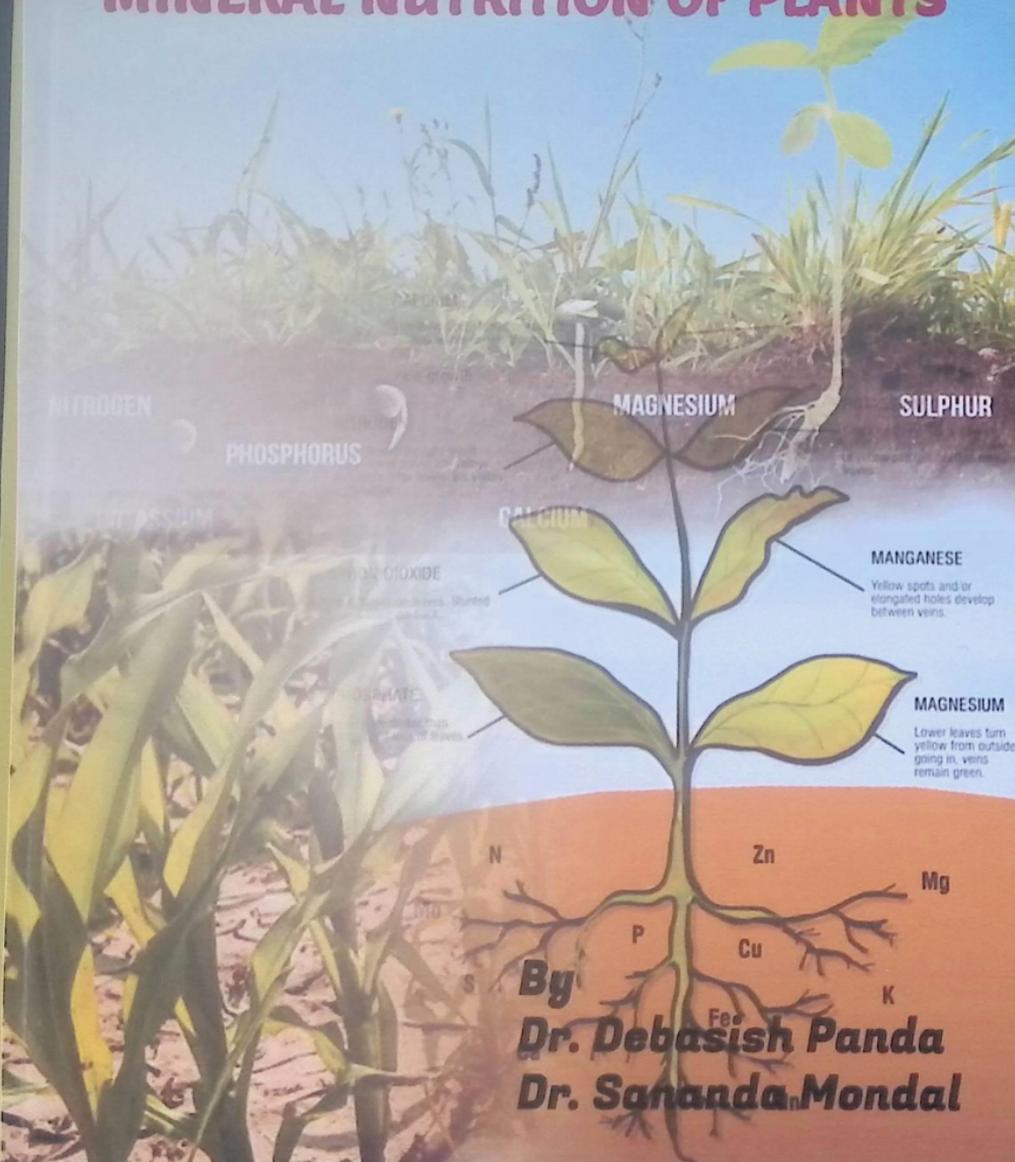


# E-LEARNING RESOURCES OF MINERAL NUTRITION OF PLANTS



NITROGEN

PHOSPHORUS

POTASSIUM

CALCIUM

MANGANESE

MAGNESIUM

SULPHUR

MANGANESE

Yellow spots and/or elongated holes develop between veins.

MAGNESIUM

Lower leaves turn yellow from outside going in, veins remain green.

N

Zn

Mg

P

Cu

K

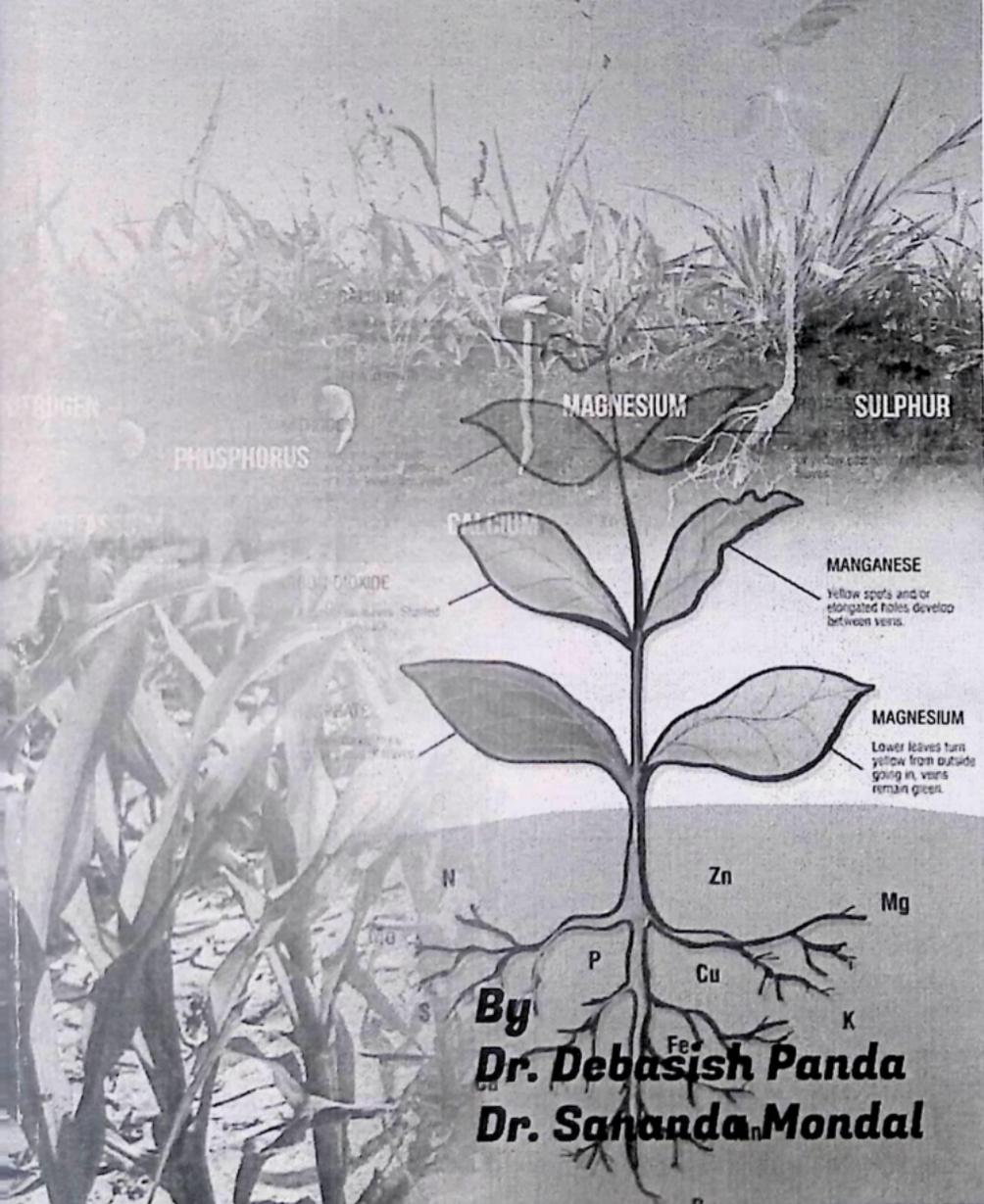
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By  
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# E-LEARNING RESOURCES OF MINERAL NUTRITION OF PLANTS



