Movement of potassium in and out of guard cells has been followed also with an electron microprobe.

# Chapter 6 Soil-Derived Nutrients

## Nitrogen

Nitrogen was discovered as an element in 1772 and was shown to be essential for plants in about 1803 by de Saussure. As far back as 1750, it was known that plants required nitre, although nitre was not identified. It could have been potassium nitrate, sodium nitrate, potassium carbonate, or sodium nitrate. The 1850s, field experiments at Rothamsted, England, showed that crops responded to applications of inorganic nitrates. Application of hydroponics confirmed that nitrogen was an essential element.

Nitrogen is usually the fourth most abundant element in plants after carbon (50%), hydrogen (5%), and oxygen (40%). Nitrogen is 1.5 to 5% of the dry weight of plants.

Nitrogen is obtained from the soil principally as $NO_3^-$ (>95%) and $NH_4^+$. Plants get only traces of N from organic compounds in solution. Plants will absorb urea. Organic nitrogen in the soil is unimportant in the direct nutrition of a crop, although 98% of the soil nitrogen is in the organic matter. The following transformations regulate the forms of nitrogen in the soil:

**Organic nitrogen ----------→ Ammonium nitrogen ----------→ Nitrate nitrogen**
**98% of soil N <0.1% of soil N ~ 1.9% of soil N**

These transformations are mediated by soil microorganisms.

Some plants, called nitrogen-fixing plants, obtain N from the air. Legumes (Family Leguminosae or Fabaceae) are the most common plants used for fixation of nitrogen in agriculture. Legumes have symbiotic relationships with bacteria – Rhizobium, Bradyrhizobium. This symbiosis fixes nitrogen in the order of 50 to 200 lb/acre/yr. Energy for fixation is from respiration of organic carbon compounds provided from photosynthesis by the host plant. The overall process, $N_2 + H_2 \rightarrow NH_4$, is the same in green plants as at a fertilizer plant. At a fertilizer plant, energy for fixation comes from petroleum products.

### Nitrogen Fixation by Nonlegumes

Nonlegume is an agronomic term to identify plants that are not legumes. Several nonlegumes are nitrogen-fixing plants. These plants live in symbiosis with actinomycetes (multicellular bacteria), plasmodial fungi, or algae. Nodules may be on leaves or on roots. Nitrogen fixation is of the range of 2 to 50 lb/acre/yr. Some nitrogen-fixing nonlegumes are listed as follows.

**Root Nodules**

***Azola***. Azolla is a nitrogen fixing fern. It is agronomically important in rice paddys. Azolla is symbiotic with Anabaena azolla (a blue-green alga) in leaf nodules.

***Gymnosperms***. Some cycads fix nitrogen in symbiosis with blue green algae.

***Alnus*** (alder). Alder is symbiotic with filamentous bacteria, *Frankia alni* (also known as actinomycetes).

***Eleagus*** (autumn olive). Autumn olive and Russian olive are members of the Eleagnaceae family and fix nitrogen in association with Frankia bacteria.

***Hippophae*** (Sea-buckthorn). This plant is a member of the Eleagnaceae family, and fixes nitrogen in association with Frankia bacteria.

***Shepherdia*** (Buffalo berry). This plant is a member of the Eleagnaceae family and fixes nitrogen in symbiosis with Frankia bacteria in root nodules.

***Comptonia*** (sweet fern). Sweet fern is a member of the Myricaceae family and fixes nitrogen in association with soil-borne actinomycetes in root nodules.

***Myrica***. Myrica is a genus of 50 or so species of bog plants in the family Myricaceae. Common plants are sweet gale, bayberry, and wax myrtle. The plants fix nitrogen in symbiosis with Frankia bacteria in nodules.

***Casuarina***. Casuarina is a genus of 17 species in the family Casuarinaceae. These plants are evergreen shrubs and trees. Nitrogen fixation occurs in root nodules after infection with the Frankia. The plants grow along riverways and on sea shores. Austrailian pine is a commonly known species in the United States.

***Ceanothus***. Ceanothus is a genus of 50 to 60 species of shrubs and small trees in the family Rhamnaceae. The genus is confined to North America with the center of its distribution being in California. It may be grown elsewhere as an ornamental plant. Puget blue or California lilac grows in poor scrubland. New Jersey Tea is another species of Ceanothus. Fixation occurs in symbiosis with Frankia in root nodules.

**Leaf Nodules**

Fixation by leaf nodules occurs with some tropical species.

***Rubcaceae***. This family is called the coffee family, the madder family or the bedstraw family. Fixation of nitrogen occurs in colonies of nitrogen-fixing bacteria (cyanobacteria, for example) on leaf surfaces.
***Myrsinaceae***. This family is the Myrsine family. These species are located in hot or warm regions tropics and subtropics. A few nitrogen-fixing species are in this family.

**Nonsymbiotic Fixation**

***Plant Associated***. Microorganisms live in rhizosphere (or on leaf surface) and obtain energy for fixation from exudates from plant. These organisms may fix 12 to 25 lb N /acre/yr.

***Free Living Organisms***. These organisms live in the soil or water and fix nitrogen usually independently of associations with plants. These organisms may fix 4 to 10 lb N/acre/year. Metabolism in the organisms provides energy for fixation. Some nitrogen-fixing free-living organisms include bacteria Azotobacter, Biejerincikia, Rhodospirillium rubrum, (photosynthetic as well), and Chlostridium (anaerobic); fungi Saccharomyces and Rhodotorula; and blue-green algae Anabaena, Nostoc, Tolyptothux, and Cyanobacteria. The nitrogen-fixing algae fix 3 to 25 lb N/ acre/yr in land and up to 15 to 70 lb N/acre/year in water of all kinds including hot, cold, fresh, or saline water.

Many of the non-legumes and free living organisms are pioneers in plant succession. They may occur as colonizers in glacial retreats, volcanic ash, swamps, dry land, sandy soils, peaty soils, cold, wet soils, or any land with low nitrogen status. These organisms live under these poor conditions and allow for establishment of other species.

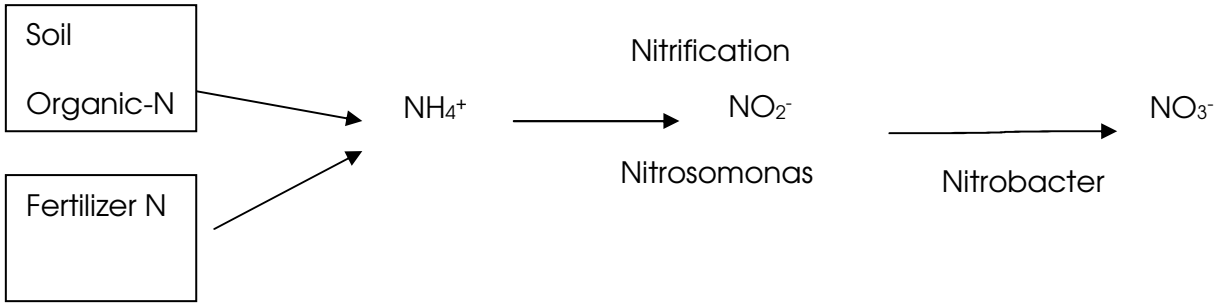
The total worldwide nitrogen fixation is about 265 million metric tons of which about 175 million tons is biological fixation (Table 6.1). Fixation on agricultural land is about 90 million metric tons of which about half is in tilled land with legumes. Industrial fixation for fertilizers is about 50 million metric tons per year. Lightning, which converts nitrogen gas to nitrate, fixes about 10 million metric tons per year.

**Table 6.1 World nitrogen fixation by type of process and location**

| Type of fixation | $N_2$ fixed (millions of metric tons per year) |
|---|---|
| **Non-biological** | |
| Industrial | about 50 |
| Combustion | about 20 |
| Lightning | about 10 |
| **Total** | **about 80** |
| | |
| **Biological** | |
| Agricultural land | about 90 |
| Forest and non-agricultural land | about 50 |
| Sea | about 35 |
| **Total** | **about 175** |
| Data from various sources, compiled by DF Bezdicek & AC Kennedy, in *Microorganisms in Action* (eds. JM Lynch & JE Hobbie). Blackwell Scientific Publications 1998. | |

### Nitrate Metabolism

In the soil nitrate ($NO_3^-$) is the principal form of available N because of nitrification. Mineralization of organic matter releases ammonium from combination in organic molecules. Nitrification by soil microorganisms converts ammonium to nitrite (by *Nitrosomonas* spp. bacteria), and the nitrite is converted to nitrate (by *Nitrobacter* spp. bacteria). Mineralization is a slow process. About 1% or 2% of soil organic matter is mineralized in one year. Nitrification is a relatively rapid process that is governed by environmental factors such as water and temperature but usually occurs in a few days. Very little if any nitrite accumulates in soils during nitrification, as the oxidation of nitrate to nitrate is rapid.



***Assimilation of nitrate***. After $NO_3^-$ enters the plant, it must be reduced to ammonia – essentially the reverse of nitrification – before it is incorporated into organic compounds.

*Nitrate reductase* reduces nitrate to nitrite.

**$NO_3^-$ ——————> $NO_2^-$**

This process occurs in the cytoplasm (cytosol) of cells and involves the transfer of two electrons. In nitrate, nitrogen has a charge of +5, and in nitrite, the nitrogen has a charge of +3. Hence, two electrons are transferred to reduce the charge from +5 to +3. The electrons and energy for this process are provided by respiration in the mitochondria.

The biochemical reaction for nitrate reductase is:

**$NO_3^-$ + NADPH + $H^+$ ——————> $NO_2^-$ + $NADP^+$**

NADPH + $H^+$ is reduced nicotinamide adenine dinucleotide phosphate. $NADP^+$ is oxidized nicotinamide adenine dinucleotide phosphate. Nitrate reductase is a large enzyme having a molecular weight of about 200,000 g or more. Molybdenum is required for the reaction along with flavin adenine dinucleotide (FAD) and cytochrome b. If molybdenum is deficient, nitrate may accumulate to toxic levels.

*Nitrite reductase* reduces nitrite to ammonium.

**$NO_2^-$ ——————> $NH_4^+$**

This process occurs in chloroplasts and involves the transfer of six electrons. The nitrogen in nitrite has an oxidation state of +3, and the nitrogen in ammonium has an oxidation state of -3. Hence six electrons are involved in the reduction from +3 to -3. This reductant and energy comes from photosynthesis.

The biochemical reaction for nitrite reductase is:

**$NO_2^-$ + Ferredoxin (reduced) ————> $NH_4^+$ + Ferredoxin (oxidized) + $H_2O$ + $OH^-$**

In the reduction, no intermediates are released between nitrite and ammonium. No hypochlorite and no hydroxylamine are released, meaning the reduction proceeds in one step. Ferredoxin formed during photosynthesis provides the reducing power. Nitrate is reduced in roots, and NADPH from respiration may provide the reductant for that operation.

Nitrate is not reduced in the bundles sheaths of C-4 plants.

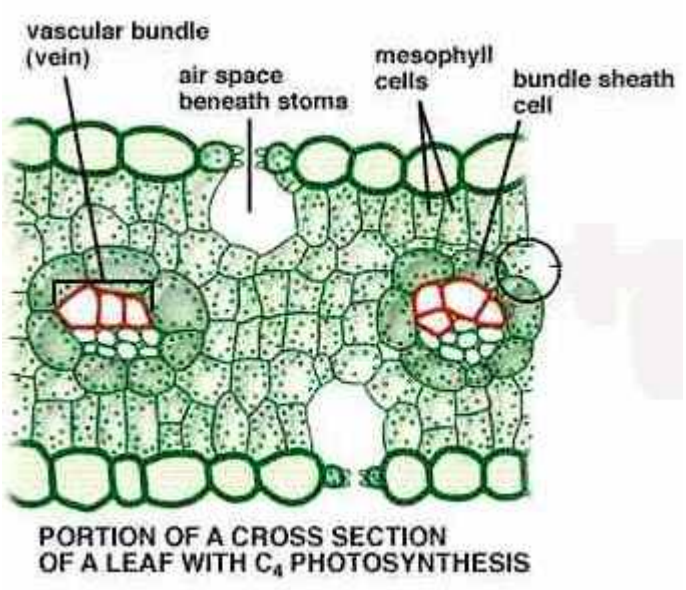



Figure 6.1 Cross-section of a leaf of a C4 plant showing the bundle sheath around the vascular tissue and the surrounding mesophyll

Nitrate is essential for activity of nitrate reductase. The half life of the enzyme is only a few hours after nitrate is exhausted. However, the enzyme can be induced again in a few hours if nitrate is added back to the system. It is unsure whether new nitrate reductase is formed or whether an existing enzyme is activated by the return of nitrate. Ammonium, amino acids, or amides also inhibit the activity of nitrate reductase.

Nitrite reductase is at high concentrations in leaves relative to nitrate reductase, and nitrite does not accumulate even though the enzymes are separated spatially. Accumulation of nitrite would be toxic to plants and the high concentration of nitrite reductase ensures that nitrite does not accumulate.

***Assimilation of ammonium***. After nitrate is reduced to ammonium, it may enter into organic compounds and become an essential constituent of:

1. Proteins and enzymes. The average protein is 16% N. Protein content of plants is estimated from the concentrations of nitrogen: N x 6.25 = crude protein. The product is called crude protein because it is a rough estimate of total protein—not a crude product.

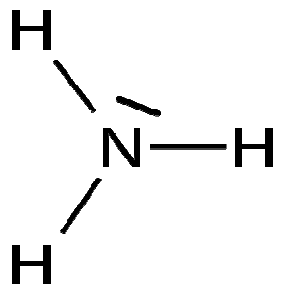
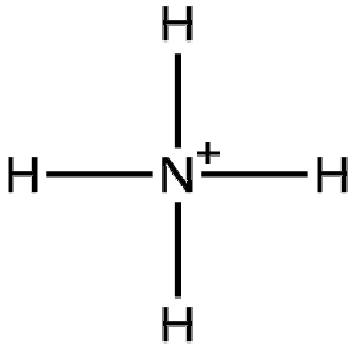
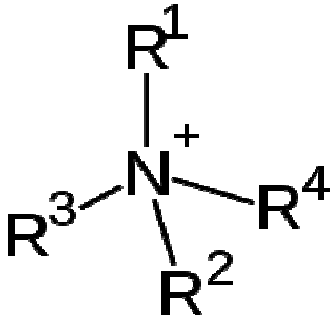
2. Nucleic acids

3. Chlorophyll

4. Vitamins, pyridine nucleotides, flavin nucleotides, lecithin, and other nitrogen-containing compounds
Organic nitrogen-containing compounds in plants are at the same oxidation state as ammonium. Whether ammonia or ammonium exists in a cell or solution is the result of the acidity of the solution.

**$NH_3 + H^+$ ----------→ $NH_4^+$**

In organic combinations, the nitrogen structure is a quaternary ammonium ion, **$RNH_3^+$**.

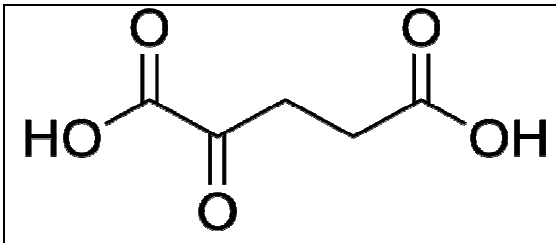
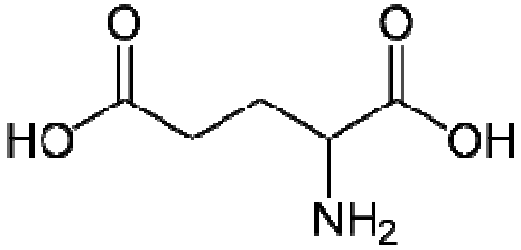
| **Ammonia** | **Ammonium ion** | **Quaternary ammonium** |
|---|---|---|
|  |  |  |
| This is written as **$NH_3$**. | This ion is **$NH_4^+$.** It is formed by protonaton of **$NH_3$**. | This ion is formed by the substitution of an organic structure for at least on H on the ammonium ion. The organic structures may be the same or may be different. This structure is that of any organic |

| | | |
|---|---|---|
| | | nitrogen-containing compound. |

***Assimilation of ammonium into organic components***

*Assimilation with high concentrations of $NH_4^+$ in cells--as with absorption of $NH_4^+$.* Glutamic acid dehydrogenase is an enzyme that assimilates ammonium into organic combinations in mitochondria. The enzyme catalyzes the reaction of a-ketoglutaric acid with ammonium and reductant to form glutamic acid.

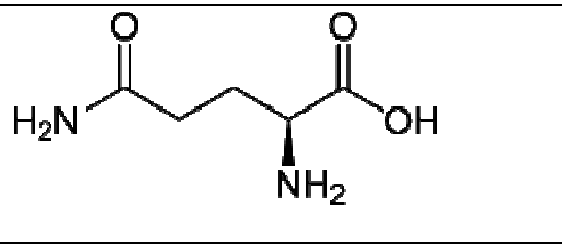
**a-Ketoglutaric acid + $NH_4^+$ + NADH + $H^+$ ----------→ Glutamic acid + $NAD^+$ + $H_2O$**

| | |
|---|---|
|  |  |
| a-Ketoglutaric acid; also known as a-oxoglutaric acid or 2-oxoglutaric acid; the ionic forms are a-ketoglutarate, a-oxoglutarate, and 2-oxoglutarate. | Glutamic acid; the ionic form is called glutamate. |

*Assimilation with Low Concentrations of $NH_4^+$- as with formation of $NH_4^+$ from $NO_3^-$.* Assimilation of ammonium following its formation from nitrate reduction occurs in the chloroplasts. This process involves two enzymatic reactions: *Glutamine synthetase* and *glutamate synthase*.

**Glutamine synthetase** catalyzes the reaction of glutamic acid with ammonium and ATP in the presence of magnesium to form glutamine. Glutamine synthetase has a high affinity for ammonium and can operate at low concentrations of ammonium.

**Glutamic acid + $NH_4^+$ + ATP ----------→ Glutamine + ATP + Phosphate**



**Glutamine**

**Glutamate synthetase** catalyzes the reaction of glutamine with a-ketoglutaric acid and reduced ferredoxin (or NADPH or NADH) to form glutamic acid. This enzyme also is known as **Glutamine oxoglutarate aminotransferase** or **GOGAT**.

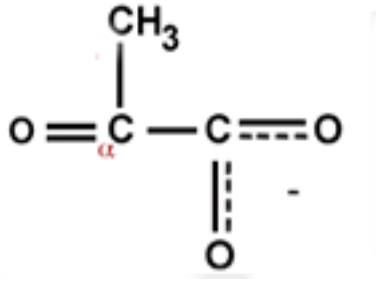
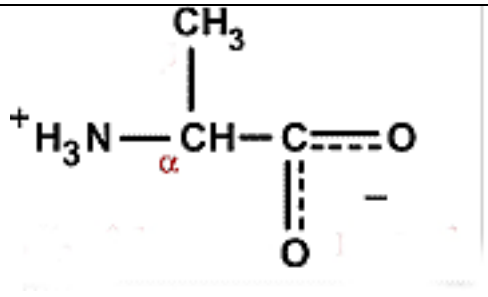
### Glutamine + a-ketoglutaric acid + Ferredoxin (reduced) ---------→ 2 Glutamic acid

The sum of the reactions of glutamine synthetase and glutamate synthetase is:

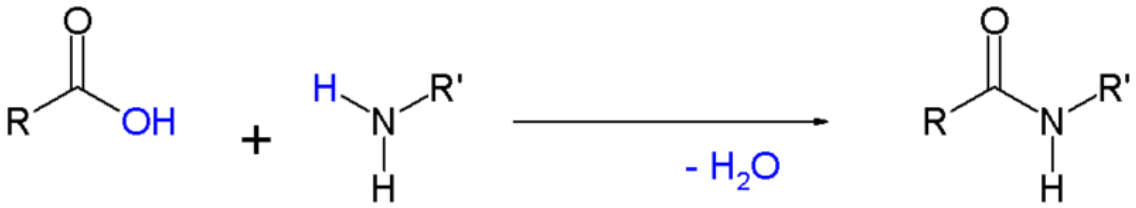
### a-Ketoglutaric acid + $NH_4^+$ + ATP + Ferredoxin (reduced) -------→ Glutamate + ADP + Phosphate

Once glutamate is formed any other amino acid can be formed by transfer of amino groups to the appropriate organic acid known as a carbon skeleton. The enzymes that catalyze these reactions are called aminotransferases or transaminases (old term).

### Glutamic acid + a-ketoacid ----------→ a-ketoglutaric acid + a-amino acid

| | |
|---|---|
|  |  |
| **a-ketoacid (pyruvate)** | **a-amino acid (alanine; ionic form, zwitterion)** |

***Amide synthesis***. Amides are formed from the reaction of a carboxylic acid with and amine (ammonia).




**Carboxylic acid** **Amine** **Amide**

Common amides in plants are glutamine and asparagines. They are formed by the following enzymatic reactions.

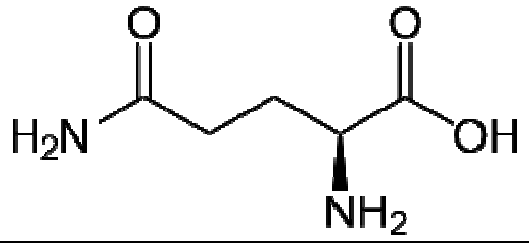
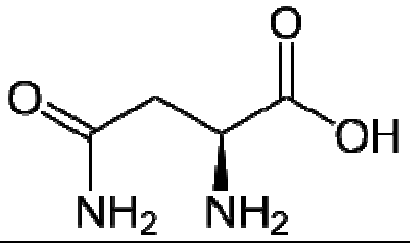
Glutamine formation:

**Glutamic acid + $NH_4^+$ + ATP ----------→ Glutamine**

Asparagine formation (The first reaction is an aminotranserase reaction)

**Glutamic acid + Aspartic acid ---------→ Aspartic acid**

**Aspartic acid + $NH_4^+$ + ATP ----------→ Asparagine**

| | |
|---|---|
|  |  |
| **Glutamine** | **Asparagine** |



Amides are nitrogen-rich compounds that are formed in plants to transport nitrogen or to store nitrogen.

***Peptides and proteins.*** Peptides are short polymers of amino acids linked together. A peptide might have three or more amino acids. Proteins are long polymers of amino acids linked together. Proteins might have sixty or more amino acids linked together. The bonds between the amino acids are *peptide bonds,* which are covalent chemical bonds formed between a carboxyl group of one amino acid and the amino group of another amino acid.