## MINERAL NUTRITION OF PLANTS (Course No. CPH502)

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### MINERAL NUTRIENTS

Nutrition may be defined as the supply and absorption of chemical compounds needed for growth and metabolism. The mechanisms by which nutrients are converted to cellular material or used for energetic purposes are metabolic processes. The chemical compounds required by an organism are termed nutrients.

#### Mengel's Classification of Plant Nutrients

Classification of plant nutrients according to biochemical behaviour and physiological function

| Nutrient elements                              | Uptake  | Biochemical function   |
|--|---|--|
| 1 <sup>st</sup> group<br>C, H, O, N, S         | in the form of CO <sub>2</sub> , HCO <sub>3</sub> <sup>-</sup> , H <sub>2</sub> O, O <sub>2</sub> , NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup> , N <sub>2</sub> , SO <sub>4</sub> <sup>2-</sup> , SO <sub>2</sub><br>The ions from soil solution and gases from atmosphere | Major constituent of organic material. Functional elements in enzymic processes. Assimilation is by oxidation reduction process.   |
| 2 <sup>nd</sup> group<br>P, B, Si              | in the form of phosphates, boric acid or borate, silicate from the soil solution  | Esterification with native alcohol groups in plants The phosphate esters are involved in energy transfer reaction.   |
| 3 <sup>rd</sup> group<br>K, Na, Mg, Ca, Mn, Cl | in the form of ions from the soil solution  | Maintain osmotic potential. Bring about optimum conformation of an enzyme protein (enzyme activation). Bridging of reaction partners. Balancing anions. Controlling membrane permeability and electron potentials. |
| 4 <sup>th</sup> group<br>Fe, Cu, Zn, Mo        | in the form of ions or chelates from soil solution  | Incorporated in the prosthetic groups, enable electron transport by valency change.  |

Plant growth and development can proceed only when plants are applied with these chemical elements referred to as essential elements. These nutrients are absorbed by plant roots from soil. The term **mineral nutrition** is generally used to refer to an inorganic ion obtained from soil and required for plant growth.

However certain higher plants grow on limbs of trees or on telephone wires obtain this supply of mineral nutrients from air borne dust particles which alight on their surfaces and then dissolve in rain water or dew. These are called **epiphytes** or **air plants**.

**Water culture** - Growing of higher plants with their roots in dilute solutions of mineral salts, instead of in soils; is called as **hydroponics**.

**Sand culture** - In a modification of water culture, plants are grown with their roots anchored in a soil inert aggregate such as sand, gravel, or vermiculite (heat expanded mica), which is wetted with a solution of mineral nutrients, also crushed quartz which is very low in available trace elements.

The plants grown in water culture must be provided with an adequate supply of oxygen, either by bubbling air around the roots or by frequent replacement of depleted solution with fresh solution. Organic compounds that react specifically with metal ions are known as **chelating agents**. The word **chelate** "key - late" (greek, chela, claw) refers to the claw like binding of a metal ion in solution by an organic molecule. Micronutrients are mostly supplied in the chelated form. Eg : Ethylene diamine tetraacetic acid (EDTA).

Micro elements are essential elements that are required in small amounts. They are otherwise called as trace elements. Macro elements are essential elements that are required in large amounts.

Macronutrients { Hydrogen,  
Carbon,  
Oxygen,  
Nitrogen,  
Phosphorus,  
Potassium,  
Calcium,  
Magnesium, and  
Sulphur.

Micronutrients { Zinc,  
Iron,  
Manganese,  
Copper,  
Boron,  
Chlorine,  
Molybdenum and  
Nickel

Elements essential for few plant

Silicon  
Cobalt  
Selenium  
Sodium  
Aluminium

**Criteria of essentiality of elements (Arnon and Stout 1939):**

Definition or criteria of an essential element (Criteria proposed by **Arnon and Stout** in 1939)

- (i) A given plant must be unable to complete its life cycle in the absence of the mineral element (Life cycle = vegetative state, flower, produce seeds)
- (ii) The function of the element must not be replaceable by another mineral element
- (iii) The element must be directly involved in plant metabolism or a component of an essential plant constituent (e.g. Nitrogen is a constituent of proteins and chlorophyll)

There are 17 essential elements for plants. The following table lists the essential elements, their source, concentration in the plant, whether they are a macronutrient or a micronutrient, and the form of the element that can be absorbed by the plant.

| Source              | Class                       | Element         | Form Absorbed by Plant  | Avg. Concentration in Plant |
|---------------------|-----------------------------|-----------------|-------------------------|-----------------------------|
| Non-Mineral         | Macronutrients (> 1000 ppm) | Carbon (C)      | $CO_2$                  | 44.0 %                      |
|                     |                             | Hydrogen (H)    | $H_2O$                  | 6.0 %                       |
|                     |                             | Oxygen (O)      | $H_2O, CO_2, O_2$       | 44.0 %                      |
|                     |                             | Nitrogen (N)    | $NO_3^-, NH_4^+$        | 1.5 %                       |
|                     |                             | Phosphorous (P) | $H_2PO_4^-, HPO_4^{2-}$ | 0.2 %                       |
|                     |                             | Potassium (K)   | $K^+$                   | 1.0 %                       |
|                     |                             | Sulfur (S)      | $SO_4^{2-}$             | 0.1 %                       |
|                     |                             | Calcium (Ca)    | $Ca^{+2}$               | 0.5 %                       |
|                     |                             | Magnesium (Mg)  | $Mg^{+2}$               | 0.2 %                       |
| Mineral - from soil | Micronutrients (< 1000 ppm) | Iron (Fe)       | $Fe^{+3}, Fe^{+2}$      | 100 ppm                     |
|                     |                             | Manganese (Mn)  | $Mn^{+2}$               | 50 ppm                      |
|                     |                             | Boron (B)       | $H_3BO_3$               | 20 ppm                      |
|                     |                             | Zinc (Zn)       | $Zn^{+2}$               | 20 ppm                      |
|                     |                             | Copper (Cu)     | $Cu^{+2}$               | 6 ppm                       |
|                     |                             | Molybdenum (Mo) | $MoO_4^{2-}$            | 0.1 ppm                     |
|                     |                             | Chlorine (Cl)   | $Cl^-$                  | 0.2 %                       |
|                     |                             | Nickel (Ni)     | $Ni^+$                  | 0.5 ppm                     |

**Beneficial nutrients** are nutrients that are not needed for the plant to complete its life cycle, but may provide other benefits such as disease resistance, etc. Examples of beneficial nutrients are silicon, cobalt and sodium.