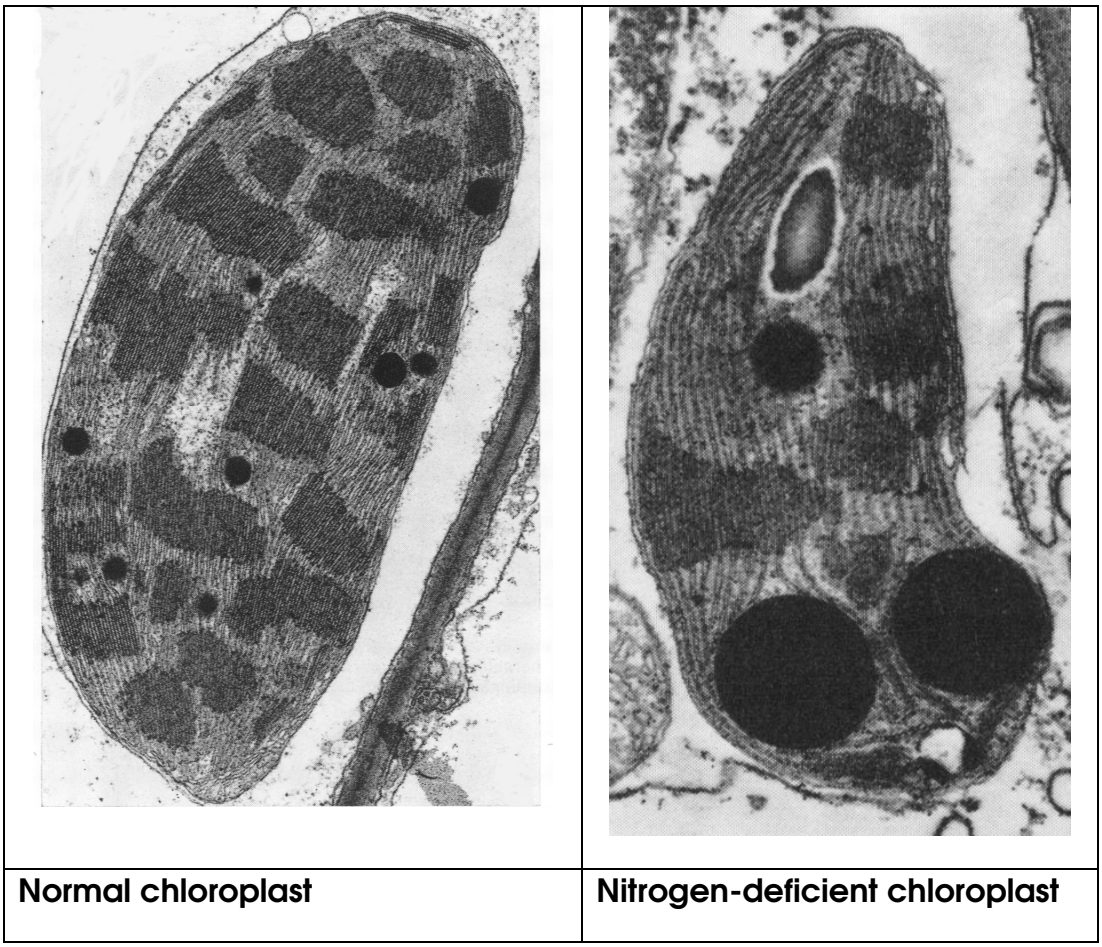
**Deficiency Symptoms**

Nitrogen-deficient plants are stunted and chlorotic. For plants that have been nitrogen deficient throughout their growth cycle, the whole plant is yellow or light green. For plants that initially had sufficient nitrogen but exhausted the supply during their growth, the lower leaves will have the chlorosis. Nitrogen is mobile in plants so that during low supplies of nitrogen, nitrogen is transported from the old leaves of plants to young leaves or fruits and the lower leaves become nitrogen deficient.

Loss of chlorophyll is associated with protein synthesis and chlorophyll synthesis. Chlorophyll is held in a complex with protein in the thylakoids (granal stacks) of the chloroplasts. With nitrogen deficiency, the chloroplast structures become altered with fewer grana and apparently smaller chloroplasts. In addition to the degraded structure of nitrogen-deficient chloroplasts, starch granules and osmiophilic granules appear more in the altered chloroplast than in normal chloroplasts with adequate nitrogen nutrition (Figure 6.2). Nitrogen-deficient chloroplasts may be smaller than normal chloroplasts.

It is normal during the maturation of a crop for lower leaves to lose nitrogen to fruits. In some plants, rate of nitrogen absorption slows with flowering.

Yield of crops will be limited by N deficiency. Restrictions in yields occur due to fewer and smaller fruits and seeds. Quantity and quality of vegetative matter will be lower with nitrogen deficiency than with well-nourished plants.



| Normal chloroplast | Nitrogen-deficient chloroplast |
|---|---|

Figure 6.2 Electronmicrographs of normal chloroplast (left) and nitrogen-deficient chloroplast of corn

**Effects of Nitrogen on Plant Growth and Quality**

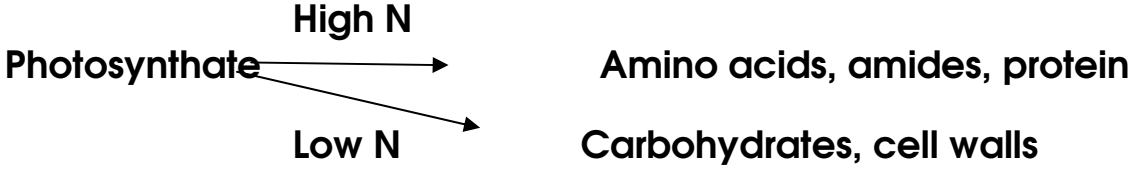
*Vegetative growth is enhanced more by nitrogen nutrition than reproductive growth, and shoot growth is enhanced more than root growth*. Effect is due to changes in hormonal balance in plants with changes in N nutrition. Nitrogen nutrition increases production of cytokinin and indoleacetic acid (IAA). Cytokinin is produced in roots, and an increase in root primordia and cytokinin by nitrogen increases leaf area. Nitrogen nutrition increases IAA synthesis, and the increased IAA enhances shoot growth and suppresses root growth.

*Nitrogen nutrition delays maturation*. Promotion of root growth by nitrogen increases number of primordial and synthesis of cytokinin, which is transported to shoots in xylem flow. A shortage of nitrogen stimulates abscisic acid synthesis

(ABA), which affects stomatal closure and partitioning of water, cytokine, and other minerals in the xylem.

With grain crops, high nitrogen supply causes crops to grow longer into the fall and to have a high water content and delay in harvest. With tomato, squash, citrus, cucumbers, and other fruit crops flowering may be delayed, and productivity may be lost due to the prolonged vegetative stages. However, for varieties of these crops that flower and fruit over a period of time, nitrogen application will prolong the period of productivity so that total yields will increase with nitrogen supply within limits.

*Nitrogen is needed for utilization of carbohydrates*. With high nitrogen nutrition, carbohydrate accumulation in plants is restricted, and proteins are increased. With low nitrogen supply, carbohydrates accumulate.




Sugar producing crops may not require much nitrogen as is illustrated from these results from an experiment with sugar beets (Table 6.2). In this experiment, beet yield increased with increased nitrogen fertilization. The concentration of sucrose in the beets decreases as nitrogen supply increased. Total sucrose yield increased also with increases in nitrogen but reach a maximum and fell with the next increment of nitrogen.

Table 6.2 Yield, sucrose concentration, and sucrose yield in response to nitrogen fertilization of sugar beets

| | Sugar beet yield components | | |
|---|---|---|---|
| N Applied | Yield | % Sucrose | Sucrose yield |
| lb/acre | T/A, dry matter | (dry weight) | T/A |
| 0 | 20 | 18 | 3.6 |
| 80 | 24 | 17 | 4.1 |
| 160 | 26 | 16 | 4.2 |
| 240 | 27 | 15 | 4.0 |

*The amount of water in plants and their succulence is increased by nitrogen fertilization*. This action results from the fact that more protoplasm with high

protein content is formed at the expense of cell wall synthesis. Protoplasm has a much higher water content than cell wall materials.

*With abundant nutrition, cells are large—stretched out with thin walls.* This phenomenon besides giving succulence to plants causes the plants to be susceptible to lodging. Lodging is the irreversible displacement of plants from an upright position. Thin cell walls in the stems give weakness to the stems, and the plants fall over thereby causing a loss in harvestable produce of grain crops in particular (Figure 6.4). The susceptibility of wheat to lodging increased in an experiment in which nitrogen supply was increased (Table 6.3).

Figure 6.4 Lodging of corn, likely from the action of wind.

Table 6.3 Susceptibility of wheat to lodging as a function of nitrogen supply

| N supply (kg/ha) | Lodging index |
|---|---|
| 0 | 2.4 |
| 80 | 4.8 |
| 120 | 5.8 |
| 160 | 6.3 |

## Phosphorus

Phosphorus was discovered as an element in about 1669. Evidence of its essentiality as a plant nutrient is attributed to Liebig, who in about 1839, experimented with bone meal. He made the first chemical fertilizer by treating bone meal with sulfuric acid. In about 1840, Gilbert and Laws at the Rothamsted

Experimental Station in England experimented with rock phosphate and superphosphate and added more evidence that phosphorus was a nutrient. Sachs and Knop in about 1960 confirmed the essentiality of phosphorus with solution culture.

Three ionic forms of orthophosphate (the most hydrated form of phosphate) are common in soils or nutrient solutions, $H_2PO_4^-$, $HPO_4^=$, and $PO_4^{3-}$:

$H_2PO_4^-$ = most prevalent in acid soils up to pH 7

$HPO_4^=$ = dominant in slightly alkaline soils, pH 7-12

$PO_4^{3-}$ = dominant in strongly alkaline solutions pH > 12

An equilibrium exists between these forms of phosphate.

**$H_3PO_4$ ---------→ $H_2PO_4^-$ + $H^+$ ---------→ $HPO_4^{--}$ + $H^+$ ----------→ $PO_4^{3-}$ + $H^+$**

| **Phosphoric acid** | **Monobasic phosphate** | **Dibasic phosphate** | **Tribasic phosphate** |
|---|---|---|---|

Hence, for the equilibrium between phosphoric acid and monobasic phosphate, $K_1$= 1.1 x $10^{-2}$, at pH 2, the ratio of $H_3PO_4/H_2PO_4^=$ = 50/50.

Likewise, for the equilibrium between monobasic phosphate and dibasic phosphate, $K_2$= 7.5 x $10^{-8}$, at pH 7.1, the ratio of $H_2PO_4^-/HPO_4^=$ = 50/50.

And, for the equilibrium between dibasic phosphate and tribasic phosphate, $K_3$= 4.8 x $10^{-13}$, at pH 12.3, the ratio of $H_2PO_4^=/PO_4^{3-}$ = 50/50.

Solubility of phosphates decreases with rise in pH and changes with the species of orthophosphate.

Monocalcium phosphate (as in triple superphosphate) is the only calcium phosphate soluble enough for use in formulating nutrient solutions (Table 6.4).

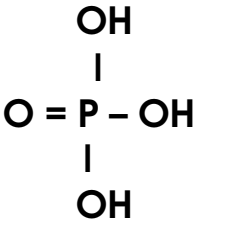
Table 6.4 Solubility of monobasic, dibasic, and tribasic calcium phosphates in water

| | Solubility in water at 25° C | |
|---|---|---|
| | g/100g $H_2O$ | Molarity of saturated solution |

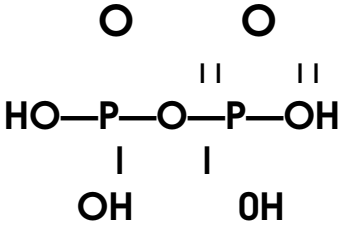
| | | |
|---|---|---|
| $Ca(H_2PO_4)_2 \cdot H_2O$ | 1.8 | 0.07 |
| $Ca\ HPO_4 \cdot 2\ H_2O$ | 0.03 | 0.002 |
| $Ca_3\ (PO_4)_2$ | 0.002 | 0. 00006 |

From solution, plants accumulate $H_2PO_4^-$ preferably over $HPO_4^{2-}$ and $PO_4^{3-}$. This effect is due to difference in solubility of the ions and the relative abundance of the ions in the pH of nutrient solutions.

Phosphorus is absorbed as orthophosphate and enters into organic combination as orthophosphates through formation of esters without any reduction. Structures of phosphoric acid ($H_3PO_4^-$) and pyrophosphoric acid ($H_4P_2O_7$, a phosphate ester) are shown below.



**Phosphoric acid ($H_3PO_4$)**



**Pyrophosphoric acid ($H_4P_2O_7$; ester)**

An ester is a compound formed by the reaction of an alcohol and an acid. They are known generally as esters of organic compounds. Compounds containing phosphate esters in plants include glucose-6-phosphate, ribulose-1,5-bisphosphate, ATP, DNA, phospholipids, coenzymes (thymine pyrophosphate, pyridoxal phosphate), and phytin among many other phosphorylated organic compounds that are important plant metabolites.

The normal concentration of phosphorus in plants is 0.2 to 0.5 % on a dry weight basis. Plants seldom accumulate more than 0.8% P and 1.2% is about as high as recorded. The high concentrations occur with plants grown in hydroponics. Excess P is stored as inorganic phosphate in leaves.

Deficiency develops if P falls below 0.15% in leaves.

Deficiency Symptoms – in order of appearance

1. Stunting. Stunting is the most frequent symptom of phosphorus-deficient plants but may not be detectable unless a standard of comparison is available.

2. Dark-green color. Phosphorus-deficient leaves are often darker green than normal leaves. Cell expansion is limited more by phosphorus deficiency than chlorophyll formation so that deficient leaves have more chlorophyll per unit area.
3. Off green color. Grey green or blue green coloration develops. This response may be due to some loss of chlorophyll and appearance of other pigments.
4. Reddish color develops. This response is due to anthocyanin formation. Anthocyanins are water-soluble glycosides, which are increased by disruption of glucose metabolism.
5. Yellowing. This action results for the loss of chlorophyll and other pigments.
6. Necrosis. Leaves die and drop off.

Sometimes enhanced rate of maturation occurs with fewer flowers being produced (perhaps resulting from less cytokinin and more abscission acid formation). This effect results in the discussions that phosphorus enhances flowering. However, once phosphorus is optimum for plant development, further applications of phosphorus do not enhance flowering. Phosphorus-deficient plants also have restricted root growth, and adding phosphorus promotes root growth. As with flowering response, once optimum phosphorus is supplied, further applications of phosphorus do not enhance rooting.

## Potassium

Potassium was discovered as an element in 1807. In 1856, it was shown to be essential for plants by Salm-Horstmar and by Knop and Sachs.

Plants absorb potassium as $K^+$. Potassium does not enter into any organic combination in covalent bonds in plants. It is held to some compounds by electrostatic bonds. All of the potassium remains as $K^+$. All of the potassium in plants can be washed from dead tissues by water.

The concentration of potassium in plant protoplasm is high ranging from about 0.05M in the cytoplasm of most cells to over 0.12M in some organelles and in guard cells. Since no organic compound contains potassium in covalent combination, scientists were puzzled about the role of $K^+$ in plant metabolism, particularly since so much $K^+$ is required in plants. Potassium has several defined roles in plant development and metabolism.

### Roles of Potassium in Plants

Osmotic effects

*Cell expansion.* The high concentrations of potassium in plant cells contribute to cell expansion. The high concentrations of potassium in solution in cells give the potential for water to move into the cells. The water in the cells imparts a turgor force on the cell walls and gives a stretching action that enlarges cells (Figure 6.5). Leaves emerging from buds contain most of the cells that they will ever have, and the observed growth of leaves results from cell expansion.

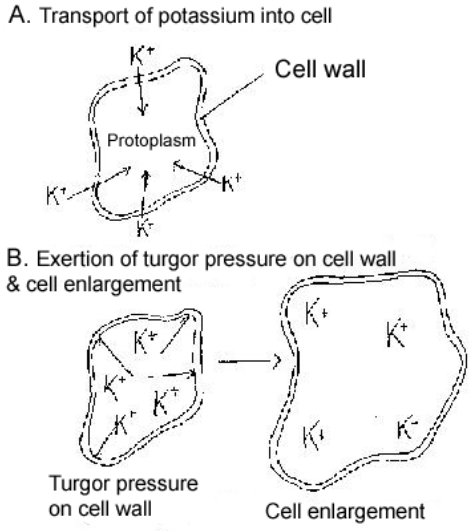
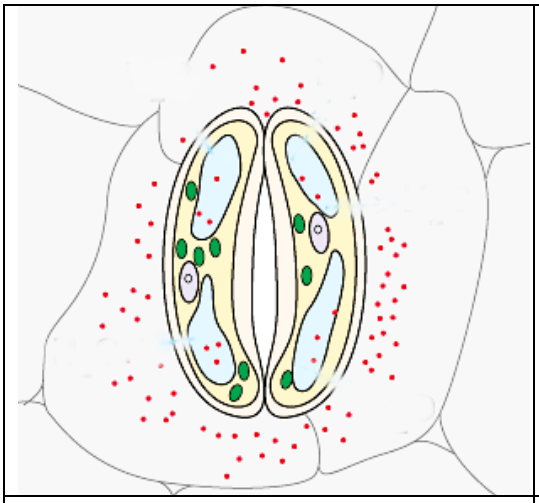


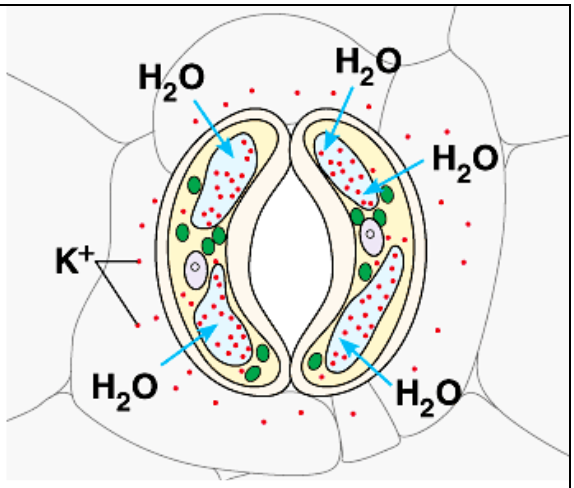

Figure 6.5 Illustration of turgor pressure resulting in cell expansion after movement of potassium and water into a plant cell

*Stomatal movement.* Guard cells are the only cells than have chloroplasts in the leaf epidermis. Stomates of epidermal strips have been observed to open when the strips are floated on KCl solutions and illuminated. In intact leaves, potassium from cells surrounding the guard cells contributes $K^+$ to guard cells. Potassium in surrounding cells enters into the guard cells in illuminated leaves (Figure 6.6). The light provides the energy for synthesis of ATP, and the ATP provides the energy for transport of $K^+$ into the guard cells. Following the transport of $K^+$ into the guard cells, water enters the guard cells through osmosis thereby producing a turgor pressure in the cells. Guard cells have an anatomical structure of cellulose microfibrils that surround the cells. These microfibrils prevent the width of the guard cells from swelling and only allow for the cells to elongate. The guard cells are held firmly in place by the surrounding epidermal cells; consequently, as the guard cells lengthen the cells bow apart and create an open pore, the stomate. Negative ions balance the influx of potassium. In some plants, chloride ions enter to balance the charge, whereas in other plants the malate is produced as the counter ion in guard cells. In darkness, ATPase activity hydrolyzes the ATP or ATP is no longer made by the chloroplasts; thus, the guard cells can no longer retain the high concentration of $K^+$, and it leaks out into surrounding epidermal cells. Closed guard cells have about 0.21M $K^+$. Opened guard cells have about 0.5M KCl. Transport of $K^+$ into guard cells has been

verified by the electron microprobe and by light microscope using sodium cobaltinitrite to precipitate $K^+$.



Light → Darkness ←




| | | |
|---|---|---|
| Closed stomata with potassium in epidermal cells surrounding guard cells | Light gives energy for production of ATP and $K^+$ transport. | Open stomata showing potassium accumulation in guard cells and water movement into the guard cells, resulting in opening of the stomate |

Figure 6.6 Diagrams of epidermal and guard cells of leaves showing closed stomata (left) and open stomata (right) following illumination leaves

*Balance of charges.* High concentrations of $K^+$ are needed to balance the negative charges of soluble organic and inorganic anions and macromolecules, such as nitrates, phosphates, organic acids, and proteins.

**$NO_3^-$ $K^+$ $H_2PO_4^-$ $K^+$ R-$COO^-$ - $K^+$ -C-C-C-C-C-C-C-C-C-C-C-**
**Nitrate Phosphate Organic acid**

**$COO^-$ $K^+$**

**Macromolecule**

*Stabilization of pH.* The participation of $K^+$ as the balancing rather than $H^+$ being the balancing ion leads to some stabilization against acidity in the cells.

*Circadian movements and thigmotropic responses.* Leaves open by day and close at night or move in response to mechanical stimuli (Figure 6.7). These

movements are turgor-related processes caused by distribution of $K^+$ in the pulvini.

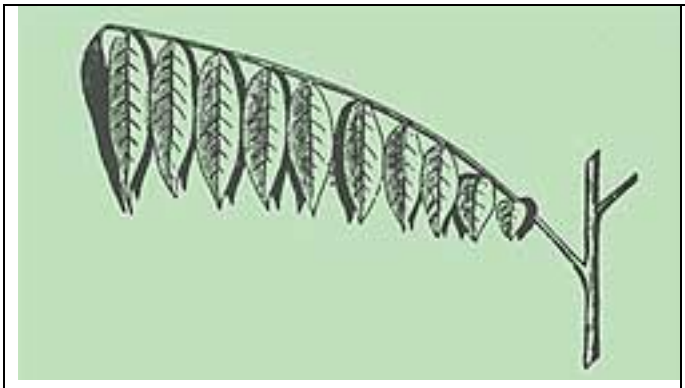
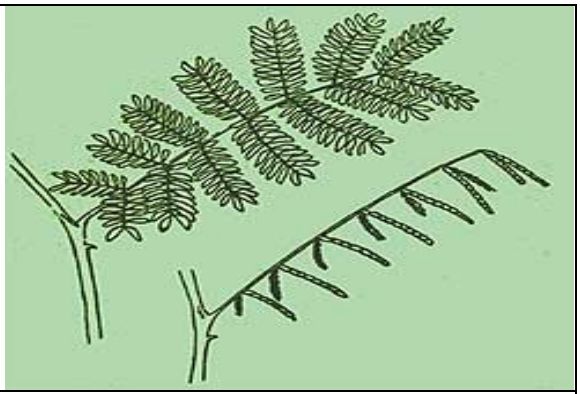
| | |
|---|---|
|  |  |
| Closed leaf in response to darkness | Open and closed leaf. Closed leaf is in response to touch. |

Figure 6.7 Leaves closed in response to darkness and open and closed leaf of sensitive plant in response to touch

Pulvini are thickenings at the base of leaves or leaflets and facilitate growth-independent movements. Pulvinar movement is caused by changes in turgor pressure leading to a contraction or expansion of the parenchyma tissue. Touch initiates a response from which sucrose is unloaded for the phloem into the apoplast of the pulvini. The increased sucrose in the apoplast decreases the water potential and initiates an efflux of $K^+$ and water from surrounding cells, resulting in a change in turgor pressure in the pulvini. The process is similar to stomatal closure in darkness. Examples of pulivnar movements include the night closure of leaves of legumes and the touch responses of sensitive plant (*Mimosa pudica*) throughout the plant.

*Transport processes*. Potassium ions may have a role in transport processes in phloem. Electroosmotic flow is a hypothesis for transport of solute in phloem. In this process, $K^+$ is loaded into the phloem setting up a potential for flow of water into the sieve tubes and flow of solute.

*Enzyme activation*. More than 50 enzymes require $K^+$ for activity. Pyruvic kinase of glycolysis is a potassium-requiring enzyme. Potassium is needed for the maintenance of the tertiary structure or folding of proteins (enzymes) for enzyme activity (Figures 6.8 and 6.9). The primary structure of proteins is the sequence of amino acids in the protein. The secondary structure is generally three-dimensional form (coiling, helices) of local segments. Quaternary structure is the arrangement of multiple folded protein molecules in a complex having a molecular weight in excess of 50,000 g/mole.

The effect of $K^+$ appears to be in maintaining the essential tertiary structure or conformation of the protein for enzymatic action. The concentration of $K^+$ required for this action is about 0.05 M.

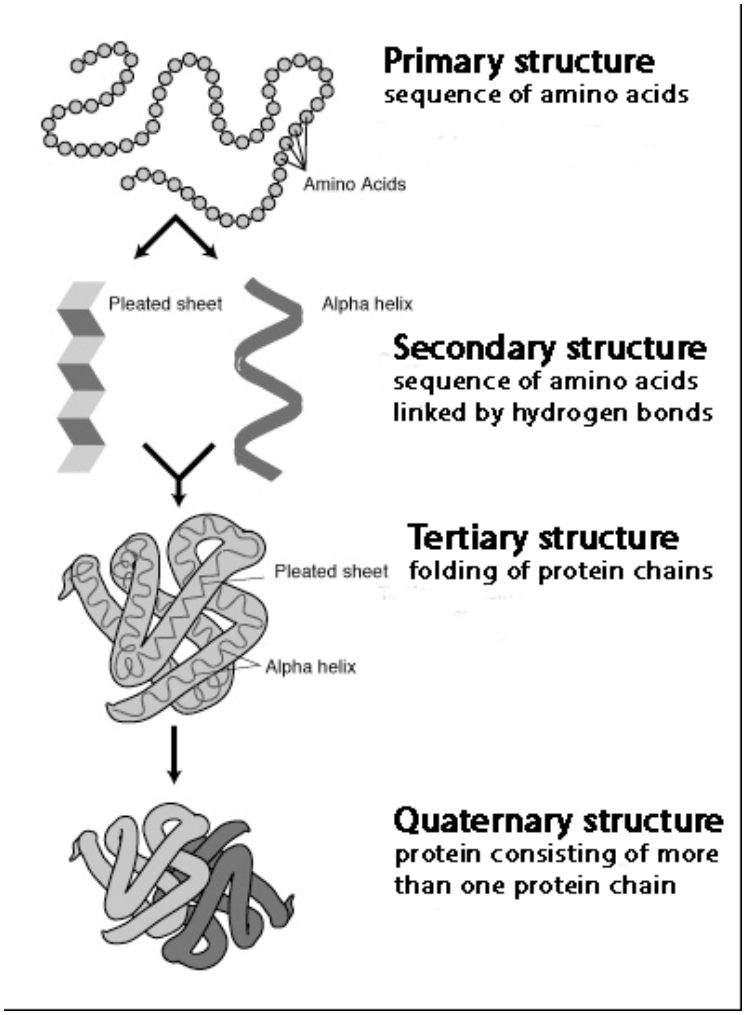



Figure 6.8 Diagrams of primary, secondary, tertiary, and quaternary structures of proteins

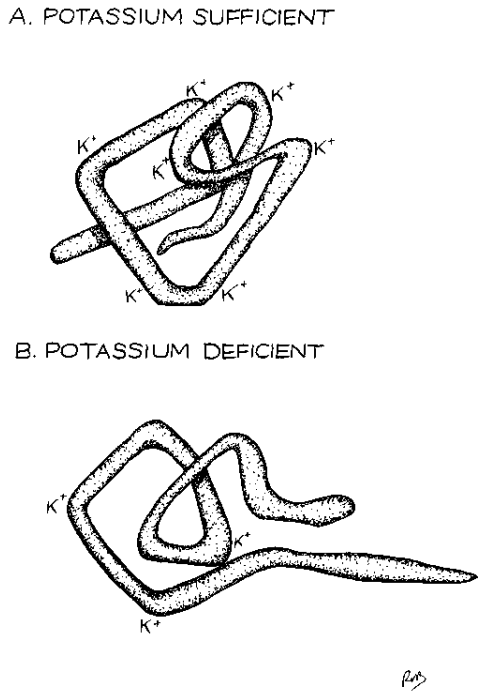



Figure 6. 9 Folded structure of potassium-sufficient protein (enzyme) and unfolding of potassium-deficient protein

*Protein synthesis.* Potassium is involved in the translation process including binding of t-RNA to ribosomes. Potassium is required also for peptide bond synthesis. Rate of protein synthesis is maximal at 0.13M $K^+$ and 0.002M $Mg^{++}$. Because of the high concentration of $K^+$ required for protein synthesis, protein synthesis is likely the first process to be affected by potassium deficiency.

**Symptoms of Potassium Deficiency**

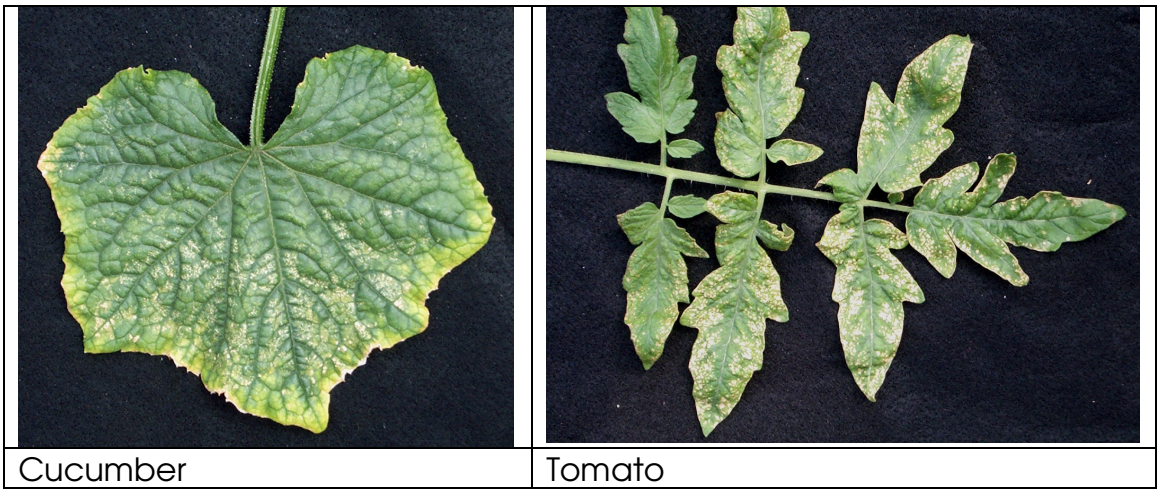
Accumulation of toxic compounds during potassium deficiency

Putrescine accumulates in potassium-deficient leaves and may be the cause of appearance of symptoms. Potassium deficiency increases the activity of certain enzymes such as arginine decarbooxylase, which leads to the synthesis of putrescine. Plants transport putrescine to the margins of leaves to eliminate the compound from leaf cells. The accumulation of putrescine at the margins causes necrosis. Putrescine is a four-carbon diamine.

$$NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2$$

**Putrescine molecule**

The first symptoms of potassium deficiency are dots along the margin. The necrosis advances to scorch (Figure 6.10).



| Cucumber | Tomato |
|---|---|

Figure 6.10 Advanced stage of potassium deficiency symptoms on cucumber and tomato leaves

Other symptoms of potassium-deficient plants include lodging as the cellular structure of stems degrades, malformed fruits, small fruits, and unfilled seeds (Figure 6.11).

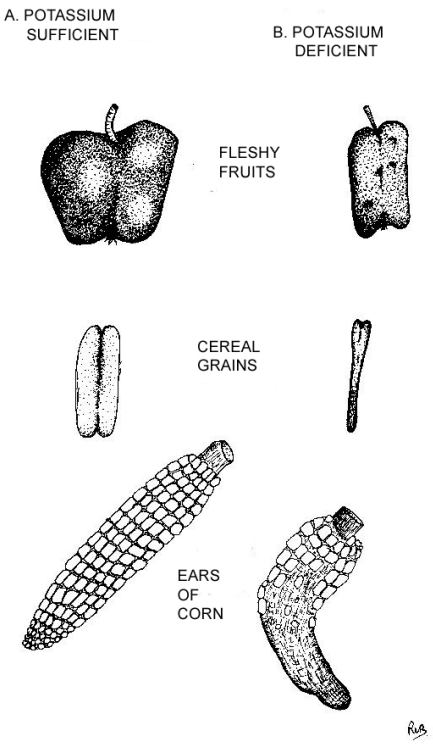


Figure 6. 11 Symptoms of potassium deficiency on apple, cereal grain, and ears of corn

The potassium concentrations in leaves range from 1.5% to 5% in normal tissue. Potassium concentrations below 1% are likely to cause deficiencies. Above 6 %, luxury consumption occurs. Toxic ranges are usually much higher than 6 %.

## Calcium

Calcium was discovered as an element in about 1808 and was shown to be essential for plants in about 1860 by Sachs. It is absorbed as $Ca^{++}$. Uptake is connected loosely with plant metabolism and is driven by electromotive forces.

The concentration of total calcium in leaves varies from 0.1% to 5% of the dry weight, differing greatly with species and plant tissues. The concentration of soluble $Ca^{++}$ is low in cells perhaps being of the order of $10^{-6}$ to $10^{-3}$ M compared to $K^{+}$, which is 0.01 M to 0.1 M or higher. The concentration of $Ca^{++}$ is similar to that of micronutrients in cytoplasm. Water-soluble calcium, however, might comprise 20% of the calcium in cells of some plants. The calcium of cells occurs in sparingly soluble compounds, such as calcium oxalate, calcium carbonate, calcium phosphate, and calcium sulfate. These compounds may comprise 25% to 50% of the calcium in cells, increasing as the external supply of calcium increases. Much of the calcium in plants is in the pectin, which surrounds each living cell. In calcium-deficient plants, half of the calcium is in the cell walls (pectin). About 30% of the calcium is in pectin in plants with sufficient calcium.

A protein (calmodulin) in the cytoplasm regulates the concentration of $Ca^{++}$ in cells. The concentration of $Ca^{++}$ may be kept low in the cytoplasm to prevent precipitation of phosphates, competition with magnesium, and perhaps enzyme inactivation.

Calcium tends to be transported to organs that have a high rate of transpiration. Large, old leaves have more calcium than young, small leaves. Wrapper leaves of heads of lettuce and cabbage have more calcium than the young tips of the heads. Fruits and storage organs are low in calcium. Often, deficiencies develop in tips and fruits, giving diseases called tip-burn, blossom-end rot, bitter pit, and poor storage.

Calcium is associated with the exchange sites of cell walls and requirements seem to be related to the cation exchange capacity of the plants. Movement of $Ca^{++}$ up the xylem appears to be by exchange along the negatively charged

walls of the xylem. A sharp gradient exists from top of plants to the bottom perhaps because of this transport.

In most plants, phloem transport of $Ca^{++}$ does not occur; therefore, no redistribution of calcium occurs among plant organs. Calcium that enters into an old leaf remains in that leaf. Calcium is not transported by phloem because $Ca^{++}$ does not enter the phloem. Calcium is said to be *immobile* in plants due to this lack of transport. The immobility of calcium is due to its distribution into sparingly soluble compounds and pectin and to the low concentration of $Ca^{++}$ in the cytoplasm (Figure 6.12). The concentration of $Ca^{++}$ in the cytoplasm is regulated by calmodulin, which is a protein (MW 20,000 g/mol) that reversibly binds $Ca^{++}$, strongly and selectively.

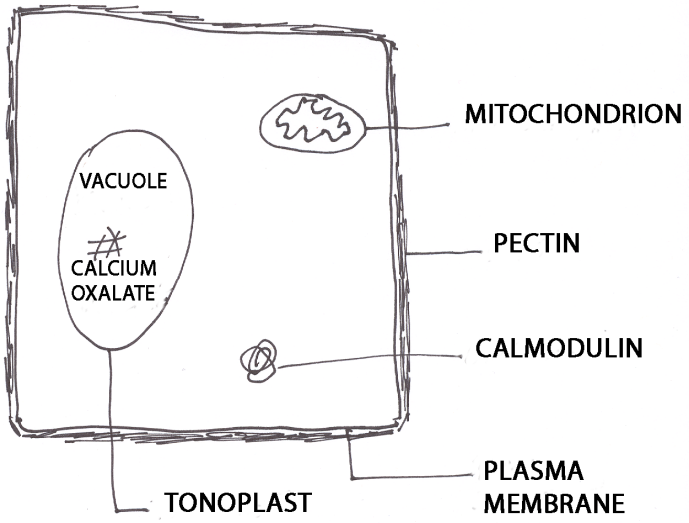



Figure 6.12 Cellular structures with potentially high concentrations of calcium

Symptoms of deficiency develop rapidly if the external supply is exhausted. Foliar symptoms of calcium deficiency appear at the tips in the leaves that are expanding and at lateral branches.

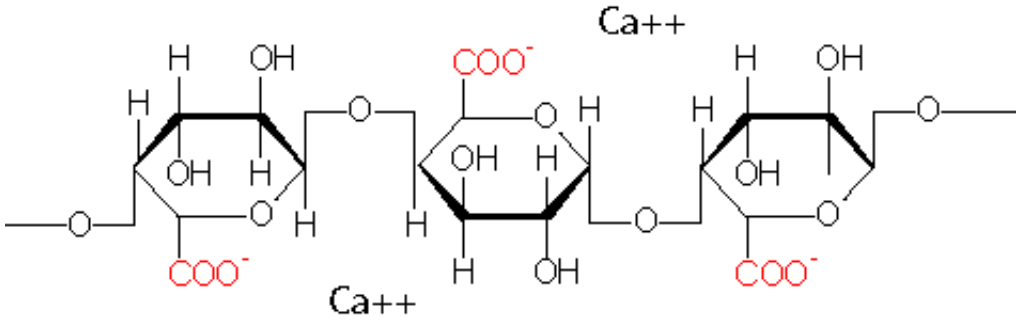
**Functions of Calcium**

***Membrane stability***. In the absence of $Ca^{++}$ membranes lose their selectivity. Solutes leak readily form cells of calcium-deficient tissues and from vacuoles into cytoplasm. Membrane structures within the cell disintegrate. Calcium forms bridges between carboxyl and phosphate groups of lipids. If the calcium is

removed from membrane by chelation the membrane appears fragmented and disorganized.

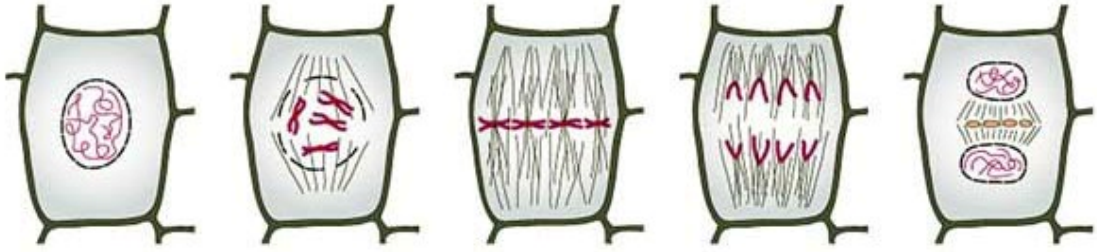
A good supply of $Ca^{++}$ must be present constantly in the external solution for maintenance of integrity of the cell membrane. No experiment involving ion absorption should be seen without $Ca^{++}$ (about 0.001M) in the external solution.

***Cell division (mitosis)***. A high proportion of the calcium of plants is in the cell walls and held to binding sites in the middle lamella (pectin). In leaf cells, 30% to 50% of the calcium is in pectin. In apple fruit, 90% of the calcium may be in the pectin. Pectin is a calcium salt of polygalacturonic acid, which is a derivative of galactose. Cell division and expansion require synthesis of pectin.




**Fragment of Pectin**

It is suggested that $Ca^{++}$ has a role in maintenance of chromosome structure (Figure 6.13). Spindle fibers do not form right if $Ca^{++}$ is deficient. Spindle fibers pull the chromosomes to the poles of the dividing cell. Spindle fibers are microtubules of globular protein. Calcium transport occurs in and out of tubular organelles and surrounding fluids. Spindle fibers lose elasticity in calcium-deficient cells, and the chromosomes do not migrate to the poles.



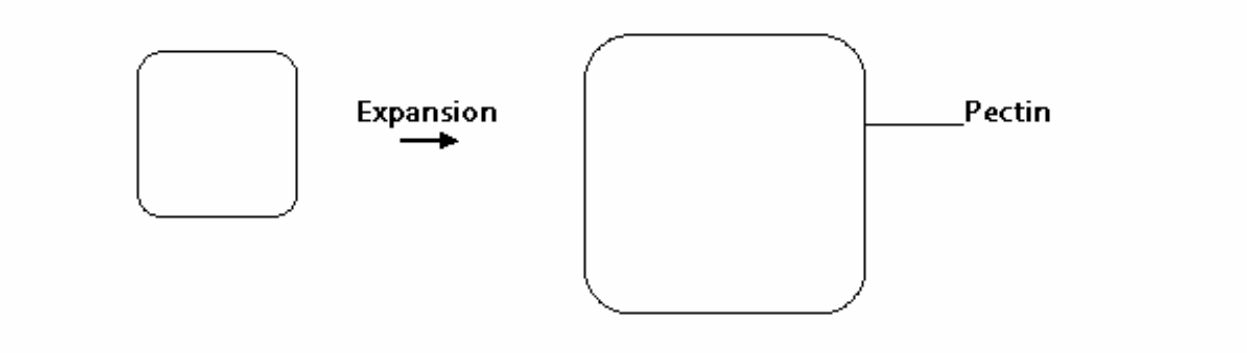
**Interphase** **Prophase** **Metaphase**
**Anaphase** **Telophase**

Figure 6.13 Cell division from prophase through telophase. Note separation of chromosomes with attached spindle fibers during anaphase

Cell division (one cell forming two cells) occurs by *cell plate* formation. Vesicles derived from Golgi bodies and endosomes carry cell wall and cell membrane components to the central plane of the cell. With the completion of the mature cell plate, cellulose and pectin synthesis are needed to complete the division into two daughter cells.

If cell division is disrupted by calcium deficiency, nuclear and cell structure can be abnormal. With no spindle formation or function, chromosomes may not divide, and cells might be tetraploid. The tetraploid cell may live. On the other hand, with no nuclear division and with cell plate formation, one of the daughter cells might have a nucleus, and the other one will not. The cell without a nucleus will die. If no plate forms, one cell may have two nuclei, and this cell will die.

***Cell enlargement***. When leaves emerge from buds, the leaves have almost all of the cells that they will ever have. The growth of the leaves is by cell expansion. Each living cell is surrounded by pectin. Pectin must be made to surround the larger cell, thus requiring calcium. If calcium is in inadequate supply, pectin synthesis will be limited. Cell expansion may cause cells to rupture or tissue to tear, resulting in death of the expanding leaves.




Symptoms of calcium deficiency occur on young leaves in which cells are expanding. The symptoms are characterized by deformation and death of the leaves (Figure 6.14).

Figure 6.14 Calcium deficient cabbage showing deformation and tipburn of leaves

**Disorders in Calcium Deficient Crops**

Calcium deficiency occurs commonly in crop production. Produce from calcium-deficient crops will be of poor quality. These disorders may be exhibited on fruits, foliage, or roots.

***Blossom end rot*** (tomato, watermelon, peppers, mango). Blossom-end rot occurs in calcium-deficient soils, dry soils, or in soils fertilized heavily with ammonium-containing fertilizers. Fruits do not transpire much; hence, water movement into the fruits is limited. Calcium flows in the plant with the flow of water in the xylem. Calcium is not transported in the phloem. Therefore, any process that limits the absorption or movement of calcium by plants may cause maladies in fruit development. In dry weather, calcium solubility in soil is restricted, and calcium may precipitate from solution. Potassium and ammonium may remain in solution and further restrict the absorption of calcium. Water is needed to transport calcium to the roots of plants in soil. Calcium does not flow into roots with the flow of water, but water is needed for calcium to move in the xylem. Any one of these factors—limited solubility, limited absorption, limited transport of calcium—can suppress calcium delivery to fruits and result in disorders in fruits, such as blossom-end rot of tomato (Figure 6.15).

Figure 6.15 Blossom-end rot of tomato

***Bitter pit of apple.*** Bitter pit of apple is a disorder that appears on the skin or just under the skin of apples (Figure 6.16). Bitter pit appears as small, brown, dried pits of collapsed tissue. Symptoms of bitter pit seldom are seen before harvest time. The majority of bitter pit occurs during storage. Symptoms normally develop within 30 to 60 days after harvest. Fruits not showing bitter pit within 30 to 60 days usually do not develop bitter pit. Growers sometimes dip apples in calcium chloride solutions or slurries to prevent bitter pit in stored apples. Internal breakdown, water core, and cork spot are other problems in apples and are associated with calcium deficiency.

| Bitter pit | Internal breakdown | Water core |
|---|---|---|

Figure 6.16 Bitter pit, internal breakdown, and water core of apple

***Tip burn***. Tip burn of the young leaves of lettuce, cabbage, Brussels sprouts, escarole, and other leafy vegetables is a disorder of calcium deficiency. Calcium transport to the young leaves is hindered by calcium supply or by

environmental conditions. If calcium is limiting in the medium, $Ca^{++}$ may fail to reach the young leaves as the old, larger leaves will receive more $Ca^{++}$ than the young leaves. In a dry atmosphere, old leaves will receive more $Ca^{++}$ through xylem transport and transpiration than young leaves, thereby depriving the young leaves of $Ca^{++}$. In humid atmospheres, transpiration will be limited, thereby limiting the flow of $Ca^{++}$ into all leaves. Young leaves will continue to grow, and the restricted flow of $Ca^{++}$ can lead to tip burn.