**Primary Nutrients** - N, P, and K- most common growth limiting nutrients-usually most deficient. **Secondary Nutrients** - S, Ca, Mg.

The **secondary nutrients** are calcium (Ca), magnesium (Mg), and sulfur (S). There are usually enough of these nutrients in the soil so fertilization is not always needed. Also, large amounts of Calcium and Magnesium are added when lime is applied to acidic soils. Sulfur is usually found in sufficient amounts from the slow decomposition of soil organic matter, an important reason for not throwing out grass clippings and leaves.

Macronutrients are usually found in plants at a concentration above 1000ppm on a dry weight basis and micronutrients are found in plants at a concentration below 1000ppm on a dry weight basis. They are also divided by the quantity or abundance of nutrients the plant needs. Plants need more macronutrients than micronutrients. Primary nutrients are usually limit plant growth the most and are the most deficient whereas secondary nutrients do not limit plant growth as much and are not as deficient in the soil.

**Micro elements can be presented in plants in different chemical forms either as**

inorganic ions or  
undissociated molecules or  
organic complexes (chelates)

**In nutrient solution they are present as undissociated molecules or ions like**

$H_3BO_3$  (Boric acid)  
 $Cl^-$  (Chloride)  
 $Cu^{++}$  (Cupric)  
 $Fe^{++}$  (Ferrous or Ferric  $Fe^{+++}$ )  
 $Mn^{++}$  (Manganese)  
 $MoO_4=$  (Molybdate) and  
 $Zn^{++}$  (Zinc)

#### **Functions of Essential Mineral Elements:**

##### **Nitrogen**

1. Present in the structure of the protein molecule.
2. It is found in important molecules as purines, pyrimidines, porphyrins and co-enzymes.
3. Purines and pyrimidines are found in the nucleic acids, RNA, DNA, which are essential for protein synthesis.
4. The porphyrin structure is found in the chlorophyll and cytochrome enzymes. These are essential for photosynthesis.
5. Co-enzymes are essential to the function of many enzymes.

##### **Phosphorus**

1. Phosphorus is found in plants as a constituent of nucleic acids, phospholipids, the co-enzymes NAD and NADP and constituents of ATP.
2. Heavy concentration of phosphorus are found in the meristematic regions of actively growing plants – its is involved in the synthesis of nucleoproteins.
3. Phospholipids along with protein may be important constituent of cell membranes.
4. Co-enzymes like NAD, and NADP are important in oxido reduction reactions. The important processes in which they involve are (i) Photosynthesis, (ii) Glycolysis, (iii) Respiration and (iv) Fatty acid synthesis.

### Calcium

1. A constituent of cell walls in the form of calcium pectate.
2. The middle lamelle of plant cells is composed primarily of calcium and magnesium pectates.
3. Calcium salt of lecithin, a lipid compound is involved in the formation of cell membranes.
4. Calcium is required in small amounts for mitosis. It is involved in chromatin or mitotic spindle organization.
5. As an activator of enzyme, phospholipase in cabbage leaves.
6. Also activator for the enzyme, like arginine kinase, adenosine triphosphatase, adenylkinase etc.
7. As secondary messenger in metabolic regulations (extra-cellular cell signaling)

### Magnesium

1. Essential role in photosynthesis and carbohydrate metabolism.
2. Magnesium is a constituent of chlorophyll molecule.
3. Enzymes involved in carbohydrate metabolism, require magnesium as an activator.
4. Magnesium is involved in the synthesis of nucleic acid.
5. Co-enzymes such as ATP or ADP could become linked to the enzyme surface through a chelate complex involving magnesium and pyrophosphate group. Mn can substitute to some extent.
6. Magnesium binds to the subunits in the microsomal particles containing RNA, protein and magnesium i.e., as a binding agent.

### Potassium

1. Special role of potassium is yet unknown
2. Highest concentrations are observed in the meristematic tissue.
3. Potassium is essential as an activator for enzymes involved in the synthesis of certain peptide bonds.
4. In addition to its role as an activator in protein metabolism, K also acts as an activator for several enzymes involved in carbohydrate metabolism.
5. Apical dominance appears to be lacking or weak under K deficient conditions.
6. It regulates water balance.

### Sulphur

1. Participate in protein structure in the formation of the sulphur - bearing amino acids, cystine, cysteine and methionine.
2. Sulphur bearing vitamins - Biotin, thiamine and co-enzyme A. Thus S is involved in metabolic activities of these vitamins.
3. Sulphur is found as sulphhydryl groups which are present in many enzymes.
4. Sulphur is present in peptide glutathione and glucosides, such as mustard oil and thiols which contribute the characteristic odor and test to plants in Cruciferae and Liliaceae families.
5. Sulphur also reduces the incidence of disease in many plants

### Iron

1. Ferrous state ( $Fe^{2+}$ ) is the metabolically active form of iron in the plant.
2. Essential for the synthesis of chlorophyll
3. Protoporphyrin is the intermediate in chlorophyll biosynthesis. Fe is incorporated into the porphyrin structures.
4. A component of flavoproteins (metallo flavo proteins)

5. Iron is found in iron – porphoryn proteins viz. cytochromes, peroxidases and catalases.
6. Iron is an important component of many plant enzyme systems such as cytochrome oxidase (electron transport) and cytochrome (terminal respiration step).

#### Manganese

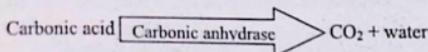
1. Essential for respiration and nitrogen metabolism. Functions as enzyme activator.
2. Essential for malic dehydrogenase (in Krebs cycle) and oxalosuccinic decarboxylase.
3. Manganese is the predominant metal ion in Krebs' cycle. It acts as a bridge for ATP and enzyme complex phosphokinase and phosphotransferase
4. Play an important role in nitrate reduction, acts as an activator for the enzyme nitrate reductase and hydroxylamine reductase.
5. Manganese is involved in the oxidation-reduction processes in the photosynthetic electron transport system. Hill reaction is suppressed under Mn deficient conditions.

#### Molybdenum

1. Essential functions in Nitrogen metabolism.
2. Also involved in P metabolism
3. Chloroplast disorganization occurs in Molybdenum deficiency.
4. Acts as a co-factor for two major enzyme systems, nitrate reductase and nitrogenase.

#### Zinc

1. Involved in biosynthesis of auxin Indole – 3 – acetic acid (IAA)
2. Zinc is involved in synthesis of tryptophan a precursor of auxin i.e. tryptophan synthetase.
3. (i) Activator of carbonic anhydrase in the metabolism of plants.



- (ii) Alcohol dehydrogenase and
- (iii) Hexose kinase or triosphosphate dehydrogenase.
4. It play an important role in protein synthesis.