***Blackheart of celery***. Blackheart is disorder similar to tip burn. Blackheart shows on the petioles of the young leaves (heart) of celery, thereby yielding an unmarketable product.

***Cavity spot***. Cavity spot of carrot and parsnip is a disorder of calcium deficiency expressed on roots. It is caused by an imbalance of $K^+$ as high $K^+$ supply suppresses $Ca^{++}$ absorption. High concentrations of ammonium as with supply from some fertilizers and manures can lead to this disorder.

***Peg (gymnophore) of peanut***. Calcium nutrition is important in peanut production. Calcium is needed for the growth and development of the peg of peanut (Figure 6.17). The flower of peanut blooms above ground, and then the peg grows into the soil where the peanuts develop. The peg does not enter calcium-deficient soils; hence, peanuts do not form in calcium-deficient soils. If the peg enters the soil, adequate calcium for seed development must be available in the pegging zone (top 3 inches of soil) because the nutrient must be absorbed directly by the peg or pod. None of the calcium absorbed by the plant roots is translocated through the plant to the developing peg or pod. A calcium-deficient peanut may show black heart, a disorder in which the embryo is black and the cotyledons are healthy. Calcium-deficient peanut seeds have poor germination. Gypsum or calcitic limestone is applied to peanut crops to ensure adequate calcium for seed development.

Figure 6. 17 Peanut plant showing pegs and peanuts underground
***Other disorders***. Brownheart of escarole, cracking of cherries, softness of mango, and internal tip burn of Chines cabbage are crop production problems that develop in calcium-deficient soils.

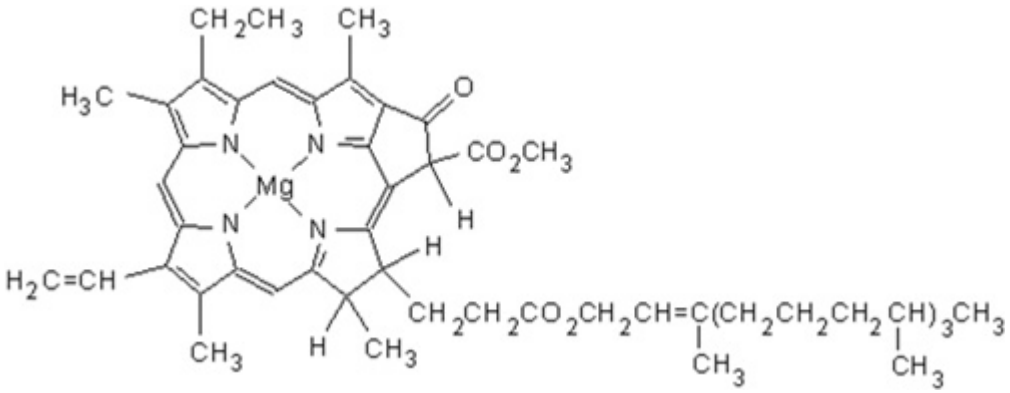
## Magnesium

Magnesium was discovered as an element in 1808. It was shown to be essential in 1860 by Salm-Horstmar, Sachs, Knop, and Mayer.

Magnesium is absorbed as $Mg^{++}$, which is mobile and readily transported in plants. About 70% of the magnesium in plants can be extracted with water as $Mg^{++}$ is held loosely to organic molecules by ionic charges.

### Functions of Magnesium

***Constituent of Chlorophyll***. About 10 to20% of the magnesium of leaves is in the chloroplasts, which occupy only about 5% of the volume of a leaf cell. About 5 to 10% of the magnesium of leaves is in the chlorophyll. Chlorophyll is held in a complex with proteins in the chloroplast. About 25% of the proteins of leaves is in the chloroplast.



**Chlorophyll *a*.** Other chlorophyll molecules (b, c, d, and f in plants and algae) differ from chlorophyll a in the carbon chain and in other structural groups attached to the ring structure.

***Adjustment of cellular acidity***. The high concentrations of $Mg^{++}$ and $K^{+}$ in chloroplasts and cytosol help to maintain a pH of 6.5 to 7.5. Vacuolar acidity in contrast is pH 5 to 6.

***Protein synthesis***. Magnesium has several functions in protein synthesis. Magnesium is needed for the synthesis of RNA polymerases (forming mRNA, tRNA, and ribosomal RNA). Magnesium has a function as a bridging element in the aggregation of ribosomal subunits (30S + 50S → 70S; S is the Svedberg unit, which is a measure of aggregate size). Aggregation is essential for protein synthesis. Under magnesium deficiency, the subunits dissociate, and protein synthesis ceases. Magnesium is needed for the activation of amino acids. Amino acids are activated by ATP to form acylamino acids with tRNA by this reaction, which requires $Mg^{++}$:

**Amino acid + ATP + tRNA →Amino acid acyl-tRNA + AMP + inorganic Pi**

***Energy metabolism***. The synthesis of ATP by ATPase (ADP + Pi → ATP) and the reverse reaction has an absolute requirement for $Mg^{++}$.

***Enzyme activation***. $Mg^{++}$ activates more enzymes than any other element. Most of the reactions involve transfers such as phosphate (ATPases, phosphatases) and carboxyl groups (carboxylases). Some other magnesium-requiring enzymes are ribulose-1,5-phosphate carboxylase (RuBP+ $CO_2$→ 2PGA), glutamine synthetase ($NH_4$+aKG+ATP +$2e^{-}$→Glu + ADP + Pi), enolase (2PGA→ PEP + $H_2O$), and isocitric dehydrogenase (Isocitric acid +NAD or NADP→ Oxalosuccinic acid + NADH or NADPH), which are important enzymes in photosynthesis, nitrogen utilization, glycolysis, and respiration. In many enzymatic reactions, $Mn^{++}$ may substitute for $Mg^{++}$.

**Magnesium Requirement by Plants**

The normal concentration of $Mg^{++}$ is 0.2% to 0. 6% of the dry weight of leaves. Symptoms of deficiency are the mottling of the lower leaves in which interveinal chlorosis or necrosis occurs with green tissues remaining near the veins (Figure 6.18). The development of the symptoms is the result of restricted protein synthesis and subsequent loss of chlorophyll. Magnesium deficiency limits protein synthesis more than chlorophyll synthesis. Protein for the protein-chlorophyll complex is inadequate resulting in a degradation of chloroplast thylakoids. Hence, magnesium deficiency is essentially an expression of protein deficiency.

Figure 6.18 Advanced symptoms in magnesium-deficient cucumber leaf

Magnesium is toxic to plants if $Mg^{++}$ is a prevalent cation on soil exchange complexes. Serpentine soils are barren. Magnesium deficiency in forage causes a disease known as grass tetany.

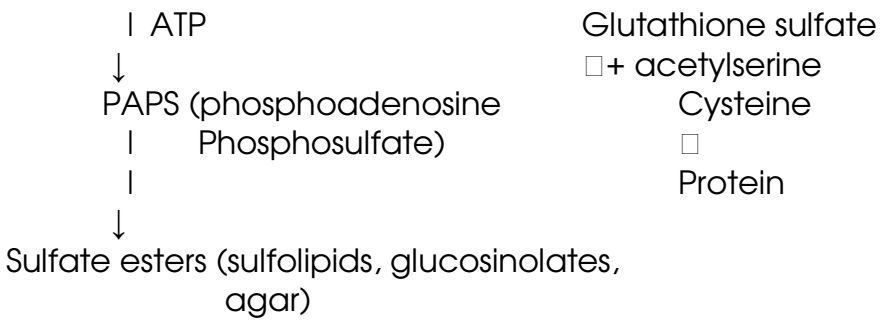
## Sulfur

Sulfur is a prehistoric element. Its essentiality for plants was demonstrated by Sachs in about 1860.

Sulfur is absorbed as sulfate ($SO_4^=$) from solutions; $SO_2$ may be absorbed from the air.
Most of the sulfur in plants is in organic compounds and in the form of reduced sulfur.

Sulfate is reduced to $S^=$ before it is incorporated into organic compounds by the following pathway in chloroplasts:

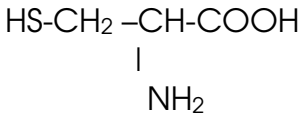
$SO_4^=$ + ATP → APS (adenosine phosphosulfate)
| + glutathione



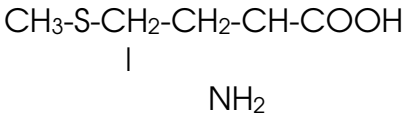

Sulfate assimilation in chloroplasts competes with use of ATP with nitrate reduction and with carbon dioxide fixation (photosynthesis).

**Functions of Sulfur**

***Constituent of proteins***. Two amino acids, cysteine and methionine, are constituents of proteins.



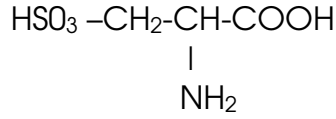
**Cysteine**



**Methionine**

In proteins, cysteine is a dimer with disulfide bonds. The dimer is called cystine (cys-S-S-cys).

Cysteic acid is formed by breaking disulfide bonds in proteins; thus, plants can oxidize as well as reduce sulfur.

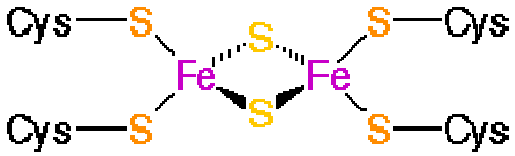


**Cysteic acid**

Thus plants can oxidize S as well as reduce it. Cysteic acid may not have a function in plants.

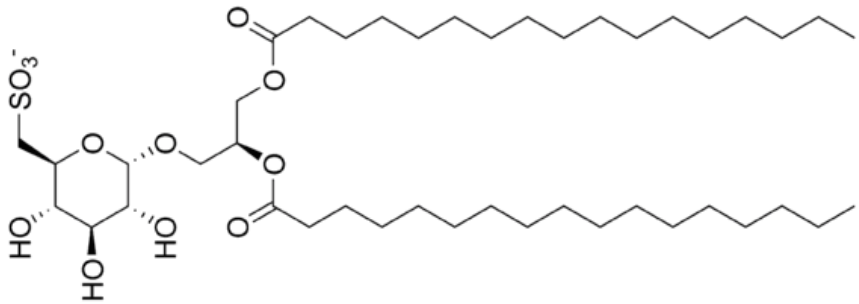
***Constituent of coenzymes (vitamins)***. Sulfur is a constituent of thiamine (thiamin pyrophosphate, vitamin B1), biotin, Coenzyme A, and lipoic acid. Thiamin is a cofactor in the decarboxylation of pyruvic acid and a-ketoglutarate in glycolysis and in respiration. Biotin is a coenzyme for carboxylase enzymes and is involved in fatty (oleic) acid and amino acid (aspartic acid) metabolism. Biotin is involved in the decarboxylation of oxaloacetic acid and oxalosuccinic acid in respiration. Coenzyme A and lipoic acid are cofactors in the decarboxylation of pyruvic acid and other a-ketoacids.

***Constituent of ferredoxin***. Ferredoxin is an iron-sulfur protein that mediates electron transfer in a variety of reactions including nitrite reduction, ammonium assimilation (GOGAT), nitrogen fixation, and photosynthesis. Ferredoxin is a nonheme protein in which the iron atoms are arranged linearly in the molecule. The number of iron atoms in ferredoxin varies with the kind and source of the protein.




**An iron-sulfur protein with two iron atoms. The iron atoms are linked to cystine in the protein.**

***Constituent of sulfolipids***. Sulfolipids are structural components of all biological membranes.




Six-carbon sugar—Glycerol—Fatty acid

**Sulfolipid**

***Constituent of mustard oils, glucosinolates, and other secondary metabolites.*** Sulfur is a constituent of glucosides, isothiocyanates (-CNS), and other constituents referred to as essential oils in plants. The pungency and flavors of brassicas (cabbage, mustard) and onions and garlic are due to these compounds.

**Plant Composition**

A healthy plant has about the same amount of sulfur as phosphorus, with 0.2% to 0.5% dry weight being the normal range and <0.15% being deficient in foliage.

**Deficiency Symptoms**

Symptoms look similar to those of nitrogen deficiency with plants being spindly and yellow overall if sulfur is deficient throughout the life cycle. If the plants exhaust sulfur from the medium during their growth, yellowing occurs on young leaves in contrast to chlorosis that develops on the old leaves of nitrogen-deficient plants. Sulfur does not have the mobility in plants that nitrogen has and, hence, transport of sulfur from old leaves to young leaves is slow. Nitrogen and sulfur are constituents of amino acids in proteins; so, the symptoms are similar because of that requirement.

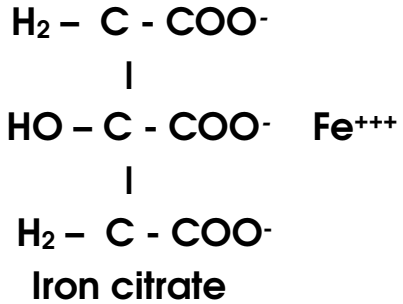
## Iron

Iron is a prehistoric element and was shown to be essential for plants by Sachs in about 1860.

As a rule, iron is absorbed as $Fe^{++}$ (ferrous iron). Ferric iron ($Fe^{+++}$) is the prevalent ionic species in soils due to the well-aerated conditions that oxidize iron. The concentration of $Fe^{+++}$ in soils is low, less than $10^{-10}$ M although soils have 10,000 to 100,000 pounds of iron per acre furrow slice (top six inches) (<50,000 is considered deficient).

At the root surface, iron is reduced by the plant ($Fe^{+++} \rightarrow Fe^{++}$) prior to absorption. Grasses may lack this capacity and absorb considerable $Fe^{+++}$ and may have difficulty obtaining sufficient iron from some media.

Iron is transported in plants as a chelate of $Fe^{+++}$ and citrate. Citrate is a chelating agent that helps to keep iron in solution in the xylem.




**Iron citrate**

### Functions of Iron

About 80% of Fe is in chloroplasts regardless of the nutritional status of plants.

***Synthesis of chlorophyll***. Iron is not a constituent of chlorophyll but is needed for chlorophyll synthesis. Iron is needed for the synthesis of δ-aminolevulinic acid, which is a precursor for chlorophyll. δ-Aminolevulinic acid is needed for the

synthesis of the ring structure (porphyrin) of chlorophyll. δ-Aminolevulinic acid is formed from succinyl CoA and glycine.

***Oxidation-reduction reactions***. Iron participates in electron transport in photosynthesis, respiration, nitrogen fixation, nitrite reduction, sulfate reduction, and some other processes that involve oxidation and reduction. Some iron is in hemeproteins, which include cytochromes, catalase, peroxidase, and leghemoglobin. In hemeproteins, the iron atom is contained in the center of a heterocyclic organic ring (prophyrin). Other iron-containing constituents participating in oxidation and reduction include nonhemeproteins (iron in a straight chain), which include ferredoxin, aconitase, and succinic dehyrogenase.

**Iron Absorption**

In dicotyledons (and monocots except grasses), the morphology of roots changes. Rhizodermal cells called transfer cells form in response to iron deficiency. Transfer cells have high metabolic activity and lead to export of $H^+$ that acidifies the medium and dissolves the iron in the rhizosphere. Transfer cells apparently have membrane bound enzymes that enhance reduction of $Fe^{+++}$ to $Fe^{++}$. The combination of increased capacity to reduce $Fe^{+++}$ and acidification of the medium permits dicots and certain monocots to grow in media low in iron. Transfer cells degenerate or return to original form after iron is restored in 1 or 2 days. This mechanism is sensitive to $HCO_3^-$ and sensitivity causes plants to develop lime-induced chlorosis in calcareous soils (soils with free lime). Grasses are particularly sensitive to development of iron deficiency in alkaline soils or media.

Grasses do not develop transfer cells. They release phytosiderophores that chelate $Fe^{+++}$. The Fe chelates appear to be absorbed by the roots of grasses. Phytosiderophores are methionine-based, complex compounds that vary with plant species. These compounds may have functions in iron transport and deposition in plants.

Dicots and non-grasses also release phenolic substances when iron is in low supply or in sparingly soluble compound. The phenolic substances may chelate $Fe^{+++}$ or $Fe^{++}$ and increase its availability.

**Iron Composition of Plants**

Sufficient Fe concentrations in plants are 50 to 100 mg/kg (ppm) (0.005 to 0.01%) of the dry weight of leaves. Iron deficiency is common in calcareous soils and in greenhouse production with peat-based media in which iron is naturally low. In nutrient solutions, iron is supplied as chelated iron, such as iron EDTA (ethylene

diaminetetraacetate), which protects the iron from precipitation as alkalinity rises.

Iron toxicity may occur in water-logged soils in which $Fe^{++}$ may be high due to anaerobic conditions. Toxicity may occur in greenhouse production in peat-based media in which acidity develops following repeated applications of iron in acid-forming fertilizers. The leaf concentration at which toxicity occurs is over 500 mg Fe/kg dry weight and depends on the presence of other nutrients such as calcium, magnesium, and manganese. Iron toxicity is associated with iron-catalyzed formation of reactive oxygen species.

## Manganese

Manganese was discovered as an element in 1774 and was shown to be essential for plants in 1920 by McHargue. Plants absorb $Mn^{++}$ and transport $Mn^{++}$.

### Functions in Plants

A relatively large number (perhaps 35 or more) of enzymes require manganese for activity. Manganese is a cofactor for enzymes catalyzing oxidation-reduction, decarboxylation, or hydrolytic reactions. Manganese has roles in the Krebs Cycle. In vitro, $Mn^{++}$ substitutes, or vice versa, for $Mg^{++}$ in many reactions, but due to the much higher concentration of $Mg^{++}$ than $Mn^{++}$, $Mg^{++}$ may be the functional ion. Manganese is more effective than magnesium for some enzymes such as RNA polymerase. An absolute requirement for manganese occurs with PEP carboxylase in the bundle sheath of C4 plants.

Superoxide dismutase (SOD), which is present in all aerobic organisms, protects cells against superoxide ($O_2^-$). Superoxide dismutase is prevalent in chloroplasts (about 90% of SOD activity) and mitochondria (about 5% of SOD activity). Superoxide dismutase enzymes differ in their metal component, which can be iron, manganese, or copper and zinc.

The reactions forming and destroying superoxide proceed as follows:

Formation:

$O_2 + e^- \rightarrow O_2^-$ superoxide

Degradation by superoxide dismutase (a dismutase reaction is one in which a substrate reacts with itself):

$$O_2^- + O_2^- + 2H \rightarrow \underset{\text{Hydrogen peroxide}}{H_2O_2} + O_2$$

Fe-containing catalase destroys the resulting $H_2O_2$ :

$H_2O_2 \rightarrow H_2O + 0.5\ 0_2$

Manganese is required for the hydrolysis of water with the evolution of oxygen in Photosystem II. Four manganese ions ($Mn^{+++}$) in a cluster with one calcium function in the splitting of water into $0_2$ and H (electrons). The $Mn^{+++}$ is reduced to $Mn^{++}$ in the process. The electrons are transferred to Photosystem II and subsequently to Photosystem I through the electron transport cahing. The hydrolysis reaction may be the most sensitive one in manganese-deficient, photosynthesizing leaves.

### Manganese in Plants

Plants require leaf concentrations of manganese of about 30 to 100 mg/kg dry weight. Symptoms of deficiency occur at concentrations of 10 to 20 mg/kg. Symptoms are interveinal chlorosis of young leaves. Deficiencies occur in alkaline soils (pH>7.5), organic soils, and highly leached mineral soils. Manganese deficiency was identified as plant diseases named marsh spot of pea and fava bean, grey speck of oats, and yellow speck of sugar beets by pioneering farmers, who were farming before manganese was known as an essential element. Manganese deficiency may occur in alkaline soilless media or alkaline nutrient solutions or in solutions in which phosphate concentration is high. Deficiencies can be corrected by application of manganese sulfate ($MnSO_4$) to soil, foliage (spray), or seeds (dusts).

In acid soils, $Mn^{++}$ may increase to toxic concentrations. In nutrient solutions, $Mn^{+}$ toxicity does not occur because $Mn^{++}$ concentration is supplied at a low level. However, manganese may accumulate in acidic, peat-based soilless media repeatedly treated with manganese-containing fertilizers. Toxicity symptoms appear as chlorosis, dieback, death, and irregular growth among plants. Plants may appear to be iron deficient or calcium deficient. The toxic concentration of manganese in leaves varies widely among plants, ranging from 200 mg/kg dry weight in corn to 5,000 mg/kg in sunflower.

## Zinc

Zinc was used in prehistoric times for medicinal purposes and for making brass and was recognized as a metal as early as 1374. It was shown to be essential for plants in 1926 by Sommer and Lipman.
Zn is absorbed as $Zn^{++}$ (or $ZnOH^{+}$ in soils above pH 7.5). Zinc is transported as $Zn^{++}$ or as a chelate with organic acids. Zinc is not oxidized or reduced in plants, and

its roles are related to its properties of a divalent cation that forms tetrahedral complexes with N, O, and S functional groups of enzymes.

**Functions**

***Enzyme action***. Zinc is required as a structural component of some enzymes. *Alcohol dehydrogenase* (Acetaldehyde→Ethanol) operates in root apices under anaerobic condition and requires zinc as a structural component. Carbonic *anhydrase* ($CO_2 + H_2O = HCO_3^- + H^+$) maintains an equilibrium between carbon dioxide and bicarbonate. Carbon dioxide is the substrate for photosynthesis in C3 plants and C4 bundle sheaths; $HCO_3^-$ is substrate in C4 mesophyll. *Superoxide dismutase* (SOD, Zn and Cu in this enzyme) protects plants against reactive oxygen species, such as superoxide. All aerobic organisms produce superoxide. As mentioned in the section about manganese, 90% of SOD is in chloroplasts and 4% to 5% of the activity is in mitochondria.