#### Boron

1. Specific role of Boron in the metabolism of plants is not clear. Boron does not seem to be connected to any enzyme system
2. Essential for transport of carbohydrates, auxins and maintenance of sugar starch balance, pectin formation, amination of sugars and protein synthesis and production of root nodules in legumes
3. Boron is believed to be important in the synthesis of one of the bases of RNA (uracil) formation and in cellular activities (i.e. division, differentiation, maturation, respiration, growth etc.)
4. Boron plays important role in maintaining of cell wall structure and ultra structural arrangement in the apoplast
5. Boron has been associated with pollen germination and growth and it improves the stability of pollen tubes. Relatively immobile in plants, B is transported primarily in the xylem.

#### Copper

1. Copper acts as a component of phenolase, lactase and ascorbic acid oxidase.
2. Copper also functions in photosynthesis. Chloroplast is known to possess a Cu – containing protein called plastocyanin which is important in photosynthesis.

### Chlorine

Chlorine is absorbed and remains in the plant as the chloride ion.

1. Although deficiency never occur, it is essential for the growth of tomato
2. Chlorine is involved in the evolution of oxygen ( $O_2$ ) in photosystem II in the photosynthetic process
3. It is required for cell division in leaves and roots.
4. It is an important osmotically active solute.

### Nickel

1. It is a cofactor of the enzyme urease in higher plants.

### Role of other micro-nutrients:

#### Silicon

Plant species may be divided into Si accumulators and non-accumulators. The accumulators include paddy rice (*Oryza sativa*), Horse tails (*Equisetum arvense*) and members of the Pinaceae, all of which contain 10-15%  $SiO_2$  in the drymatter. Other cereals, sugarcane and a number of dicots with 1 to 3%  $SiO_2$  are also included in this category. The non-accumulators are most of the dicots including the legumes with less than 0.5%  $SiO_2$ .

There is little biochemical evidence to justify Si as an essential element for higher plants, however it shows a number of well-established beneficial effects on plant growth. Important silica fertilizers are soluble silicates, sinter Phosphates and Ca silicate slags.

1. In plants well supplied with Si, cuticular water loss is lowered because of the epidermal accumulation of silica.
2. In cereals the presence of silicon is important for keeping the leaves erect and decreasing susceptibility to lodging.
3. In rice, a significant relationship is observed between the Si content of the straw and yield of rice.
4. Si especially promotes the formation of reproductive organ in rice.

#### Cobalt

The Co concentration in the dry matters of the plants grown in soil normally lies around 0.02 to 0.5 ppm. In soils the content varies from 1 to 40 ppm. Cobalt is not readily mobile in the plant.

1. Co is essential for symbiotic  $N_2$ -fixation. Increasing the supply of Co increases rhizobial growth,  $N_2$ -fixation and formation of leghaemoglobin in nodules.
2. Cobalt is essential component of vitamin cyanocobalamin.

### Foliar nutrition

Foliar nutrition is a useful method of fertilizing certain crop plants which can tolerate the aerial spray without damage. Tolerant plants like orchard trees have a heavy waxy cuticle layer.

1. Foliar nutrition may serve as a mean of applying supplemental macronutrients during critical growth periods when it is unpracticable to apply fertilizers to soil. Ex: Unusual period of dry weather.
2. Foliar nutrition may afford a remedy for the time lag between soil applied and plant absorbed. Time is too long because of fast growing rates.
3. Most economical in forest trees.

## Mechanism

Penetration through diffusion by cuticle – the layer of polymerized waxy esters and hydrocarbons which occurs on outer surface of epidermal cells of leaves. After penetration in the cuticle, further penetration takes place mostly through fine, thread – like, semi microscopic structures called ectodesmata. This extends through the outer epidermal cell walls, from the inner surface of the cuticle to the plasma membrane. When a substance reaches plasma membranes of an epidermal cell it will be absorbed by mechanisms similar to those which operate in root cells.

## Hydroponics

The practice of growing plants in nutrient enriched water without soil called as Hydroponics. The term hydroponics was coined by Gericke (1937). The solution used in culture contains all essential elements in proper proportions for normal growth of plants. This practice has become quite popular during the recent past even for large scale commercial growth of plants such as roses, tomatoes, lettuce, carrots and potatoes.

### *Advantages:*

1. a controlled chemical composition of nutrient solution may be provided,
2. there are no soil colloids present to immobilize any of the nutrients through adsorption,
3. frequent replacement of culture solution prevents the accumulation of toxic organic decomposition products.
4. the growth of bacteria and higher fungi is minimised which may otherwise cause diseases of crop plants,
5. the culture solution is kept aerated and well circulated ensuring better environment for plant growth.
6. no tillage is required, there is no weed growth, and natural calamities such as floods, droughts, erosion etc. can be avoided.
9. growth and reproductive phases can be manipulated up to a certain extent by supplying modified nutritional medium etc.

### *Disadvantages:*

1. The cost of setting up the system is very high
2. It requires skill and knowledge for its operation
3. Fruit trees cannot be grown hydroponically

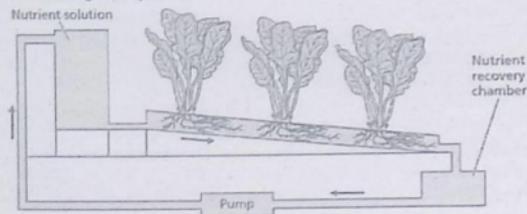
## Aeroponics

It is a system for growing plants with their roots supplied with moisture in the air. The rooted plants are placed in a special type of box with their shoots exposed to air and roots inside the box with computer controlled humid atmosphere. It is however, a recent method developed particularly for research purposes because plants show a very good growth of root hairs. Plants like Citrus and olive have been successfully grown through this method.