In some enzymes, zinc is not a component but is required as a cofactor in the enzymatic reaction. Dehydrogenase enzymes such as glutamic dehydrogenase (α-KG +NH3 +NADH +$H^+$→Glu +$NAD^+$) which is involved in ammonium assimilation in mitochondria are activated by zinc. Aldolase of glycolysis (fructose-1,6-diphosphate → glyceraldehyde-3-phophate + dihydroxyacetone phosphate) and RNA polymerases ($Mg^{++}$ also needed) require zinc ($(RNA)_n$→ $(RNA)_{n+1}$). In absence of $Zn^{++}$, ribosomes disintegrate, and protein synthesis stops. Processes resume after $Zn^{++}$ restored to system.

Zinc may be involved in auxin metabolism and is reported to be needed for the maintenance of auxin (indoleacetic acid, IAA) or formation of IAA. The most accepted role is in the synthesis of tryptophan, which is suggested as a precursor of IAA.

**Zinc in Plants**

The normal concentration of zinc in leaves is 20 to 100 mg/kg. Below 15 to 20 mg/kg zinc may be deficient. In sandy soils, zinc may be leached; in calcareous soils, zinc may be precipitated in sparingly soluble compounds or held to calcium carbonate; and in peaty soils, zinc has been leached or is held to the organic matter. With soils high in phosphate, zinc may be precipitated by phosphates or may be immobilized by phosphate in plants.

Soils high in phosphate (precipitated by phosphate or adsorption to Fe Al oxides and hydroxide or immobilization in plant)

**Symptoms of Deficiency**

Rosetting caused by shortening of internodes possibly due to the lack of auxin occurs in zinc-deficient dicotyledons. Little leaf of citrus and other fruit trees is a symptom of zinc deficiency. These symptoms often occur in conjunction with mottling of broad leaves and striping of grasses.

| | | |
|---|---|---|
|  |  |  |
| Little leaf of pear (left) | Striping of corn | Mottling of citrus |

Figure 6.19 Symptoms of zinc deficiency on pear, corn, and citrus

To correct zinc deficiency, zinc sulfate or other zinc salts are sprayed on plants or banded in soils.

## Copper

Copper is a prehistoric element and was shown to be essential for plants in 1930 by Sommer.

Plants absorb the nutrient as $Cu^{++}$ or as copper chelate. In soils almost all (98%) of $Cu^{++}$ is held in complexes of low molecular weight organic compounds that are essentially unavailable to plants. The $Cu^{++}$ in soils is bound to humic and fulvic acids with not much copper being held in inorganic complexes. $Cu^{++}$ is held also to exchange sites on inorganic colloids. The concentration of $Cu^{++}$ in the soil solution is about $10^{-8}$ to $10^{-7}$M.

### Functions of Copper

Most of the functions of copper in plants are based on copper bound to enzymes that catalyze oxidation-reduction reactions. Copper is held tightly to functional groups of proteins, and its concentrations in the cytoplasm are very low. About half of the copper in plants is in the chloroplasts.

***Superoxide dismutase***. This enzyme is the same as the one discussed under functions of zinc.

***Plastocyanin***. Plastocyanin is a component of electron transport in photosynthesis. Plastocyanin delivers electrons to Photosystem I from Photosystem II.

***Cytochrome oxidase***. Cytochrome oxidase is a protein in the membranes of mitochondria. It is a terminal oxidase in the electron transport chain of oxidative phosphorylation. This enzyme is poisoned by cyanide.

***Amine oxidases***. These enzymes catalyze the aerobic degradation of diamines such as putrescine.

***Polyphenol oxidases***. These enzymes catalyze the oxygenation of plant phenols and require molecular oxygen. These enzymes are abundant in cell walls and in thylakoids and are called phenolases and laccases. Phenolases and are involved in the formation of lignin and brown melanotic substances. Browning reactions occur in wounded tissues (apples, potatoes). The melanotic substances of browning reactions may be antimicrobial against spores and fungi. In thylakoids, laccase may lead to the synthesis of plastoquinones, which are constituents of electron transport in photosynthesis.

### Copper in Plants

The critical concentration of copper in leaves is 1 to 5 mg/kg dry weight. Deficiencies occur in media that are inherently low in copper or high in organic matter.

Symptoms of copper deficiency are stunting of growth and distortion of young leaves shown by chlorosis or bleaching of young leaves and marginal necrosis resulting in dieback. Enhanced tillering of cereal grasses occurs with copper deficiency, commonly in organic soils. With pioneering farmers, these maladies were called reclamation disease, white tip, and summer dieback. Auxillary shoots (branching) develop on dicotyledonous plants giving a distortion referred to as witch's broom. Wilting is common due to insufficient water transport in xylem vessels that are not sufficient in lignin. Rough bark and exudation of gum occurs with copper-deficient fruit trees.

## Boron

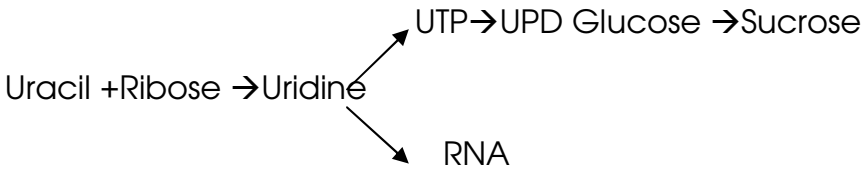
Boron was discovered as an element in 1808 and was shown to be essential for plants in 1923 by Warington. Boron is absorbed in as boric acid ($H_3BO_3$, sometimes written as $B(OH)_3$). Boron is transported only in the xylem, and distribution of boron from nutrient solution to shoots depends on the flow of water. Boron is complexed strongly to cell wall constituents. This complex may render B immobile

**Function of Boron in Plants**

The function of boron in plants is not defined. No organic boron-containing compound has been isolated from plants. However, more boron is required on a molar basis by plants than any other micronutrient. Fungi and fresh-water algae do not require boron. Boron deficiency causes a wide range of anatomical and physiological symptoms. These symptoms include death or inhibition of apical growth, brittleness, abortion of flowers, corky fruits, and fruit drop.

Lignification and cell wall formation are apparent functions of boron. Prominent symptoms of deficiency are associated with primary cell wall development and include thickened walls and brittle walls of cells that do not expand properly. Boron, however, does not appear to be involved directly in cell wall synthesis. In boron-deficient plants, disorders such as corky stems, cracked stems, and hollow stems develop. Most anatomical disorders in plants are associated with abnormal cell walls. Physiological disorders are interpreted as resulting from cell wall damage. Dicotyledonous plants require more boron (about 30 mg/kg dry weight) than monocotyledons (3 to 5 mg/kg), possibly because of differences in lignification and complexation of boron in cell walls.

***RNA and sucrose synthesis***. Uracil is involved in the synthesis of RNA and sucrose. Boron has been associated with these processes.




***Carbohydrate transport***. Because of some of the symptoms that develop during boron deficiency, boron has perceived roles in carbohydrate transport. A direct connection between boron requirement and carbohydrate transport has not been demonstrated.

***Protein synthesis***. Any effect of boron on protein synthesis is indirect, possibly related to effects of boron on membrane structure and to the accumulation of soluble amino acids during protein deficiency.

***Tissue differentiation***. These effects are likely due to the actions of boron deficiency on cell wall formation.

***Phenol metabolism***. Under boron deficiency, phenols accumulate in plants possibly in response to impaired cell wall and lignin synthesis.

### Symptoms of Deficiencies

Boron deficiency is widespread. Boron is low in leached soils, and availability decreases with increasing soil alkalinity. The requirements of plants for boron vary widely with crops. Grasses such as wheat and corn have low requirements with about 6 to 10 mg B/kg leaf dry weight being adequate. Sugar beets on the other hand may need 100 mg B/kg leaf dry weight for optimum yield. Other crops have boron levels that are between these concentrations, with about 30 mg/kg being a common value.

Symptoms include discoloration or death of terminal leaves and buds, resetting, brittle stems, and a number of disorders associated with production of specific crops. Cracked stem of celery, crown rot of sugar beet, black neck and brown curd of cauliflower, internal cork of apple, tip burn, brown or black heart, and water soak of lettuce, and thick peel of citrus are disorders that appear commonly in crop production. Often these disorders develop because growers have not considered the boron requirements of different species and cultivars.

Varying with plant genotype (species and cultivars), the critical concentration of boron in leaves is 6 to 100 mg B/kg dry weight with grasses requiring between from 6 to 30 mg/kg and dicotyledons requiring from 30 to 100 mg/kg. Boron can be toxic to plants with overapplication from fertilizers or composts or from irrigation water with high boron (1 to 10 mg/L). Toxic concentrations vary with crops, but leaf concentrations of 200 to 300 mg B/kg dry weight and higher are likely to be toxic. Toxicity symptoms occur along margins of leaves with chlorosis, necrosis, drying, and flaking as boron is translocated to the leaf edges for elimination from the plant.

## Molybdenum

Molybdenum was discovered as an element in 1781 and was shown to be essential for plants by Arnon and Stout in 1939. The requirement of plants for molybdenum is low. Less than 1 mg Mo/kg dry weight (10 ppb to 1 ppm) is

required for plant growth. Plants may accumulate as much as 1,000 mg/kg without toxic effects. However, high concentrations in forage are not good for livestock health. In contrast to most micronutrients, the availability of molybdenum decreases in acidic soils (below pH 5.3). Molybdenum is absorbed as molybdate ($MoO_4^=$). It is suggested that in acid soils forms sparingly soluble polymers of molybdic acid or that molybdate is bound to sesquioxides in reactions similar to phosphorus fixation. Soils average about 2 mg/kg with a range of 0.1 to 3.5 mg/kg with 0.1 being enough. The concentration of $MoO_4^=$ in soil solution is $10^{-8}$M.

**Functions**

*Nitrate reductase.* Molybdenum is a cofactor in nitrate reductase. Without molybdenum, plants accumulate nitrate to toxic levels and appear to be nitrogen deficient. Plants supplied with ammonium nitrogen may not require molybdenum, but the toxicity of ammonium hinders the assessment of this requirement.

*Nitrogenase.* Nitrogenase is a key enzyme in nitrogen fixation. It consists of two iron-containing proteins, one of which is a FeMo protein, in nitrogen-fixing legumes and nonlegumes. Free-living, nitrogen-fixing organisms also have a requirement for molybdenum. Plants will grow on mineral nitrogen (nitrate in particular) and will have no molybdenum requirement.

*Other enzymes.* Xanthine oxidase, aldehyde oxidase, and sulfite oxidase are molybdenum-containing enzymes. These enzymes are important in plants exposed to biotic and abiotic stresses, such as pathogens, cold, drought, or sulfur dioxide.

**Deficiency symptoms**

Early symptoms resemble those of nitrogen deficiency, since nitrate is not reduced and metabolized. Deficiency symptoms show on the old and middle leaves. Molybdenum has moderate mobility. Young leaves may be spared but often show chlorosis. Molybdenum-deficient legumes appear to be nitrogen deficient. Accumulation of nitrate at leaf margins can lead to marginal necrosis and failure of leaves to expand properly.

Whiptail of cauliflower is a disorder that results from nitrate accumulation in leaves. This disorder was recognized on cauliflower grown in Long Island, New York, in the 1920s in land previously cropped to potatoes in acid soil (pH 5.3 to prevent scab disease). Liming of the land from pH 5.3 to 7.0 cured the disorder. This diagnosis was made before it was known that molybdenum was an essential element. Whiptail disease is considered to be caused by accumulation of

nitrate in leaf blades. Nitrate may accumulate to 15% of leaf dry weight during molybdenum deficiency. Marginal burn of leaves from nitrate accumulation also occurs with nitrogen-deficient plants, such as cucumber.

Critical levels are between 0.1 and 1.0 mg Mo/kg of dry weight of leaves. Molybdenum is not highly toxic to plants and may accumulate to 1000 mg/kg without toxicity, although high concentrations of molybdenum are not healthful in forages that livestock, particularly ruminants, consume, giving a disease called molybdenosis. Conditions of high molybdenum in plants usually occur in soils with naturally high concentrations of molybdenum. Fertilization is by molybdenum salts or oxides ($MoO_3$) that may be applied to the soil or as pelleting of seeds (100g Mo/ha) to correct deficiencies. Seeds from molybdenum-sufficient plants may contain enough molybdenum for crop nutrition.

## Chlorine

Chlorine was discovered as an element in 1774 and was shown to be essential for plants in 1954 by Broyer.

The concentration of chlorine in leaves ranges from 2,000 to 20,000 mg/kg (0.2 to 2%) dry weight, since $Cl^-$ is prevalent in the environment, especially in fertilized fields (KCl in commercial fertilizers). The critical concentration of $Cl^-$ is low but could be of the order of 300 to 1000 mg/kg in some plants. Deficiency has been demonstrated only in situations in which water, air, and chemicals are chlorine free; hence, the critical concentration must be very low. Demonstrating the essentiality of chlorine was challenging since chlorine is present widely and abundantly in the environment. Special precautions were necessary to remove chlorine from chemicals, water, and air to induce deficiency symptoms. Deficiency is difficult to demonstrate in large seeded crops because of the $Cl^-$ in the seeds. Chlorine deficiency in nature is rare. A field of wheat fertilized with potassium sulfate showed symptoms that were corrected with applications of potassium chloride.

### Symptoms of Deficiency

In controlled experiments, wilting and browning of foliage and restricted root elongation, but proliferation of lateral roots developed.

### Functions

The Hill Reation, which is the evolution of oxygen by illuminated chloroplasts, requires $Cl^-$, hence, giving support to the role of chlorine in photosynthesis.

**$H_20$ light, $Cl^-$, isolated chloroplasts $O_2$**

**Hill Reaction**

Chloride is much higher in chloroplasts than in rest of cell, being of the order of 3,500 mg/kg compared to 300 mg/kg overall. Bromide may substitute for $Cl^-$, but $I^-$ does not. Sugar beets may have a high requirement for $Cl^-$ as NaCl enhances yields of sugar beets, but this effect may be due to the $Na^+$ or to the $Cl^-$. In some plants, such as onion, $Cl^-$ may have a role in stomatal opening and closing.

## Nickel

Nickel was discovered as an element in 1751 and was shown to be essential for plants in 1987 by Brown. To demonstrate the essentiality, plants needed to be grown in a nickel-free environment for two generations to eliminate nickel from the seeds. The nickel-depleted seeds did not germinate, but germination was restored with the addition of nickel. Vanadium did not substitute for nickel.

Nickel is required for urease activity: **~~Urea~~ → $NH_3 + CO_2$**

Nickel is part of the structure of the enzyme. Urea can be absorbed from nutrient solutions; hence nickel is required for the assimilation of urea. Also, urea is made in plants by the urea cycle and other metabolic precursor (ureide, purine, and amine catalyses). Accumulation of urea in plants is toxic.

The critical concentration of nickel is about 0.05 mg/kg dry weight of leaves. No field-grown crop has been recorded with less than 0.2 mg Ni/kg dry weight.

## Beneficial Elements

Beneficial elements are chemical elements that improve the growth of some plants or that are essential for the growth of some plants. Plants that benefit from these elements will complete their life cycles without the elements, and no symptoms of deficiency develop in their absence. A number of elements are considered beneficial. Improvements in analytical chemistry and in purifying solutions may lead to some beneficial elements being classes as essential. Nickel was once considered as beneficial.

***Cobalt***. Cobalt is required for nitrogen fixation as Vitamin $B_{12}$. It is needed for formation of leghemoglobin and nodule growth. Leghemoglobin carries oxygen to the bacteroids of the nodule. Elemental oxygen is toxic to nitrogen fixation. Cobalt may have other roles in the growth of bacteroids in the nodule, as it is associated with methionine synthesis. The critical concentration of cobalt in

nodules is between 20 and 170 mg/kg fresh weight. In large-seeded legumes, the seeds may carry enough cobalt to meet the nitrogen-fixing needs of the plants. Some authors list cobalt as an essential element. But, cobalt is not essential since nitrogen-fixing plants will grow well on mineral nitrogen.

***Sodium***. Some plants benefit from sodium, but on the other hand, many plants have a toxic response to sodium. Sodium does not appear to be a macronutrient for any species. Sodium might substitute partially for $K^+$ in plants, but this substitution is not complete. The halophyte, *Atriplex* spp., requires $Na^+$ at low concentrations (0.02 mM). Below 0.00001 mM, atriplex became chlorotic and did not grow. At supplies higher than 0.02 mM, sodium seemed to function as a substitute for potassium.

Sodium may have a function in C4 plants in the shuttling of metabolites from the mesophyll to bundle sheath and in the conversion of pyruvate to phosphoenol pyruvate.

Plants are classified into four groups based on the substitution of $Na^+$ for $K^+$. Group A includes plants, such as sugar beets, for which considerable substitution may occur with stimulation of growth by $Na^+$. This stimulation in growth cannot be achieved by increasing the potassium supply. These plants are considered as natrophilic. In Group B plants, growth responses to $Na^+$ are not as distinct as with Group A plants, and the substitution for $K^+$ is small. In Group C plants, substitution is very limited, and no growth response to $Na^+$ occurs. In Group D plants, such as corn, bean, and soybean) no substitution occurs, and these plants may be considered as natrophobic.

The growth response to sodium depends on the transport of $Na^+$ to shoots. In natrophilic species, $Na^+$ is transported readily to shoots. Sugar beets may accumulate 6% sodium in leaves. The growth increases may be related to leaf expansion with increased succulence and thickening. Natrophobic plants have limited transport of sodium to shoots with leaf concentrations being about 0.01% of the dry weight. Group D plants also may have limited absorption of $Na^+$. The majority of agriculturally important crops is natrophobic.

***Silicon***. All plants growing in soil accumulate some silicon. Silicon is absorbed as uncharged silicic acid ($HSiO_3$ sometimes written as $Si(OH_4)$). The concentration in leaves ranges from 1 to 100 mg Si/kg dry weight, varying greatly with species. Gymnosperms and angiosperms accumulate little silicon whereas species such as horsetails are high in silicon. Rice is an exception in flowering plants and may have about 40 mg Si/kg dry weight in leaves. Silicon-deficient rice shows symptoms of suppressed growth and grain production and symptoms of wilting and necrosis, but silicon is not required for the life cycle of rice. Silicon helps to

keep some plants erect and enhances the resistance of plants to fungal and bacterial diseases and to some insects. The effects may be symplastic (biochemical responses) or apoplastic (barrier to infestation). Some grasses form phytolyths, which are deposits of silicon dioxide and which may be abrasive to rumens and to hands.

***Selenium***. Selenium is nonessential and toxic to most plants. The chemistry of selenium is similar to that of sulfur. Selenate is a chemical analog of sulfate and competes with sulfate for uptake by plant roots. Plants differ greatly in their capacities to accumulate selenium and to tolerate selenium in the soil. Astragalus (locoweed, tumbleweed) is a legume that accumulates as much as 4,000 mg Si/kg in leaves, whereas corn accumulates only 10 mg/kg and sunflower accumulates only 2 mg/kg. The concentration of selenium in plants is associated with the capacity of plants to accumulate sulfate with sulfate accumulators being selenium accumulators. Selenium is essential for mammals but also is highly toxic. Animals require 0.1 to 0.3 mg Se/kg dry weight in diets. Some foods are fortified with selenium to ensure adequate intake of selenium. Selenium toxicity to livestock is called loco disease, blind staggers, or alkali disease. This accumulation to toxic concentrations occurs in selenium-rich soils. Selenium accumulation in some plants such as locoweed may give some protection against herbivory.

***Aluminum***. Aluminum is an abundant element in the crust of the earth. Interest in aluminum in plants is stimulated by its toxicity and its accumulation to high concentrations in some plants. Aluminum at 0.07 to 0.18 mM stimulates growth of corn and sugar beets. Concentrations of 1 mM or higher stimulate growth of tea. Tea leaves accumulate aluminum to high concentrations (1 to 3%), but no metabolic function is known. Aluminum in leaves may help to alleviate the toxicities from other elements, such as phosphorus, copper, zinc, and iron. The toxic effects of aluminum are much more common than the beneficial effects.

***Other Beneficial Elements***. Iodine and vanadium are required by certain marine and freshwater algae, but beneficial effects on higher plants are rare and unclear. Rare earth elements such as cerium and lanthanum may enhance crop growth. Their use in fertilizers at micronutrient levels is substantial in China. The accumulation of an element such as cadmium, chromium, lead, and mercury in plants is no evidence of essentiality, and these elements have no apparent role in plant nutrition.

# Chapter 7. Accumulation of Ions by Plants

## Ion Uptake

### Sites of Uptake

*Roots*. Salt absorption is most rapid in region of root where growth by cell expansion and vacuolation is dominant, not in zone of cell division where cells are not vacuolated. Old roots absorb if ions can reach cells that absorb nutrients. The coating of suberin on root surfaces limits the absorption by old roots. Peak region is 0.5 to 2cm behind the root tip. Root hairs are probably not any more active in absorbing ions than other root cells exposed to the same medium. Root hairs are single-celled cells in the root epidermis. Root hairs, however, increase the surface area exposed to the soil solution. Root hairs have important function is increasing the absorption of water.

Roots with large surface area are effective in absorbing nutrients especially phosphorus. Efficiency of nutrition is related to root morphology with fibrous roots being better than taproots. Selection of crops with fibrous roots is a factor in developing crops with good absorption of nutrients from soil and nutrient-use efficiency.

***Leaves***. Salts enter through stomates, cuticles, or both structures. Absorption of ions by cells inside leaves is similar to that of root cells. Solute that is transported up the xylem is unloaded into the free space of the mesophyll and must be absorbed again to be used by the leaves.

### Mechanisms of Uptake (Absorption)

No single mechanism is responsible for all cases of salt absorption. Absorption is said to occur by passive processes and by active processes. *Passive processes* involve no metabolic activity directly in absorption of ions. No metabolically generated energy is not to be mistaken for no energy requirement. Passive processes are physical processes driven by kinetic energy. For *active processes*, energy of metabolism (ATP) is involved in accumulation of ions. Ions are accumulated against an electrochemical gradient (sometimes understood somewhat inaccurately as a concentration gradient).

### Passive mechanisms

***Diffusion***. Simple diffusion occurs when two liquids, gases, solutions, or colloidal suspensions are mixed in a heterogeneous manner. Diffusion occurs until uniform distribution of the materials is reached. No selectivity operates in diffusion. Net movement ceases at equilibrium concentrations of the materials in the media.

The cell wall is no barrier to diffusion, but the plasmalemma or cell membrane is not passed by simple diffusion. The space of the root subject to diffusion is extracytoplasmic, that is outside the plasmalemma. The volume of the root entered by diffusion is called *free space*, *apparent free space*, *water free space*, or *outer space* or more scientifically as the *apoplasm*. The volume of the free space is about 8% to 11% of the volume of the root. The volume inside cells is referred to collectively as the *symplasm*.