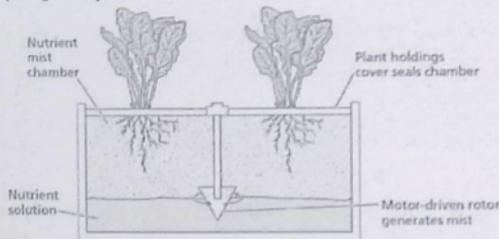
Hydroponic growth system



Nutrient film growth system



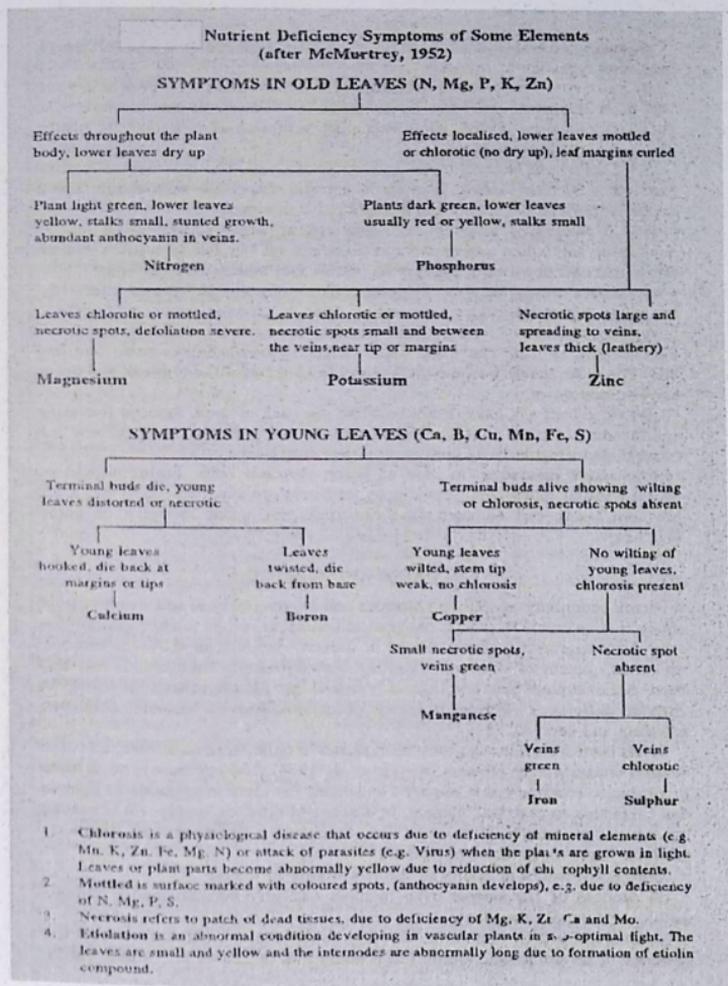
Aeroponic growth system



### Nutrient Film Growth Technique

Nutrient film technique (NFT) is a hydroponic technique where in a very shallow stream of water containing all the dissolved nutrients required for plant growth is re-circulated past the bare roots of plants in a watertight gully, also known as channels. In this technique the plant roots are suspended in channels called gullies where a thin film of nutrient solution passes through thus keeping the roots moist but not logged. The nutrients are mixed accordingly in a primary reservoir from which it flows through the system continuously feeding the plants. The system can be adjusted with automation for aeration. The main principle of the NFT is the principle by which nutrient solutions are recirculated for crop production. The system is widely adjusted for a variety of crop production and is ideal for short term crops such as lettuce, leafy crops and herbs. Larger NFT systems are can be used for long term production of crops such as cucumbers and tomatoes.

**KEY FOR IDENTIFICATION OF NURIENT DEFICIENCY SYMPTOMS IN CROPS**  
(Mc Murtrey, 1952)



**DEFICIENCY SYMPTOMS OF MINERAL ELEMENTS AND CORRECTONAL MEASURES**

Specific deficiency symptoms of various nutrient elements and their correction measures are given below:

### 1. Nitrogen (N)

- i. Plant growth is stunted and poorly developed (because protein content, cell division and cell enlargement are decreased)
- ii. N deficiency causes yellowing (chlorosis) of leaves. Older leaves are affected first
- iii. Flowering and fruiting are reduced
- iv. Protein and starch contents are decreased
- v. Prolonged dormancy and early senescence appear
- vi. Root gets more lengthened as in wheat
- vii. Veins turn purple or red due to development of abundant anthocyanin pigment (e.g. tomato, apple)
- viii. The angle between stem and leaves is reduced
- ix. Plants look so sickly and conspicuously pale that the condition is called as general starvation.
- x. Symptoms first occur on the older leaves due to its mobility

### 2. Phosphorus (P)

- i. Young plants remain stunted with dark blue green, or some times purplish leaves
- ii. P deficiency may cause premature leaf fall
- iii. Dead necrotic areas are developed on leaves and fruits
- iv. Leaves sometimes develop anthocyanin in veins and may become necrotic
- v. Cambial activity is checked
- vi. Tillering of crops is reduced
- vii. Dormancy is prolonged
- viii. P deficiency may cause premature fall of leaves
- ix. Growth is retarded
- x. Sickle leaf disease is caused in P deficiency, which is characterised by chlorosis adjacent to main veins followed by leaf asymmetry

### 3. Potassium (K)

The deficiency symptoms vary with the degree of shortage of the element.

- i. In mild deficiency cases,
  - a. thin shoots may develop and
  - b. there may be restricted shoot growth
- ii. In acute deficiency cases,
  - a. shoots may die back, eventually plant may die
  - b. plants may become stunted with numerous tillers and
  - c. there may be little or no flowering
- iii. Leaf will be dull or bluish green in colour
- iv. Chlorosis occurs in interveinal regions (interveinal chlorosis)
- v. In older leaves, browning of tips (tip burns), marginal scorching (leaf scorch), or development of brown spots near the margins occur
- vi. Necrotic areas develop at the tip and margins of the leaf which curve downward
- vii. In broad leaved plants, shortening of internodes and poor root system are important
- viii. Two diseases are common:
  - a. Rosette: In beet, celery, carrot, pea, potato and cereals, bushy growth or rosette condition develops due to K deficiency

- b. *Die back*: In acute deficiency cases, there is a loss of apical dominance and regeneration of lateral buds, which results in bushy growth. In prolonged cases, die back of laterals is also resulted.

#### 4. Magnesium (Mg)

- i. Mg deficiency causes interveinal chlorosis. The older leaves are affected first and proceeds systematically towards the younger leaves
- ii. Dead necrotic spots appear on the leaves
- iii. Severely affected leaves may wither and shed or abscise without the withering stage.
- iv. Defoliation is quite severe.
- v. Carotene content is reduced
- vi. Stem becomes yellowish-green, often hard and woody
- vii. *Sand-drown disease* is common in tobacco due to its deficiency, which is characterised by the loss of colour at the tips of lower leaves and between the veins (interveinal). The veins remain green but in acute cases, entire leaf becomes nearly white.

#### 5. Calcium (Ca)

- i. Ca deficiency appears on the younger leaves and near the growing points of the stem and root
- ii. Deficiency causes disintegration of growing meristematic regions of the roots, stem and leaves
- iii. Malformation of younger leaves also takes place
- iv. Margins of leaves appear irregular in form or show brown scorching
- v. Growing points are often killed (as in tomato)
- vi. Thin chlorotic marginal bands develop
- vii. Young leaves may be severely distorted with the tips hooked back and the margins curled backward or forward or rolled
- viii. Roots are poorly developed, lack fibre and may appear gelatinous
- ix. Two common diseases are found:
  - a. *Tip hooking*: It is found in cauliflower, beet and tobacco where characteristic hooking of leaf tip is found. This condition arises because of unequal growth in marginal and central regions of the leaf.
  - b. *Blossom end rot*: Commonly found in tomato; the disease is characterised by presence of a depressed region near the distal end of the youngest fruit. The depressed region remains surrounded by dark green tissues and flesh is orange coloured.

#### 6. Sulphur (S)

- i. Sulphur deficiency causes yellowing (chlorosis) of leaves. Young leaves are affected first
- ii. Tips and margins of leaves roll inward
- iii. Marked decrease in leaf size, general paling with red or purple pigmentation is general symptoms
- iv. Necrosis of young leaf tips develop
- v. Internodes are shortened

- vi. Apical growth is inhibited and lateral buds develop prematurely
- vii. Young leaves develop orange, red or purple pigments
- viii. Leaf tips are characteristically bent downwards. The leaf margins and tips roll inwards (e.g. tomato, tobacco and tea)
- ix. Leaf fall is rapid
- x. Fruit formation is suppressed
- xi. Sclerenchyma, xylem and collenchyma formation gets increased and hence the stem becomes unusually thick due to S deficiency
- xii. Disease: The Tea Yellow disease is caused in tea plants growing in sulphur deficient soils.

#### 7. Iron (Fe)

- i. Interveinal chlorosis of the younger leaves occurs. The veins remain green.
- ii. Leaf chlorosis may produce a mottled appearance
- iii. Leaf may show complete bleaching or often becoming necrotic
- iv. In extreme conditions, scorching of leaf margins and tips may occur
- v. Lime induced chlorosis is the common disease found in fruit trees like citrus. It is also found in beet, spinach, brassicas and cereals. The younger leaves become white or yellowish white.
- vi. Iron deficiency leads to Khaira disease in rice.

#### 8. Manganese (Mn)

- i. Deficiency causes interveinal chlorosis and necrotic spots of the leaf
- ii. Dead tissue spots are found scattered over the leaf
- iii. Severely affected tissues turn brown, the brown areas may also twist in the form of spirals and they may wither also
- iv. Root system is often poorly developed and badly affected and the plants may die
- v. Grain formation is also reduced and the heads may be blind (as in sulphur)
- vi. Four diseases are found due to its deficiency:
  - a. Grey Speck also called as grey stripe, grey spot or dry spot found in oats, barley, rye and maize is the common disease of Mn deficiency. Grey spots or chlorotic spots appear on the lower half of the leaf which fuse together and form elongated brown streaks, found mostly in third or fourth leaves.
  - b. Pahla blight of sugarcane: Chlorotic spots develop as long streaks, commonly in young leaves. These chlorotic spots fuse together and turn red and coalesce to form long streaks from which lamina may split.
  - c. Marsh spot of pea: Brown, black spots or cavities develop on the internal surface of cotyledons and thus the disease appears in the seeds.
  - d. Speckled yellow of sugar beet: It is characterised by interveinal chlorosis in the leaves and leaf margin may curl upward over the upper surface of leaf.

#### 9. Copper (Cu)

- i. It causes necrosis of the tip of the young leaves

- ii. Both vegetative and reproductive growth are retarded
- iii. Wilting of terminal shoots occur which is followed by frequent death
- iv. Leaf colour is often faded due to reduction of carotene and other pigments
- v. Foliage shows burning of the margins or chlorosis or rosetting and multiple bud formation
- vi. Gummy may also occur (gummosis)
- vii. Younger leaves wither and show marginal chlorosis (yellowish grey) of tips.

It is called as yellow tip or reclamation disease.

- viii. Following two diseases are common:

- a. Exanthema or die back of fruit trees: It is commonly found in citrus, plum, apple and pear. The symptoms include formation of strong water-shoots bearing large leaves, gummosis tissue or the bark and longitudinal breaks. Fruits become brown, glossy and splitted. Affected shoots loose their leaves and die back and lateral shoots produce bunchy appearance.
- b. Reclamation disease: It is also called as White Tip disease and is found in legumes, cereals, oats and beet. The tips of leaves become chlorotic followed by a failure of the plants to set seed.

#### 10. Zinc (Zn)

- i. Older leaves show chlorosis, which starts from tips and the margins
- ii. Leaves become leathery
- iii. Plants show rosetting due to shortening of internodes and premature shedding
- iv. Whitening of upper leaves in monocots and chlorosis of lower leaves in dicots are often found
- v. Leaf margins distorted, become twisted or wavy which later curl and look sickle shaped (*sickle leaf*)
- vi. Seed production and fruit size is greatly reduced
- vii. The following diseases are commonly noticed:
  - a. Khaira of paddy: The entire older leaves show rusty brown appearance (due to chlorosis) and ultimately die (this disease also occurs in paddy in case of Iron deficiency).
  - b. White bud (tip) of maize: Unfolded newer leaves are often pale yellow to white. There is appearance of light yellow streaks between the veins of older leaves followed by white necrotic spots.
  - c. Rosette of fruit trees: It is also called as **little leaf disease**. Yellow mottling of leaves, reduction of leaf size with rosette appearance (due to reduced internodal distance) and die back of the affected branches are symptoms of the disease.
  - d. Frenching of citrus: Initially, yellow spots develop between the veins. Leaves become progressively smaller and develop chlorophyll at the basal end of mid rib.

#### 11. Molybdenum (Mo)

- i. Deficiency causes chlorotic interveinal mottling of the older leaves
  - ii. Leaves often show light yellow chlorosis and leaf blades fail to expand
  - iii. In acute deficiency cases, necrosis of leaf tissues occurs
  - iv. Flower formation is inhibited
  - v. Failure of grain formation occurs (as in oats)
  - vi. Its deficiency cause
- s two diseases:

a. Whiptail of Cauliflower and Brassica: The symptoms begin as appearance of translucent areas near the midrib which become ivory tinted or necrotic. The leaf margins become ragged with upward curling. Before the death of the growing point, the leaf elongates and lamina remains suppressed thus gives a typical whiptail condition.

b. Scald of legumes: The leaf shows paling, wilting, marginal rolling or scorching.

## 12. Boron (B)

- i. It causes death of shoot tip
- ii. Flower formation is suppressed
- iii. Root growth is stunted
- iv. Leaves become coppery in texture
- v. Plants become dwarf, stunted with apical meristem blacken and die followed by general breakdown of meristematic tissue
- vi. Terminal leaves become necrotic and shed prematurely
- vii. Leaves show symptoms like distortion such as cupping and curling, appearance of white stripe, scorching, pimpling, splitted midrib and reduced growth.
- viii. Stem shows symptoms like die-back of apex, abnormal tillering, appearance of various forms of deformities such as curling and brittle lesions, pimpling etc.
- ix. Fruits are severely deformed and develop typical cracking or splitting.
- x. Following diseases are commonly found due to B deficiency:
  - a. Heart rot of sugar beet and marigold
  - b. Canker and internal black spot of garden pea
  - c. Browning of cauliflower
  - d. Yellow top of lucerne
  - e. Top sickness of tobacco
  - f. Hard fruit of citrus

## INDICATOR CROPS

Some of the crops are known to be specific for the occurrence of symptoms of a particular deficient nutrient element exhibiting characteristic symptoms. Such crops are called as Indicator Crops for the deficiency of that particular element(s). This is mainly due to the greater demand of the element in the respective Indicator Crops. Some of the Indicator Crops are furnished in the following Table along with the element:

Table. Indicator plants for deficiencies of some nutrients

| S.No | Nutrient Element | Indicator Crops                                |
|------|------------------|--|
| 01.  | Nitrogen         | Cereals like, maize, sorghum and pulses        |
| 02.  | Phosphorus       | Tomato, maize, cereals, Leucerne               |
| 03.  | Potassium        | Potato, banana, cotton, Leucerne               |
| 04.  | Magnesium        | Cotton (leaf reddening)                        |
| 05.  | Zinc             | Maize, paddy ("khaira" disease), citrus, beans |
| 06.  | Sulphur          | Cereals, leucerne, tea                         |

|     |            |  |
|-----|------------|--|
|     |            | (yellowing)  |
| 07. | Copper     | Citrus, cereals  |
| 08. | Iron       | Sugarcane, sorghum,<br>citrus, ornamental plants                 |
| 09. | Manganese  | Citrus, sunflower,<br>sugarbeet                                  |
| 10. | Calcium    | Cauliflower, tomato<br>(blossom end rot of fruits),<br>sugarbeet |
| 11. | Molybdenum | Cauliflower (whiptail)   |

## CORRECTION OF NUTRITIONAL DISORDERS

Correction measures of deficiency symptoms of various nutrient elements are given below:

### 1. Nitrogen (N)

For correcting N deficiency, fertilizers like ammonium sulphate, calcium nitrate, urea etc. are supplied. Foliar spray of 1-2% urea is a quick method of ameliorating N deficiency.