The importance of diffusion in ion absorption is that it brings the ions into contact with cortical cells that can do the absorbing into the symplasm. Absorption is not limited to that by root hairs on epidermal cells. Diffusion into free space brings cortical cells into contact with the nutrient solution

Rate of diffusion is governed by concentration difference between two points and path lengths for diffusion.

Rate of diffusion, $\mathbf{Q = D\ (C_{ext} - C_{int})/\ path\ length}$

For plants and this equation, **D** is the diffusion constant that varies with the solute (ion); $\mathbf{C_{ext}}$ is the concentration of solute in the external solution; $\mathbf{C_{int}}$ is the concentration inside the root; and path length is the distance between $\mathbf{C_{ext}}$ and $\mathbf{C_{int}}$. Free space (apoplasm) of root is an extension of the external solution into the epidermis and cortex up to the endodermis.

Diffusion into cells is limited by plasmalemma, and diffusion into stele is limited by casparian strip of the endodermis.

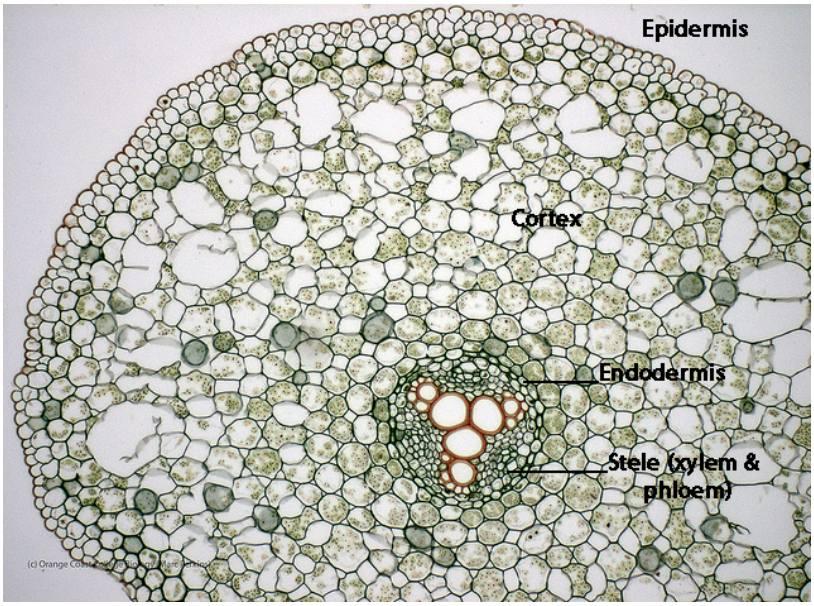


Figure 7.1 Cross section of root showing the epidermis, cortex, endodermis, and stele.

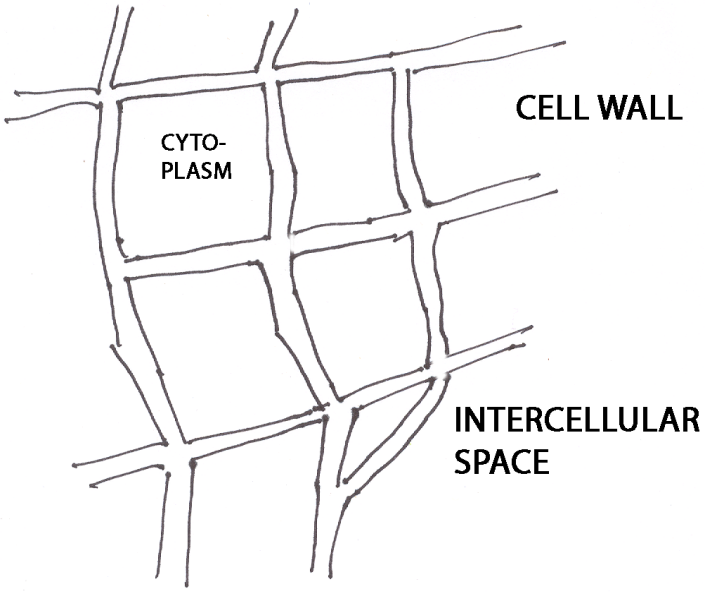



Figure 7.2 Drawing of cross section of cortex showing cell walls and intercellular space, which are entered freely by diffusion.

***Facilitated diffusion***. Some material, perhaps a protein or peptide, in membranes facilitates transport through the cell membrane. These compounds are macrocyclic antibiotics, which are low molecular weight polypeptides. They possess specific binding sites and lipiphilic properties allowing them to act as carriers. Cyclic antibiotics such as valinomyocin, nonactin, gramicidin, and enniatin B can bring about selective transport of inorganic ions through biological membranes. These antibiotics have been isolated from bacteria and fungi. They appear to be selective for $K^+$. For example, valinomycin is highly selective for $K^+$ over $Na^+$ within the cell membrane. This discrimination is due to differences in the ionic radius of the two ions with $K^+$ fitting into the geometry of the transporter and $Na^+$ being too large. Valinomycin facilitates the movement of potassium ions through lipid membranes down an electrochemical potential gradient. Enniatin B acts as a charged disk with a lipophilic exterior. The nonactin $K^+$complex is described as a ball with a lipophilic exterior and with the $K^+$ at the center of the ball.

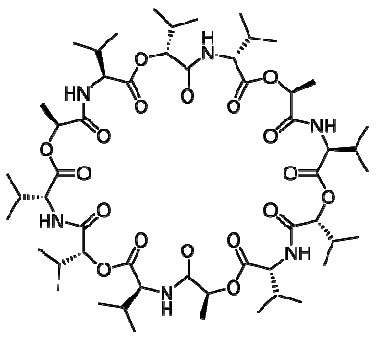
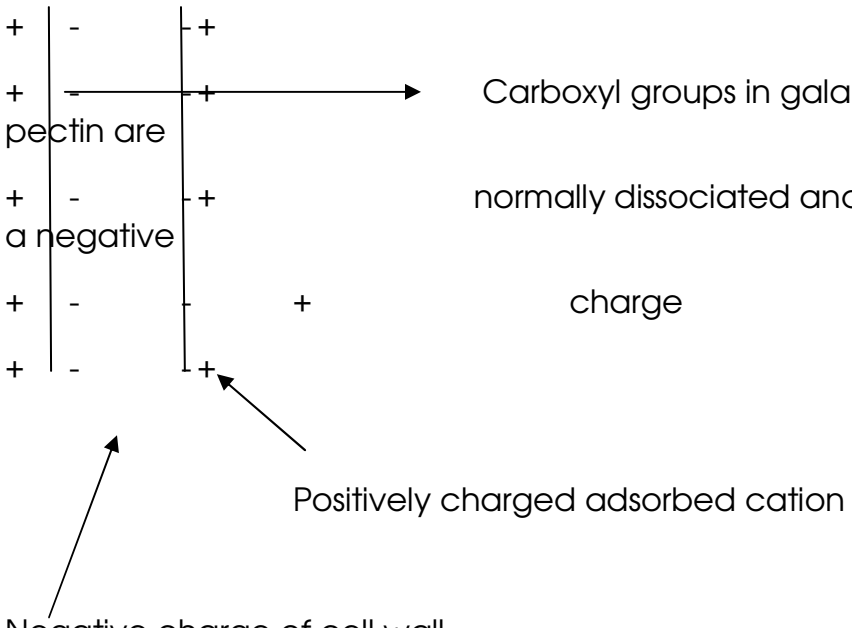


Figure 7.3 Cyclic structure of valinomycin, a cyclic antibiotic that may function in facilitated diffusion across cell membranes.

***Passive absorption by exchange***. Some of the ions that diffuse into the free space are held by cation exchange. Plant tissues are predominantly negatively charged.




An electrical double layer, like that around clay particles exists.

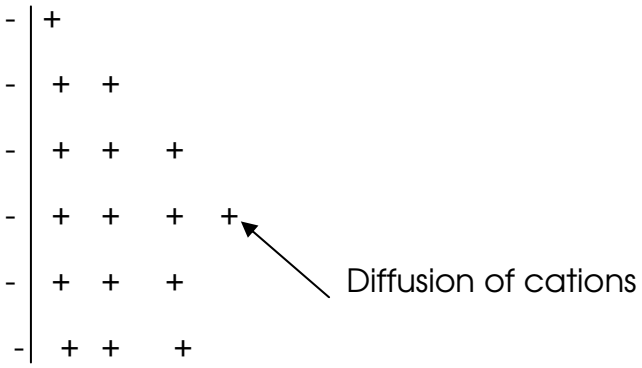


Figure 7.4 Proposed electrical double layer at cell wall, creating sites of adsorption of cations

Attraction for adsorption is by columbic force or charge attractions. Force of adsroption varies with charge of ions and distance between charges by Coulomb's Law.

$$\textbf{Force} = (q^-)(q^+)/r^2$$

Force is measured in dynes. $\mathbf{q^-}$ is the charge of the negative ion; $\mathbf{q^+}$ is the charge of the cation; $\mathbf{r^2}$ (cm) is the distance between centers of the two charges. Little or no selectivity exists although all ions are not held by equal strengths. Hence, multivalent cations will be held with greater force than univalent cations. Large ions will be held with less force than small ions of the same charge. For roots in soils, the predominant cations on exchange sites are $Ca^{++}$, $Mg^{++}$, $Mn^{++}$, $Fe^{++}$, $Al^{+++}$, and $H^+$. Monovalent cations do not seem to be held ($K^+$, $Na^+$, $NH_4^+$). But differences in forces of adsorption are too small to give much selectivity ions in exchange sites. Ions can be replaced easily by other ions depending on their size, charge, and abundance in solution. Binding of ions follows a Donnan Equilibrium (equilibrium between diffusible ions and a nondiffusible ion). The cell wall is the nondiffusible ion, and the cations in the soil solution are the diffusible ions.

The cation exchange capacity of roots may have an effect on plant composition by leading to a greater adsorption of ions to roots. The CEC of dicot roots is higher than that of monocot roots, for example, the CEC of wheat roots is 23 meq/100 g dry weight; for corn roots, CEC is 29; for bean roots, CEC is 54, and for tomato roots CEC is 62. Concentration of ions in exchange zone may exceed that of the surrounding ions in the free space or in the nutrient situation.

***Ion absorption by direct exchange***. This process is absorption without intervention of the soil solution. This concept states that ions are exchanged directly from soil colloids to the surface of roots. However, dimensions between colloids and roots are too great for direct exchange to occur. Absorption might occur from swarms of ions in solution around colloids (double layer). It is suggested that this mechanism might be important in absorption of difficulty soluble materials (such as, $Fe^{+++}$).

***Donnan equilibrium (Passive Transport across a membrane)***. The Donnan Equilibrium takes into account the situation in which diffusible ions are on one side of a membrane and non-diffusible ions are on the other side. In the case of plants, the non-diffusible ions are inside the cells, that is, inside the cell membrane, which separates the cytoplasm from the external solution.

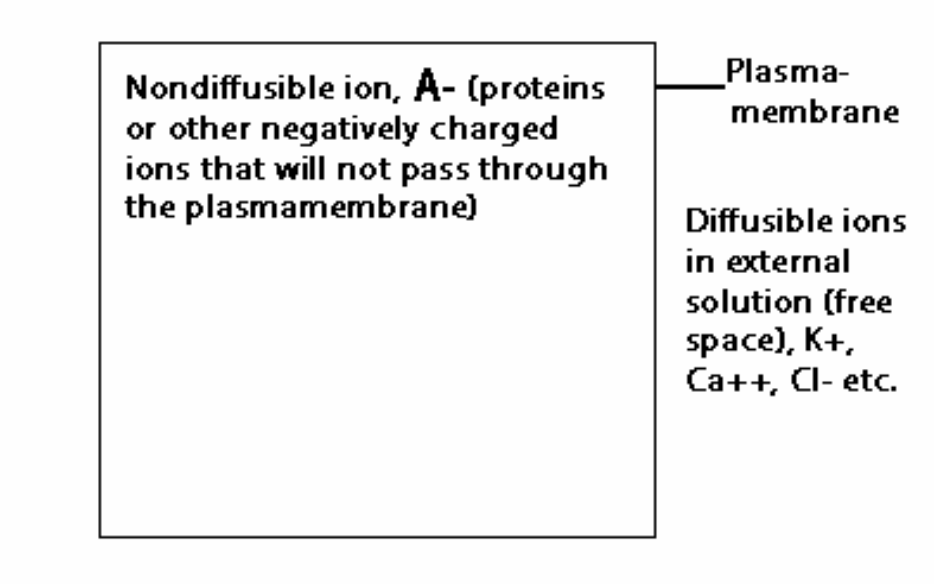



Figure 7.5 Illustration of a cell showing the nondiffusible ion inside the plasmamembrane and diffusible ions in the free space of a root

The Donnan Equilibrium establishes an electromotive force that will lead to an accumulation of cations. External $K^+$ or other cations move into cells in response to electrochemical gradients generated by the nondiffusible ions, which are too large to pass the membranes. Some $Cl^-$ also crosses the membrane, but transport is less than $K^+$, giving an equilibrium as follows:

**$(K^+)_i = (Cl^-)_I + (A^-)_i$**

Inside, non-diffusible

The inside of cells is typically 10 to 150mV more negative in potential than the external solution, represented by the following equation.

$$\Delta\epsilon = \epsilon_i - \epsilon_o = -10 \text{ to } -150\ mV$$

$\Delta\epsilon$ is the difference in potential between $\epsilon i$ (potential inside cell) and $\epsilon o$ (potential outside cell). Potential varies by the Nernst Equation.

**$\Delta\epsilon = \epsilon i - \epsilon o = -(RT/ZF)\ \ln\ ((M^+)i/(M^+)o)$**

**$= (RT/ZF)\ \ln\ ((M^+)o/(M^+)i)$**

**$= (0.059/Z)\ \log\ ((M^+)o/(M^+)i)$**

where **R** is the gas constant; **T** is the absolute temperature (degrees Kelvin); **Z** is the charge of the ion; **F** is the Faraday constant; **ln** is the natural logarithm; and **(M)i** and **(M)o** are the concentrations of the specific ion inside and outside the

cell. Equilibrium is reached when to concentrations of the ion inside the cells satisfy the Nernst equation

$(M^+)i/(M^+)o$ is a ratio that balances the electropotential. If this ratio is the same or less than that needed to balance the electopotential, cation accumulation is said to be passive. That is to say, the entrance of ions into cells is in response to energy set up by electropotential. If the ratio exceeds that needed to balance the electropotential, absorption is said to be active.

In nature, equilibrium concentrations as predicted by the Nernst equation are not reached (except for $K^+$). Research suggests that predicted $Na^+$, $Ca^{++}$, and $Mg^{++}$ concentrations do not exceed equilibrium levels in cells. Major anions ($NO_3^-$, $Cl^-$, $SO_4^=$) appear to be accumulated actively.

**Active Transport**

Several phenomena in plants provide evidence for active uptake of ions into cells. The processes include.

***Accumulation against an electropotential gradient***. This phenomenon refers to the accumulation of ions in cells beyond the concentration predicted by the Nernst Equation. This transport requires energy to drive the accumulation to a level higher than that predicted by the energy of the electropotential gradient. In conversation, it might be acceptable to refer to this accumulation as against a concentration gradient, meaning that higher concentrations accumulate inside a cell than exist in the external solution. A physical comparison is pumping of water uphill or lifting of an object to a higher plane. Energy must be imparted into the water or object to raise the material to a higher energy level.

***Failure of accumulation to reach equilibrium***. With active absorption, accumulation continues to increase without reaching equilibrium, since metabolic energy continues to drive accumulation (Figure 7.6). With passive accumulation, only so much energy is available to drive the process, and when that energy is expended accumulation ceases at an equilibrium level.

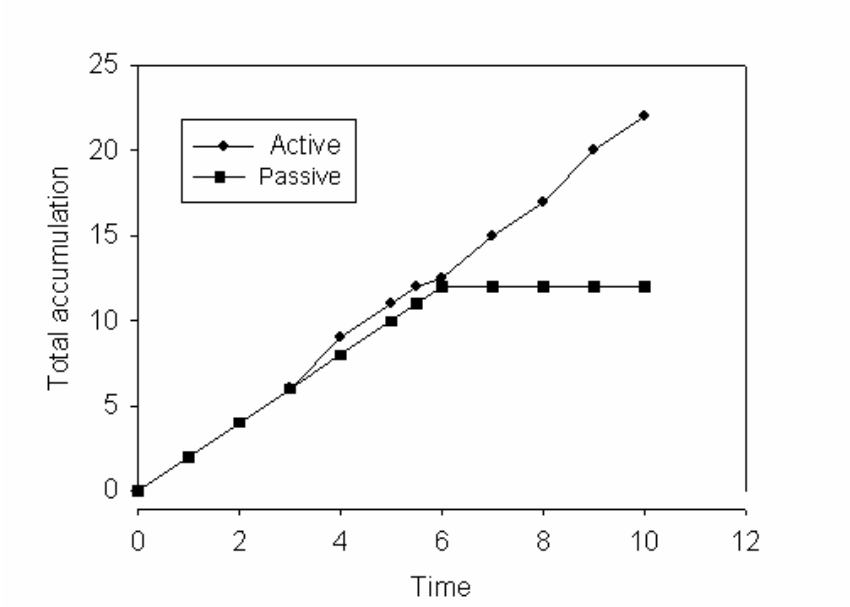



Figure 7.6 Accumulation on ions by active or passive processes as a function of time. Accumulation and time are in arbitrary units.

***Requirement for $O_2$***. Oxygen is needed for uptake and retention of ions. This requirement notes that respiration is needed for accumulation of ions. Respiration gives the energy to drive the process.

***Inhibition by metabolic poisons***. Fluoride, which is an inhibitor of aconitase, in the Krebs Cycle, inhibits ion uptake. Dinitrophenol, which is an uncoupler of oxidative phosphorylation (ATP production by respiration), inhibits ion uptake. Chloramphenicol, which is an inhibitor of protein synthesis, inhibits ion uptake. These materials inhibit energy metabolism and protein synthesis, suggesting that ATP synthesis and protein synthesis are vital for ion accumulation.

***Effect of temperature***. $Q_{10}$ refers to the increase in rate of a reaction with a 10 degree Centigrade rise in temperature. The $Q_{10}$ for ion uptake is 2 or greater, indicating that uptake is driven by chemical reactions, hence, by metabolic processes in plants. The $Q_{10}$ of physical processes is 1.1 to 1.2.

***Effects of sources of energy***. Applying light or supplying sugar to plants absorbing ions increases the rate of uptake. The sugar gives a direct source of substrate for metabolism to produce ATP, and the light by photosynthesis provides the sugars for metabolism.

***Selectivity of uptake***. All ions are not accumulated equally. Selectivity is exhibited in ion absorption so that some are absorbed preferentially over others.

***Nonexchangeability of uptake***. Once an ion enters a cell, the cell retains the ion as long as the cell is alive. Ions do not leak from cells. If cells are poisoned by metabolic inhibitors, the integrity of membranes is destroyed, and materials will lead from cells. Generally, the movement of materials from inside to outside the cells is rare and must be mediated by an active process.

**Processes of Active Uptake**

***Salt respiration (anion respiration)***. Lundegarth and Burstrom observed in about 1933 that respiration of roots increased if the roots were placed in a salt solution. This process was called salt respiration. Lundegarth and Burstrom suggested that anion transport was mediated by a cytochrome system and that cation uptake was a passive, concomitant process. Problems with the theory were that cation accumulation stimulated respiration, that sites of accumulation were not limited to mitochondria (the site of respiration), that stoichiometry of $O_2$ consumption was not followed (Theory predicted 4 ions/$O_2$ but observed 16 was observed), and that dinitrophenol (DNP) as an uncoupler of ATP synthesis for electron transport did not stimulate uptake (but actually decreased it, indicating the need for ATP).

This theory was important in that it demonstrated a link between respiration and salt (ion) absorption. The use of pop-up fertilizers was developed from this theory. The placement of salts such as KCl near germinating seeds was practiced to increase the rate of respiration of seeds and to accelerate their emergence from the soil.

***Carrier concept***. In this theory, ions are transported across membranes by carriers. A specific subunit of membrane or a carrier (identified as a protein) is needed for each ion, although some ions have common carriers. The subunits bind the ions at the external face to form a carrier-ion complex. The carrier traverses the membrane and brings the ion in contact with the inside surface, where the ion is released. Membrane is not permeable to free ions but is permeable to the complex. Ions cannot diffuse out of a cell because of impermeability of the membrane and because configuration of carrier at the inside of the membrane is not right.

Membranes are composed lipids in which proteins are associated in or on the lipid matrix. The proteins in the membranes are the transporters or carriers for ions to cross the membranes. The carriers are considered to be of three types: (1) Active transporters (pumps) which are coupled directly to metabolism of an energy substrate such as with ATP hydrolysis; (2) active transporters that harness the energy of an electrochemical gradient of $H^+$; and (3) passive transporters which are driven by the electropotential gradient.

**Kinetics of Ion Absorption**

The rate of ion or solute uptake by plant cells and tissues rises and saturates with increasing ion or solute concentration in the external solution. It was suggested by Emanuel Epstein that this relationship follows enzyme kinetics (Figure 7.2).

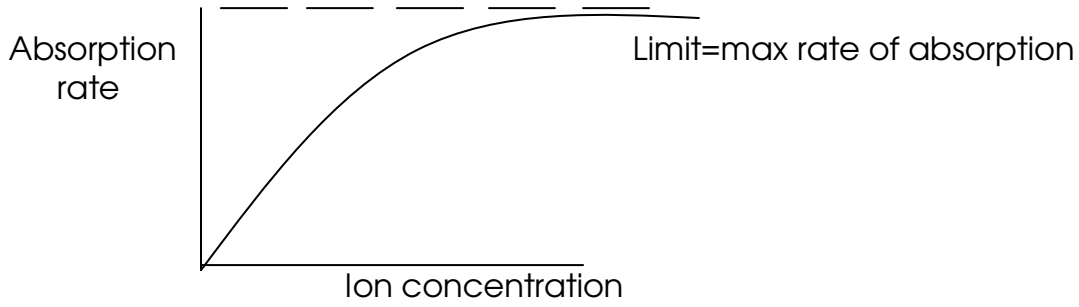



Figure 7.2 Rate of ion absorption as a function of ion concentration in the external solution

In this analogy, the carrier (transporter) is a protein that catalyzes the movement of substrate (ions, solute) from outside the cell membrane into the cell.

**Enzymatic reactions** proceed as follows:

$$E + S \underset{}{\overset{k_1}{\rightleftharpoons}} ES \rightarrow E + P$$

where **E** is the enzyme; **S** is the substrate; **ES** is the enzyme-substrate complex; **P** is the product, and $k_1$ is the rate constant.