### 2. Phosphorus (P)

Foliar sprays of 2% DAP or application of phosphatic fertilizers will correct the deficiency.

### 3. Potassium (K)

Supply of muriate of potash or foliar spray of 1% potassium chloride is commonly used to overcome K deficiency.

### 4. Magnesium (Mg)

Magnesium sulphate is usually applied for redressing the deficiency. The malady can be readily corrected as foliar spray @ 2% of  $MgSO_4$ .

### 5. Calcium (Ca)

Calcium Ammonium Nitrate (CAN) or superphosphate is supplied in deficient soils.

In Indian soils, Ca deficiency is not a serious problem.

### 6. Sulphur (S)

Common fertilizers used for supplying nitrogen and phosphorus contain appreciable amount of sulphur sufficient to meet the crop requirement.

In case of severe deficiency, gypsum is added to the soil @ 500 kg/ha.

### 7. Iron (Fe)

Foliar spray of 0.5% ferrous sulphate along with lime (50% requirement) will remove the deficiency in the plant and soil. Chelated iron compounds such as Fe-EDTA, give a very good response in ameliorating Fe deficiency.

### 8. Manganese (Mn)

Foliar spray of 0.5% manganous sulphate plus 50% lime requirement is quite effective and it should be applied in the early stage of the crop. Soil application of 15 - 30 kg  $MnSO_4$  per ha (mixed with sand) is sufficient.

#### 9. Copper (Cu)

Foliar spray of 0.5% of  $CuSO_4$  is recommended for the correction of Cu deficiency in crops.

#### 10. Zinc (Zn)

Foliar spray of 0.5%  $ZnSO_4$  twice at 7-10 days interval during early stages of growth will alleviate the problem. Also, soil application of 25 kg  $ZnSO_4$  per ha is also found beneficial.

#### 11. Molybdenum (Mo)

The Mo deficiency is commonly found in cauliflower, legumes, oats and other brassicas which can be corrected by soil application of 0.5 to 1.0 kg/ha sodium or ammonium molybdate or by its foliar spray @ 0.01 - 0.02% conc. The deficiency can be avoided with the seed treatment @ 0.03% sodium or ammonium molybdate.

#### 12. Boron (B)

Foliar spray of 0.2% borax or boric acid will be effective for quick recovery. Liming of soil should be strictly avoided when boron-containing fertilizers are applied.

### ABSORPTION AND TRANSLOCATION OF MINERAL SALTS

*The uptake of minerals by the plants through the root system from the soil is called absorption of mineral salts.* The minerals are absorbed by plants in the form of ions present in the soil solution. The ions may be positively charged *cations* or negatively charged *anions*. The common cations are K, Mg, Ca, Fe, Mn, Cu, Zn, Co etc.; the common anions are P, B, S and Cl.

The ions are absorbed mainly through the *elongation zone* of the root. They are transported through the *xylem and phloem*. The transport of minerals inside the plant is called *translocation* of mineral salts.

#### Soil Solution

*The soil water containing minerals is called soil solution.* The soil solution contains the minerals in the form of *ions*. *Ion is an electrically charged atom.* The ions exist in the soil solution in two forms, namely *cations* and *anions*. Cations are *positively charged* atoms and anions are *negatively charged* atoms. The important cations required for plants' and present in the soil are K, Mg, Ca, Fe, Mn, Cu, Zn and Co. The important anions are P, B, S and Cl.

The ions may be *monovalent* or *bivalent* or *polyvalent*.  $H^+$ ,  $K^+$ ,  $Na^+$  etc are monovalent ions.  $Ca^{++}$  and  $Mg^{++}$  are bivalent ions. Mineral salts are found either as soluble fractions of soil solution or as adsorbed ions on the surface of colloid particles. The clay crystals are in the form of *colloids*. They contain a central core called *micelle*. The micelle contains negatively charged ions and hence the micelle is *negatively charged*. In order to maintain an electrostatic

balance, it attracts *positively charged ions* on the surface of the clay crystals. There will be always equal number of + and - ions on the crystal.

#### Site of Mineral Absorption

Minerals are absorbed by the *roots*. The young root consists of *root cap*, *root apex*, *meristematic zone*, *elongation zone*, *root hair zone* and *maturation zone*. Bar - Yosef (1972) and others believed that mineral absorption occurs effectively through *elongation zone* and *maturation zone*. But, *Neyand Tinker* (1977) believed that mineral absorption occurs through the *entire surface of root*. In the root, minerals are absorbed by the cells of *epiblema* and not by the root hairs.

#### Mechanism of Mineral Absorption

*The uptake of mineral salts from the soil by the plant through the root system is called mineral absorption*. The minerals required by plants are present in the *soil solution*. The minerals are present in the form of *ions* in the soil solution. The important ions absorbed by plants are  $H^+$ ,  $K^+$ ,  $Na^+$ ,  $Ca^{++}$ ,  $Mg^{++}$ ,  $Cl^-$ ,  $P^-$  etc.

From the soil solution, the ions penetrate into the root cells. The entry of one type of ion into the cell must be accompanied by the entry of another type of ion of equal electrostatic charge. That is, equal amount of anions and cations are absorbed. The monovalent cations such as  $K^+$ ,  $Na^+$  etc. are more rapidly absorbed than salts of bivalent or polyvalent cations such as  $Ca^{++}$ ,  $Mg^{++}$  etc.

Sometimes, plants take up different amounts of anions and cations. In such cases of unbalanced ratio of cations and anions; the equilibrium is maintained by certain changes in the ionic composition of cell sap and the salt solution. For example, when cation uptake is more, the cell produces organic acids ( $R-COO^+$ ). The anions ( $R-COO^-$ ) of organic acids remain in the cell and the cations ( $H^+$ ) of organic acids move out into the external medium to compensate for the cations taken in.

In some cases, the equilibrium is maintained by  $H^+$  and  $OH^+$  ions of water available in the cell. *Root* is the site of absorption. The mineral ions pass through the *epiblema* or *epidermis* of roots. From the epidermis, the minerals pass through the *cortical cells* and reach the xylem vessels. On the way to the xylem, minerals have to cross the *cell walls (apoplast)*, the *cytoplasm (symplast)* and the *vacuoles*.

The absorption of minerals takes place in two main methods. They are *passive absorption* and *active absorption*.

#### I. Passive Absorption

Passive absorption is defined as the absorption of minerals according to *ordinary laws of diffusion without the expenditure of energy*. It is also called *physical absorption*. Passive absorption does not require the expenditure of energy. The ions move from the region of higher concentration of ions to the region of lower concentration of ions. Hence, the absorption is *along the concentration gradients*.

There are four concepts to explain the mechanism of passive absorption.