The analogous presentation for ion absorption is:

$$C + I_{out} \overset{k_1}{\longrightarrow} CI \longrightarrow C + I_{inside}$$

where C is the carrier; $I_{out}$ is the ion outside the cell; CI is the carrier-ion complex; $I_{inside}$ is the ion inside the cell, and $k_1$ is the affinity of the carrier for the ion.

Accumulation of ions is related to:

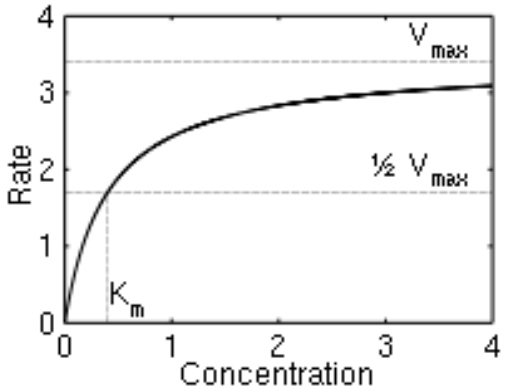
1. Amount of carrier (transporter)
2. Concentration of Ion (substrate) up to 0.001 M
3. Rate constant (affinity of carrier for ion)

At low concentrations of external ion absorptions increases rapidly but as concentrations increase the increase in rate of absorption decreases until at high concentration absorptions becomes essentially constant. Rate of absorption asymptotically reaches a maximum in a hyperbolic function. In enzyme kinetics, this function is known as the Michaelis-Menten equation.

$$v = \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m + [S]}.$$

The equation describes the rate, $v$, of enzymatic reaction, **dP/dt**, (formation of product as a function of time. The rate of reaction,$v$, is related to concentration of substrate **(S)** by the equation. $\mathbf{V_{max}}$ is the maximum rate achieved by the reaction at saturating substrate concentrations. The Michaelis constant is **Km**, which is a dissociation constant for the enzyme-substrate complex.

Experiments are run to collect much data to determine the rates of reaction at many different concentrations of substrates to obtain the plot of reaction rate as a function of substrate concentration (Figure 7.4).




Rate of transport (or rate of enzymatic reaction) is limited by $V_{max}$ and by the degree of saturation of the carrier (enzyme) at a given time. Many enzymatic reactions are transfer reactions (phosphate, amino, and keto groups) and carrier may function similarly to their enzymes.

For ion uptake, $\mathbf{K_m}$ is the dissociation constant for the carrier-ion complex.

| | | | | |
|---|---|---|---|---|
| CI | = | C | + | I |
| Carrier ion | | Carrier | | Ion |

The $\mathbf{K_m}$ can be presented as follows:

$$K_m = \frac{[C][I]}{[CI]}$$

Further development of the equation for ion absorption, including the factors $\mathbf{V_{max}}$ ($V_{max}$ = maximum rate of absorption) and □ (□ is the degree of saturation of the carrier), leads to the equation in which transport is a factor of $V_{max}$ and □:

v = $V_{max}$ x □

□ is presented as a Langmuir adsorption equation.

$$\theta = \frac{[I]_o}{Km + [I]_o}$$

where $\mathbf{I_o}$ is the concentration of ions outside the plasmalemma.

The product of $\mathbf{V_{max}}$ x □, yields the equation v= $\mathbf{V_{max}}$ ($\mathbf{I_o}$)/ $\mathbf{K_m}$ + $\mathbf{I_o}$ so that

$$transport = V = \theta \cdot V_{max} = \frac{V_{max} \cdot [I]}{K_m + [I]}$$

$\mathbf{V_{max}}$ is estimated experimentally by direct plot of data (Figure 7.3).

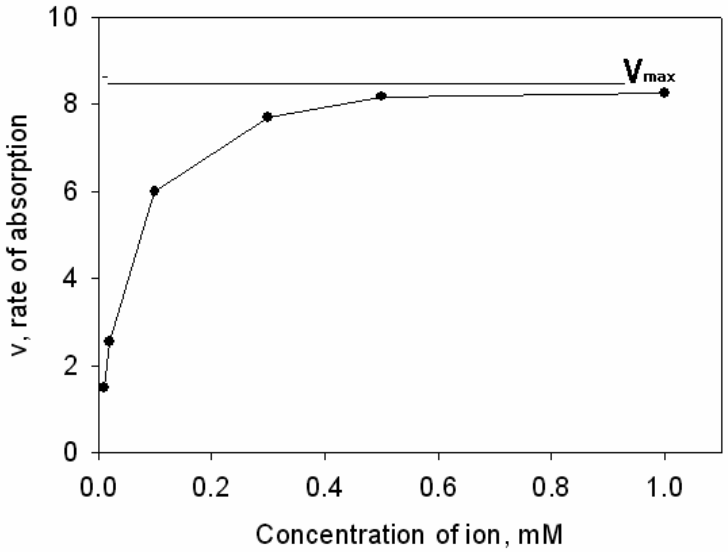



Figure 7.3 Plot of rate of ion absorption by plant roots as a function of ion concentration in the external solution. Plot notes $\mathbf{V_{max}}$, which is the maximum rate of absorption of the ion

$\mathbf{V_{max}}$ is estimated from what appears to be the maximum rate of ion absorption in the plot. If a competing ion is present, the rate of absorption

may be slowed, but the maximum rate will be unaltered, providing nothing has happened to change the amount of carrier (Figure 7.4).

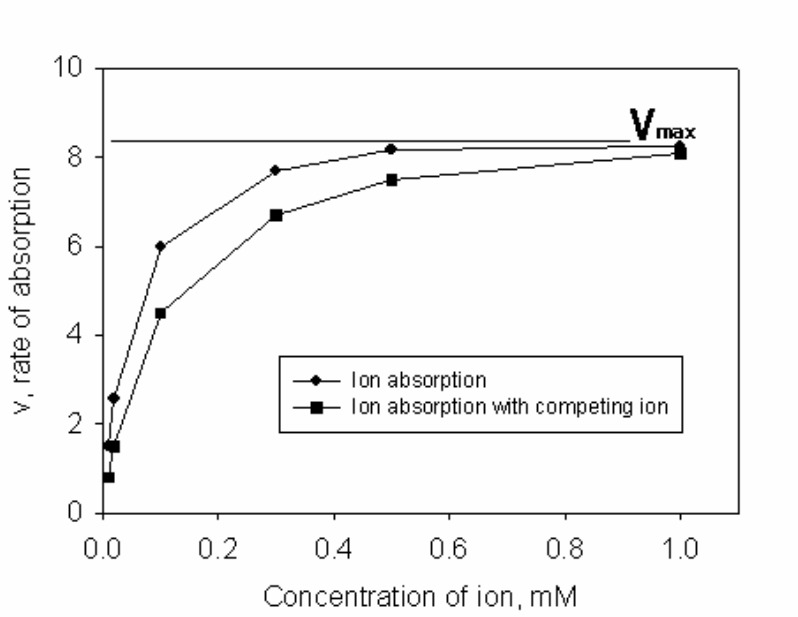



Figure 7.4 Rate of ion absorption by roots as a function of concentration of the ion in the external solution and in the presence of a competing ion

The affinity constant $\mathbf{K_m}$ estimated by determining ½ $\mathbf{V_{max}}$ , drawing a line to intersect the plots of absorption and then drawing a line to the x-axis to determine the $\mathbf{K_m}$ (Figure 7.5).

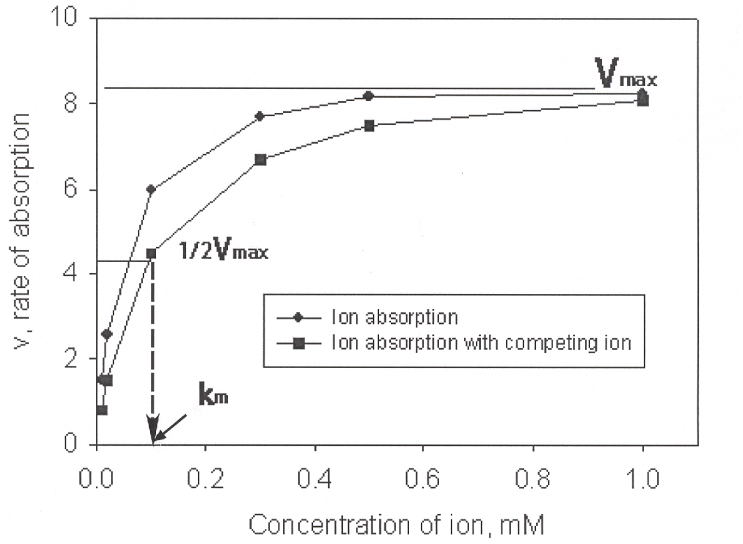



Figure 7.5 Plot of rate of ion absorption as a function of ion concentration in the external solution and showing determination of $k_m$ from the plot. Only one $k_m$ is illustrated.

These relationships can be shown algebraically. For example to show that when $v = \frac{1}{2} V_{max}$ , $K_m = (I)$ externally:

**$v = V_{max}(I)/k_m + (I)$; when $v = \frac{1}{2} V_{max}$ , $\frac{1}{2} V_{max} = V_{max}(I)/k_m + (I)$;**

**then, $(\frac{1}{2} V_{max})( k_m + (I)); = V_{max}(I)$; proceeding by dividing by $\frac{1}{2} V_{max}$, $k_m + (I) = 2(I)$,**

**and hence, $k_m=(I)$**

Direct plots given only estimates of the maximum rates and affinity constants. Many determinations must be made to obtain sufficient data the proper plots. Errors made in determination of the maximum rate will impart errors to the determination of the affinity constants. Reciprocal plots are used to facilitate determination of the parameters of maximum rate and affinities (Figure 7.6). In reciprocal plots, 1/rate (1/v) is plotted against 1/ion concentration in the external solution (1/(I)). This plot gives a straight line that intersects the x-axis at $-1/k_m$ and intersects the y-axis at $1/V_{max}$. This plot gives more precision in estimating the equilibrium constant and the maximum rate of absorption than the direct plot. The slope of the line is $k_m/V_{max}$. This plot is called a Lineweaver-Burk plot.

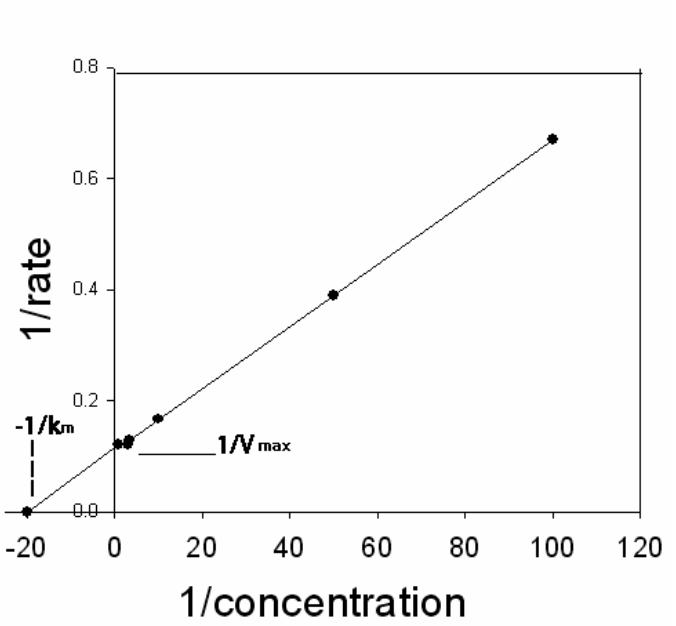



Figure 7.6 Reciprocal plot of rate (1/v) as a function of reciprocal of concentration of ion (1/(I)) in the external solution

This relationship can be shown algebraically from the Michaelis-Menten equation:

**$v = V_{max}(I)/k_m + (I)$**; the reciprocal is **$1/v = k_m + (I)/V_{max}(I)$**;

which can be written as **$1/v = k_m/V_{max}(I) + (I)/V_{max}(I)$**;

or **$1/v = (k_m/V_{max})(1/(I)) + 1/V_{max}$**;

his equation has the format of y = mx + b, which is the equation for a straight line; m = $k_m/V_{max}$ and b = $1/V_{max}$, the intercept on the y-axis.

Use of the Lineweaver-Burk plots enables determination of ions transported by a common carrier. Ions competing for the same carrier will have a common $V_{max}$, but the affinity ($k_m$ ) of the carrier for an ion will be changed by the competing ion (Figure 7.7).

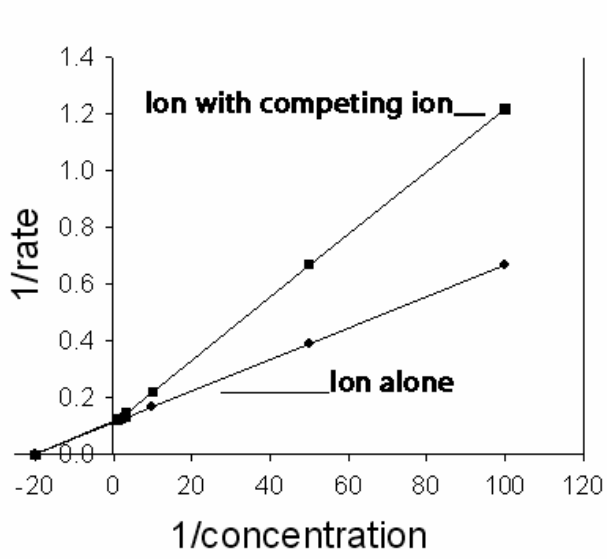



Figure 7.7 Reciprocal plots of absorption of an ion alone and in the presence of a competing ion

The following is a list of ions that have been shown to have common carriers:

| | |
|---|---|
| $K^+$, $Rb^+$, $Cs^+$ | $Cu^{++}$, $Zn^{++}$ |
| $Br^-$ , $Cl^-$ | $H^+$, $Zn^{++}$ |
| $SO_4^=$ , $SeO_4^=$ | $H^+$, $Cu^{++}$ |

$PO_4^{3-}$ , $AsO_4^{3-}$ $Fe^{+++,}$ $Zn^{++}$

$Na^+$ , $Li^+$ $Fe^{+++}$, $Cu^{++}$

$Ca^{++}$, $Ba^{++}$, $Sr^{++}$

The concept of common carriers and specific competition applies generally in dilute solutions with one of the ions is under 0.001M. If both ions are in concentration >0.001M another system applies. Ion absorption follows saturation kinetics as described by the Michaelis-Menten equation up to about 0.001M, but above about 0.001M, complex kinetics, which have not been modeled apply. The system operating below 0.001 M is called *Mechanism 1* and the system operating above 0.001M is called *Mechanism 2*. This system has been shown to occur for every nutrient and some other ions for which the absorption kinetics has been studied, including $K^+$, $Rb^+$, $Cs^+$, $Na^+$, $NH_4^+$, $Ca^{++}$, $Sr^{++}$, $Mg^{++}$, $Fe^{++}$, $Cl^-$, $I^-$, $Br^-$, $H_2PO_4^-$, $SO_4^=$, and $H_3BO_3$.

The following table shows characteristics of Mechanism 1 and Mechanism 2.

Table 7.1 Characteristics of Mechanism 1 and Mechanism2

| Characteristics of Systems | | |
|---|---|---|
| | Mechanism 1 | Mechanism 2 |
| Operating concentration | <0.001M | > 0.001M |
| Specificity | High: carrier has high affinity for specific ion. | Low: no specificity apparent. |
| Competition | Specific; e.g., $K^+$ and $Na^+$ do not compete. | General; $K^+$ and $Na^+$ compete. |
| Kinetics | Saturation; Michaelis-Menton equation applies. | Complex or linear |
| Effects of anions and cations | None | Anions or cations have an effect; $K^+$ absorption greater from KCl than $K_2SO_4$; $Cl^-$ absorption is greater from KCl than from $CaCl_2$. |
| Response to $Ca^{++}$ | $Ca^{++}$ essential | $Ca^{++}$ competes with absorption of other cations. |

**Importance of Mechanisms**

Mechanism 1 allows for ions to be absorbed from dilute solutions. If ions were absorbed only from concentrated solutions, much of cropland would be unproductive. No selectivity would operate, and much more attention would have to be given to balance of cations and anions.

Mechanism 2 allows for rapid uptake when some anions are high. This mechanism likely does not operate in natural soils. It may be important in fertilized fields where the concentrations of some ions in soil solution may be high. It would be important when nutrient supply is abundant. It would be important also in saline conditions where the maintenance of a balance of osmotic pressure inside and outside the cell is needed.

## Long Distance Transport

This transport refers to transport of ions across roots, in the xylem, and in the phloem.

***Uptake along roots***. Roots vary anatomically and physiologically along their longitudinal axis from tip to mature cells. The root cap is dead cells and does not absorb solute. The nonvacuolated cells at the immediate tip behind the root cap is a region of cell division and is not highly active in uptake; otherwise, the rate of ion uptake declines with distance from the root tip, meaning that old sections of roots are less active in ion absorption than young sections. The most active zone for uptake is about 1 to 3 cm from the root tip in the region of cell enlargement (Figure 7. 8).

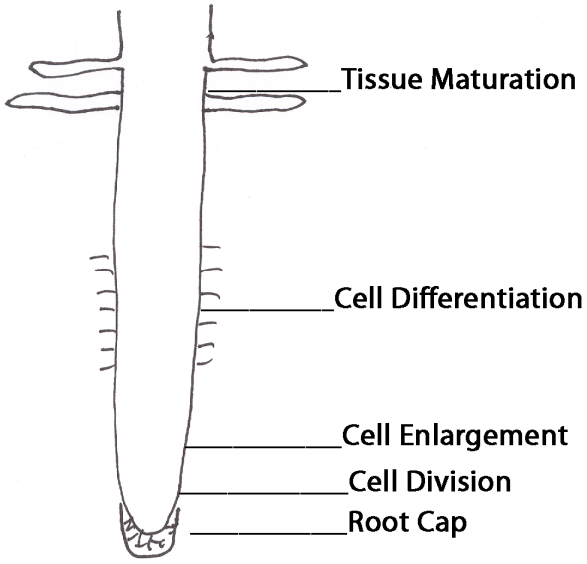



Figure 7.8 Longitudinal section of plant root showing the root cap and regions of cell division, enlargement, and differentiation and tissue maturation

***Transport across root***. Transport across a root is by two pathways. Water and solute can move in the apoplasm (free space) of the cell walls and intercellular spaces by diffusion to the epidermis, which limits transport into the stele. Transport in the apoplasm is stopped at the endodermis by the Casparian Strip, which is suberin (Figure 7.9).

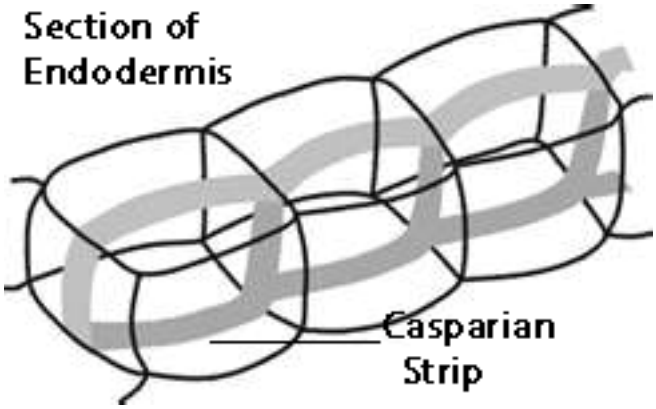



Figure 7. 9 Drawing of a section of endodermis showing the Casparian Strip surrounding individual cells of the tissue

The Casparian Strip prevents diffusion of solutes into the stele; thus, solute that is transported upward in the xylem must be absorbed by plant cells external to the endodermis. Casparian Strips are typically smaller than the cell wall on which they are deposited. In young sections of roots, Casparian Strips are analogous to the mortar between the concrete blocks in classroom walls. In old endodermal cells in the mature, basal part of roots, suberin may be extensively deposited extensively on all cell wall surfaces, and the cells can become lignified, forming a completely waterproof layer.

The symplastic pathway is through root tissues after solute has been absorbed into cells by crossing the plasmalemma. The mechanism of cell-to-cell symplasmic transport is principally by diffusion. Symplastic transport can begin at the epidermis, root hairs, or cortex (Figure 7. 10). Cell-to-cell transport across the root occurs through *plasmodesmata*. Plasmodesmata are microscopic pores filled with protein from protoplasm leading from cell to cell. The number of plasmodesmata varies with cell type and species. The interface of the cortex and endodermis may have numerous plasmodesmata. Plasmodesmata may restrict or dilate, thus giving some regulation of transport between cells. But, generally once solute (ions) enters cells no barrier in symplasmic transport occurs.

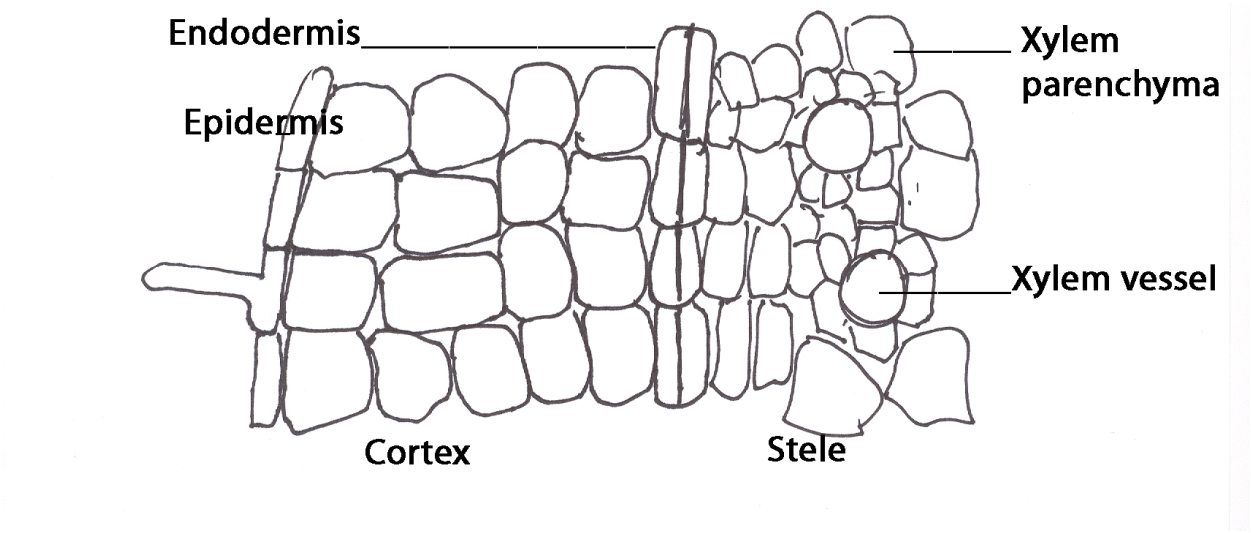



Figure 7.10 Drawing of cross-section of root from epidermis to stele

Endodermal cells may absorb solute from the free space, but most of the transport into the endodermis is by symplastic transport. Solute can move from the endodermis into parenchyma of the stele by diffusion through plasmodesmata. Solute may enter into the vacuoles of roots for storage. Storage capacity of the vacuoles may be increased by precipitation, but the capacity of symplastic system of the protoplasm far exceeds that of the vacuolar system.

After solute reaches the parenchyma of the stele, solute may be loaded into the xylem vessels (or tracheids) by xylem parenchyma surrounding the vessels. Xylem vessels and tracheids are dead cells. Loading of the vessels and tracheids is an active process driven by ATP through ATPases and transporters for specific ions.

Analysis by the electron microprobe showed that concentrations of $K^+$ increased near xylem vessels relative to the concentration in the cortex. This evidence supports the theory that solute is loaded actively into xylem vessels (or tracheids). Previous theories suggested that solute leaded into the vessels due to shortage of oxygen for respiration in the parenchyma of the stele.

**Xylem Transport**

Root pressure may be a phenomenon of the active loading of xylem. The active loading, and perhaps the concentrated solute in the apoplasm of the stele, induce a flow of water and solute into the vessels with the flow directed toward the shoots. Evidence of root pressure is observed with *guttation* from leaves in humid atmospheres, which lower transpiration rates and the evaporation of water that is exuded at hydathodes at leaf margins (Figures 7. 11 and 7.12). Exudation from bleeding stumps of cut plants also is evidence of root pressure

(Figure 7.12). Sometimes the exudate is collected from the stumps to obtain information of the transport of materials in xylem. Root pressure may be important in the delivery of elements to low-transpiring organs such as fruit.

Figure 7.12 Guttation from a leaf

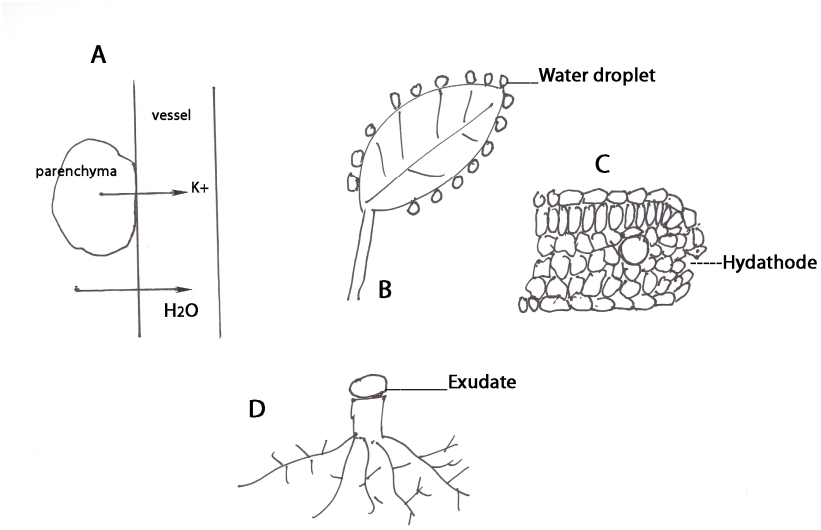



Figure 7.12 Drawing of A. Active loading of xylem to establish force for root pressure; B. Guttation from a leaf; C. Hydathode of a leaf; and D. Exudate from a bleeding stump of a cut plant

Selectivity may occur in loading of xylem vessels (Figure 7.13). For example, $K^+$ is loaded and $Na^+$ is retained in xylem parenchyma, thereby retaining $Na^+$ in the roots. If $Na^+$ enters xylem, $Na^+$ may be withdrawn selectively and not be transported to the shoots.

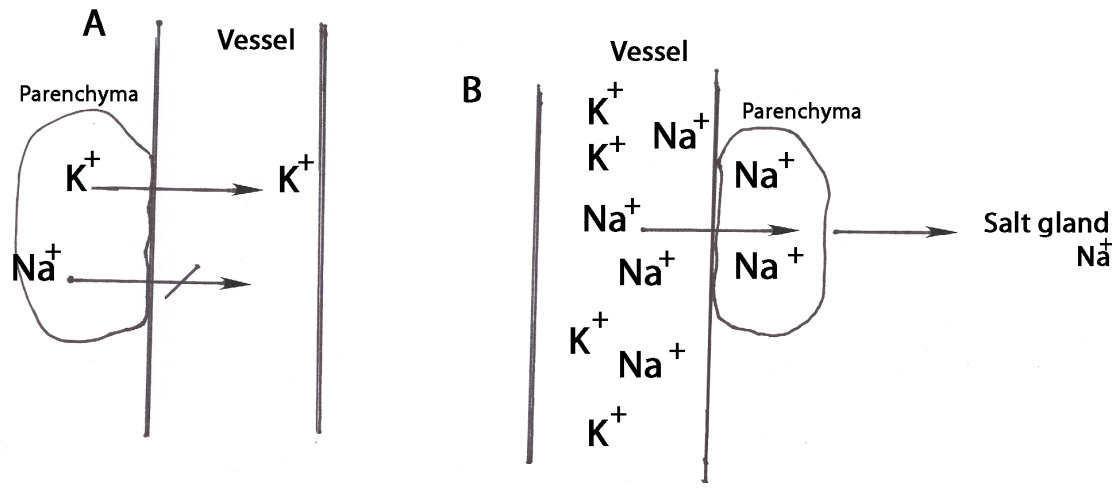



Figure 7.13 Illustration of A. Selective loading of xylem vessel with $K^+$ from xylem parenchyma and B. Selective unloading of xylem vessel with $Na^+$ to xylem parenchyma or further transport to salt gland

The fate of ions in xylem varies with kind of ion and plant species. Nutrients may be drawn off by tissues as ions move up the xylem. Some ions such as $Na^+$ may be removed from the xylem and placed in the surrounding parenchyma or translocated to salt glands to prevent the movement of $Na^+$ into the shoots.

Calcium ($Ca^{++}$) and $Zn^{++}$ are reported to move up the xylem by exchange along the negatively charged cell walls of the vessels or as chelates. Iron moves as a chelate as iron citrate and not by exchange. Magnesium ($Mg^{++}$) and $Mn^{++}$ move as cations or as complexes with organic acids. Nitrogen is transported in xylem mainly in inorganic forms ($NO_3^-$, $NH_4^+$) although transport of amino acids and amides also occur, particularly if nitrogen assimilation occurs in roots. Phosphorus and sulfur move as phosphate and sulfate.

**Unloading of xylem into leaves**

Once ions reach leaves they are delivered into the outer space of the leaves. Most of the water and solute in the xylem vessels or tracheids are transported into the leaves. Water moves preferentially from the major veins to sites of rapid evaporation or transpiration. This action may lead to accumulation of salts at the margins of leaves and necrosis of the margins or tips of leaves. In some plants, precipitation of difficultly soluble salts may prevent salt accumulation in the leaves. This process may occur in some gymnosperms, for example.

In most cases, the concentration of solute in the xylem declines from root to shoot and from base to tip of leaves so that salt accumulation is not a problem. Solutes are absorbed from the apoplasm by mesophyll cells. The electropotential and $H^+$ gradient across the plasmalemma drives solute uptake by the mesophyll. Transporters and channels for influx facilitate absorption of

ions. Nutrients are absorbed at least twice by plants—once by the root cells and again by the leaf cells.

Solute in the free space of leaves is subject to leaching from leave as the solute is accessible to losses through stomates, cracks in the cuticle, or perhaps the hydathodes. Solute that is mesophyll cells is not subject to leaching as the solute is retained by the cell membrane.

The rate of flow of water and solute in the xylem is governed by root pressure and rate of transpiration. In young plants, transpiration has little effect on the distribution of solute in the shoots. Root pressure affects the delivery in these plants. As plants grow, the importance of transpiration for translocation of elements increases. Large leaves may obtain more nutrients than small leaves because of differences in transpiring surface areas. This action can lead to deprivation of shoot tips of nutrients. Tipburn of lettuce develops from calcium deficiency under conditions in which transpiration to large, outside leaves greatly exceeds transpiration to small, inside leaves. Transpiration rates fall at night as the stomates close. This action can restrict the delivery of solute to shoots at night. In humid atmospheres, translocation by xylem can be limited. Tipburn of lettuce can develop in this circumstance because transpiration is not delivering sufficient calcium to the young leaves that continue to grow. Differences in manganese distribution in plants have been associated with whether the leaves are in the sun or shade with the leaves in sun receiving more manganese than the shade leaves because of the higher transpiration rates in the sun-exposed leaves.

The distributions of calcium and boron are correlated closely with rates of transpiration of organs. Typical rates of transpiration of organs of shoots decline in this order, leaves, fruits (pods), seeds. Fruits and seed are typically much lower in calcium and boron than leaves. Calcium and boron are not translocated in phloem of most plants; so, their distribution is heavily dependent on transpiration. The effect of transpiration on distribution of potassium is negligible, and the effects on distribution of magnesium is small. Nitrate distribution follows transpiration patterns, whereas ammonium transport does not. Very little nitrate is transported in the phloem.

## Phloem Transport

Direction of transport is generally said to be downward in the phloem, but direction depends on the sink or plant part having the demand for transport or material for transport and the source of material. Therefore, movement can be up and down in the phloem, and transport occurs in both directions in the same sieve tubes. Sources (donors) include storage organs, roots, stem, and old leaves. Sites of demand (sinks) include storage organs, fruits, seeds, roots, and young leaves.

In the xylem no barrier to transport usually exists, for xylem vessels and tracheids are dead cells with no living protoplasm. Hence, ions move with the transpirational pull or root pressure unless something is plugging the xylem (tyloses) or exchange or withdrawal occurs during transport. The pathway for transport in phloem is the sieve tubes (Figure 7.14). These cells are alive and have barriers to transport in the sieve plates. The protoplasm of the sieve tubes is sparse and has few mitochondria, which are undeveloped relative to the mitochondria in the companion cells. Companion cells may have a role in supplying energy to drive transport in the sieve tube.

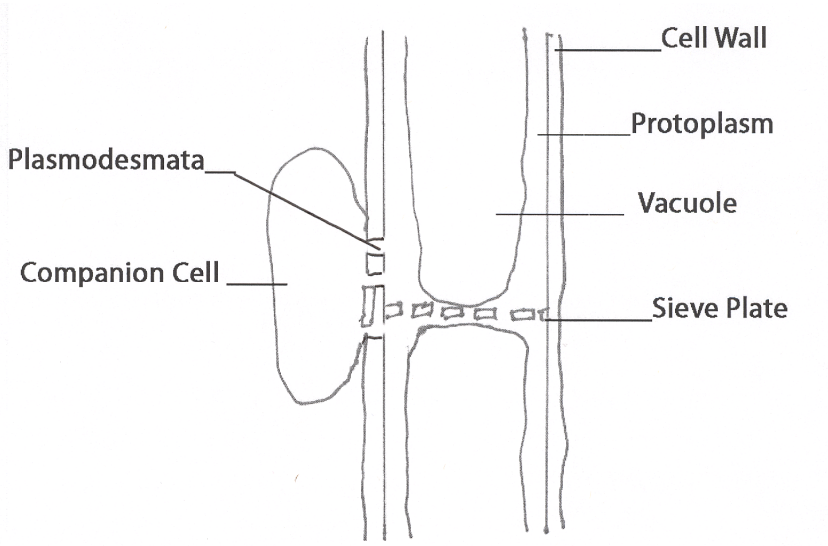



Figure 7.14 Drawing of a sieve tube of angiosperms, showing various components of the sieve tube and an adjacent companion cell

The mechanism of transport is unclear although several hypotheses have been presented to explain transport in the phloem. In 1926, The *Pressure Flow Hypothesis* (Münch Hypothesis; Source-Sink Hypothesis) was proposed. This hypothesis presents the principal mechanism that is accepted by plant physiologists to explain transport in the phloem. This hypothesis suggests that solute, such as sucrose, at a source is concentrated (loaded) into the sieve tubes. Water then enters into the sieve tubes thereby creating a pressure. This pressure induces mass flow in the sieve tubes to sites called sinks, where solute is removed from the phloem. The rate and direction of flow are governed by loading at the source and unloading at the sink.

At the sink, unloading is by active excretion. In the sink, solute may be changed into a sparingly soluble material to maintain the demand for solute. Sucrose may be changed into starch. Amino acids may be changed into proteins.

Other hypotheses to explain phloem transport include the *Contractile Filament Hypothesis* (and the *Electroosmotic Hypothesis*. The concept of Contractile

Filament Hypothesis proposed by Thaine in 1969 suggests that protoplasmic streaming mixes the contents of sieve tubes and that protein filaments in the cytoplasmic strands of sieve plates contract and pump ions in the sieve tubes. Energy of ATP drives the pumping action. The Electroosmotic Hypothesis proposed by Spanner in about 1958 suggests that electrokinetic mechanisms operate. In this hypothesis, metabolic loading (for example, of $K^+$) occurs at sieve plates setting up mass flow in the sieve tubes. The hypothesis of the contracting filaments and electroosmotic pumping are not accepted generally among plant physiologists because these mechanisms do not explain some of the phenomena of phloem transport, such as transport of the same solute in two directions in the same sieve tubes.

**Composition of Phloem Sap**

The sap of phloem has a high pH of about 7 to 8. It has a high concentration of solids, which are about 15% to 25% dry matter. Sucrose may comprise 90% of the dry matter. Amino acids, particularly glutamine and asparagines, are substantial components of phloem sap. Nitrate may be undetectable, and ammonium is also low. Organic acids, such as citrate, succinate, and malate, are relatively abundant in the sap. A number of other sugars, hormones, proteins, and other metabolite may be transported in the phloem. Potassium is the highest among the mineral elements. The following table (Table 7.1) shows relative mobility of nutrients in the phloem. Mobile elements move readily in the phloem and can be redistributed around the plant. Elements of intermediate mobility may move slowly and may not be redistributed sufficiently rapidly to meet the needs of organs, such as young leaves. Immobile elements generally do not move in the phloem.

Table 7.1 Relative mobility of mineral plant nutrients in the phloem

| Mobility | | |
|---|---|---|
| Mobile | Intermediately Mobile | Immobile |
| Potassium | Iron | Calcium |
| Nitrogen (organic forms) | Manganese | Boron |
| Magnesium | Zinc | |
| Phosphorus | Copper | |
| Sulfur | Molybdenum | |
| Chlorine | Nickel | |

Calcium does not get loaded into the phloem, since the concentrations of $Ca^{++}$ are low in the cytoplasm. A principal amount of calcium is outside the cells in the pectin. In the cells, calcium may be precipitated as oxalates and phosphates in the vacuole and held to calmodulin. Boron might enter the phloem but is suggested to leak out in transport.

# Chapter 8. Genetic Specificity of Plant Nutrition

Mineral nutrition of plants is one of the most important factors governing dry matter production, and the efficiency of utilization of nutrients is related to the genetics of plants. *Nutrient-Use Efficiency* (NUE) is defined in several ways:

For example, the accumulation of dry matter per unit of nutrient accumulated is used commonly by scientists,

NUE = dry matter mass of tissue/mass of nutrient in tissue.

Or by the dry matter mass accumulated as the result of fertilization, a concept that is used widely by farmers,

NUE = dry matter mass of tissue/ mass of nutrients added to the medium.

Or by the recovery of nutrients from fertilizers, which is a concept used by scientists and farmers,

NUE = amount of nutrient removed by crop/amount of nutrient applied as fertilizer.

Recovery includes the nutrients delivered by the soil and fertilizer, but calculations are made as if all of the nutrients were from the fertilizer. Some recovery values for nitrogen, phosphorus, and potassium are given in Table 8.1.

Table 8.1 Nutrient recoveries from crops such as corn, wheat, rice, barley, and forages

| Nutrient | Recovery % of application |
|---|---|
| Nitrogen | 30 to 70 |
| Phosphorus | 10 to 25 |
| Potassium | 50 to 60 |

With corn in the USA, yields are increasing about 1.75 bushels per acre per year. Since 1935, efficiency of nitrogen use has increased by 39%, whereas the use of nitrogen has increased by 12% and corn yields have increased by 40%. Much of the increase in efficiency has arisen from new technology in farming, including

placement of fertilizer, timing of application, improved recommendations for use of N, and use of controlled-release fertilizers.

The need for increasing nutrient-use efficiency is evident from consideration of several factors. In 1960, about 80% of the soils of the World were of low fertility due to conditions of nutrient deficiency, acidity, alkalinity, salinity, and erosion. Since 1960, use of fertilizers has increased substantially (Tables 8.2, 8.3, and 8.4). The general trend today is for use of fertilizers to increase in underdeveloped countries and to decrease in developed countries.

Table 8.2. Use of nitrogen-containing fertilizers in selected regions of the World in 1960 and 2000 and by economic development (millions of metric tons of N/year)

| Year | W. Europe | E. Europe | N. America | S. Asia | Middle East |
|---|---|---|---|---|---|
| 1960 | 3.35 | 0.76 | 2.82 | 0.31 | 0.26 |
| 2000 | 9.29 | 2.55 | 11.93 | 14.40 | 4.25 |

| | World | Developed | Developing |
|---|---|---|---|
| 1960 | 10.83 | 8.55 | 2.28 |
| 2000 | 80.80 | 28.19 | 52.61 |
| 2006 | 90.86 | 27.17 | 63.69 |

Table 8.2. Use of potassium-containing fertilizers in 1960, 2000, and 2006 by economic development of the World (millions of metric tons of $K_2O$/year)

| $K_2O$ | World | Developed | Developing |
|---|---|---|---|
| 1960 | 8.48 | 7.96 | 0.52 |
| 2000 | 21.86 | 10.91 | 10.95 |
| 2006 | 26.44 | 9.62 | 16.82 |

Table 8.2. Use of phosphorus-containing fertilizers in 1960, 2000, and 2006 by economic development of the World (millions of metric tons of $P_2O_5$/year)

| $P_2O_5$ | World | Developed | Developing |
|---|---|---|---|
| 1960 | 10.73 | 9.64 | 1.09 |
| 2000 | 32.48 | 11.29 | 21.19 |
| 2006 | 36.78 | 9.89 | 26.89 |

Increased nutrient-use efficiency would improve use of the natural fertility of soils and the used of fertilizer-applied nutrients. Increases in the nutrient-use

efficiency are also necessary to limit costs of production of crops. In 1960, nitrogen costs were about $0.15 per pound, and now costs are about $0.70 per pound on average, with costs varying widely with source of the nitrogen. Nitrogen from organic fertilizers can range from $1.00 to $5.00 or more per pound.

Another factor in increasing nitrogen-use efficiency is the conservation of resources. The nitrogen supply of the World essentially is unlimited considering the amounts of nitrogen in the atmosphere and the rate of nitrogen use in crop production. But, to get the nitrogen from the air into fertilizer nitrogen consumes a lot of energy from fossil fuels. About 1% of the energy consumption in the United States is for nitrogen fixation at fertilizer plants. Furthermore, nutrient-loading of soils through fertilization is considered to be a major factor in pollution of lakes, rivers and streams, and reservoirs. Of the nitrogen not recovered from crops, a substantial fraction may leach or wash by runoff into these bodies of waters. Losses of potassium and phosphorus by leaching and runoff into bodies of water are small but still could enrich these bodies with nutrients.

Interest is modest in breeding of plants with high nutrient-use efficiency. Little attention is given in selecting for efficient absorption, efficient utilization, efficient transport, and efficient mobilization. Attention is given to disease resistance, appearance, and flavor. Plant species and cultivars differ in nutrient-use efficiency, however.

Genetic differences in efficiency are related to many morphological and anatomical features as well as physiological and biochemical processes.

*Root morphology*. Type of root affect several morphological properties that can affect use efficiency. Fibrous roots will have much more absorptive area than tap roots. So, selection for fibrous roots would be a principal factor to improve nutrient-use efficiency. Roots with extensive cortex volume will have more free space and more absorptive surface than slender roots. Mycorrhizal associations help to increase absorption of difficultly soluble nutrients, such as iron and phosphorus. In containers, pot-bound roots will extract more nutrients from the medium than sparse roots. In poor soils, more nutrients may be allocated to roots than to shoots.

*Stem morphology*. Stems of wide diameter may have more conductive elements (xylem, phloem) than slender stems. Long stems impart a long distance of transport form source to sink.

*Leaf morphology*. Factors to consider in selection for efficiency based on leaf morphology include leaf size, shape, thickness, and position. These properties affect the amount of metabolic tissues in the leaves and their capacity to utilize nutrients and to intercept sunlight.

*Shoot/root ratio*. Generally, a wide root/shoot ratio is effective.

*Rate of growth***.** Certain wild plants or native species may grow slowly in soils of low fertility and not develop symptoms of deficiency. Some of these plants may have efficient mechanisms for nutrient absorption but generally the slow growth puts little demand for nutrients on the medium, and symptoms of deficiency do not appear. Wild plants may or may not respond with increased growth in soils of enhanced nutrient supply. Crop plants may have much more efficient in nutrient utilization than wild plants especially in fertile soils.

*Physiological processes*. Photosynthesis, transpiration, respiration, distribution, and mobility of nutrients can affect nutrient-use efficiency. Generally, increased activity and specificity of these processes increases use efficiency. However, assessment and selection of these factors will be more difficult than for selection of the morphological factors.

*Biochemical processes*. Active metabolic pathways can improve efficiency and might be factors for selection in plant breeding. Rate of nitrate reduction, capacity of plants to reduce iron at root surfaces, and selectivity in nutrient absorption are properties that may be considered. Selection for these processes may have limitations. Traits for efficiency in these processes may not be passed to progeny. High nitrate reductase activity and rates of absorption may not be associated with high dry matter production. Favorable traits for some nutrients may affect availability of other nutrients adversely. Favorable phosphorus efficiency may affect the efficiency of copper, iron, or zinc.

Differences in efficiency may disappear with increased supplies of the nutrient in question. Much of the efficiency occurs from use of the nutrient in the plant and not in acquisition. No single mechanism applies to all nutrients.

In experiments to assess nutrient-use efficiency, the plants must be genetically uniform. Identical nutrient solutions or soil types must be used. Ecological conditions and growing seasons must be the same in all experiments. Assessments must be made at the same phases of ontogenesis of a plant species. The same specific plant organs or parts must be used in assessments. Forms of nutrients supplied must be considered. Results must be considered on the basis of concentrations in plants and on total accumulation in plants.