1. Outer free space theory
2. Mass flow theory
3. Ion exchange theory
4. Donnan equilibrium

#### 1. Outer Free Space Theory or Diffusion Theory

*Salt absorption through the intimate contact of the root system with the soil solution is called outer free space theory*. The term *outer free space* refers to the volume of a plant tissue into which the mineral ions can move by diffusion. It includes all *primary cell walls* and

*intercellular spaces* in the cortical tissue of the root. The ions move freely in and out of this tissue. Because of this *free diffusion*, the ion concentration of this tissue and the soil solution will be the same.

## 2. Mass Flow Theory

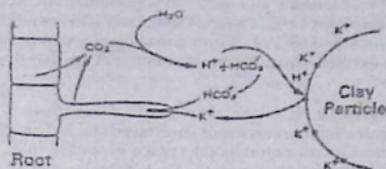
According to this theory, ions are absorbed by the roots along with the mass flow of water under the influence of transpiration. Kramer (1956) proposed this theory of passive absorption.

## 3. Ion Exchange Theory

According to ion exchange theory, root absorbs cations or anions from the soil solution in exchange of its own ions. It is a *passive absorption*. The ions adsorbed on the surface of the walls or membranes of root cells may be exchanged with the ions of same sign from external solution. For example the cation,  $K^+$  of the external soil solution may, be exchanged with  $H^+$  ion adsorbed on the surface of the root cells. Similarly an anion may be exchanged with  $OH^-$  ion. There are two theories explaining the mechanism of ion exchange. They are (a) Contact exchange theory, (b) Carbonic acid exchange theory.

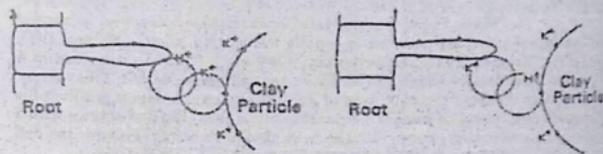
(a) **Contact Exchange Theory:** According to contact exchange theory, the ions attached on the clay particles and root surface are absorbed into the root in exchange of ions between them. The ions adsorbed on the surface of root cells and clay particles are not tightly bound. They are oscillating within small volume of space. These ions may be exchanged between the root cell membrane and clay particles if the root and clay particles are within the oscillating volume. Suppose  $H^+$  is present on the root cell surface and  $K^+$  is present on the clay particle, both the ions oscillate. This will result in the transfer of  $H^+$  to the clay particle and  $K^+$  to the root surface. Thus in contact exchange, the attached ions are absorbed and ions dissolved in the soil solution are not absorbed.

(b) **Carbonic Acid Exchange Theory:** According to carbonic acid exchange theory, the carbonic acid ions dissolved in the soil solution are absorbed by the roots in exchange of ions present in the root. Here, the dissolved ions are absorbed. The  $CO_2$  released during respiration of root cells combines with water to form carbonic acid ( $H_2CO_3$ ) in the soil solution. It dissociates into a cation,  $H^+$  and an anion  $HCO_3^-$  in soil solution. These  $H^+$  ions can be exchanged for cations adsorbed on clay particles. These cations released into the soil solution from the clay particles may be adsorbed on root cells in exchange for similar ions.



Mode of ion absorption through carbonic acid exchange.

Ionic exchange operates at the root end to absorb ions from the soil. Two of the theories are (i) Carbon dioxide hypothesis and (ii) Contact exchange hypothesis (Fig. 12.5 & 6).



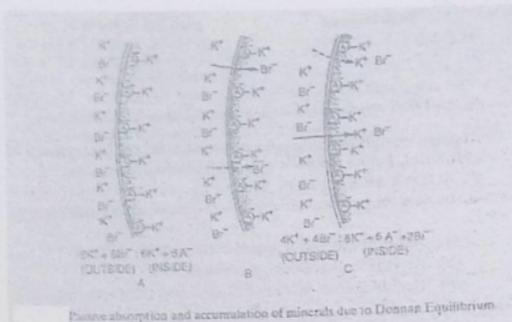
Mode of ion absorption through contact exchange.

#### 4. Donnan Equilibrium:

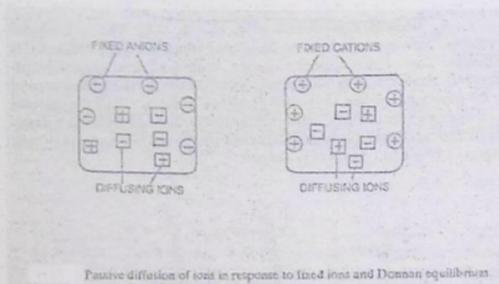
*Donnan equilibrium is a kind of equilibrium in which the products of the same pair of ions on two sides of the permeable membrane become same.* It explains how difference of ions can be established on two sides of a membrane. At Donnan's equilibrium, the number of positive ions inside equals the number of positive ions outside or vice versa.

There are certain pre-existing ions inside the cell which cannot diffuse outside through membrane. Such ions are called as *indiffusible or fixed ions*. These fixed ions may be cations or anions. However, the membrane is permeable to both anions and cations of the outer solution. When the cell is in contact with the *soil* solution containing anions and cations, equal number of anions and cations diffuse into the cell. If the cell contains fixed anions, to balance the fixed anions, more cations will diffuse into the cell. In this case, there would be an accumulation of cations inside the cell.

If there are fixed cations inside the cell, more anions will diffuse into the cell resulting in the accumulation of anions inside the cell. Thus, the donnan equilibrium brings out the accumulation of ions inside the cell against a concentration gradient with out the involvement of metabolic energy.



In Donnan Equilibrium the multiple of cations and anions in the outside solution becomes equal to that present in the inside.



In this particular example  $(K^+)_o X (Br^-)_o = (K^+)_i X (Br^-)_i$

$$\text{or } \frac{(K^+)_o}{(K^+)_i} = \frac{(Br^-)_i}{(Br^-)_o}$$

Donnan equilibrium is a good mechanism for accumulation ions. However it does not allow selectivity between similar ions.

#### Objections to Passive Absorption

1. The cell sap in many plants accumulates large quantities of mineral ions *against* the concentration gradient. In the alga *Nitella*, the cell sap accumulates  $K^+$  and phosphate ions to concentrations thousand times greater than in the pond water. This can not be explained by diffusion or other theories of passive absorption.
2. Further, ion absorption was found to be higher even when the rate of transpiration is low.

#### II. Active Absorption

The absorption of ions against the concentration gradient and with the expenditure of metabolic energy is known as active absorption. The active absorption is controlled by the activity of the cell. It is related to metabolism, respiration and the expense of energy.

Active absorption is similar to an uphill transport. The uptake of minerals by active absorption is evidenced by a number of facts.

1. The diffusing substance is at a higher concentration within the cell and is at a lower concentration outside the cell.
2. The concentration of K in the cells of *Nitella* is 1000 times more than that of the

surrounding water.

3. The rate of absorption is too rapid which cannot be explained by passive absorption.
4. A number of factors which affect metabolism also affect transpiration. E.g. Low temperature, low levels of  $O_2$  etc.
5. When a plant is transferred to a salt solution, the salt up - take is increased along with increased respiration. This increased rate of respiration in salt solution is called *salt respiration*.

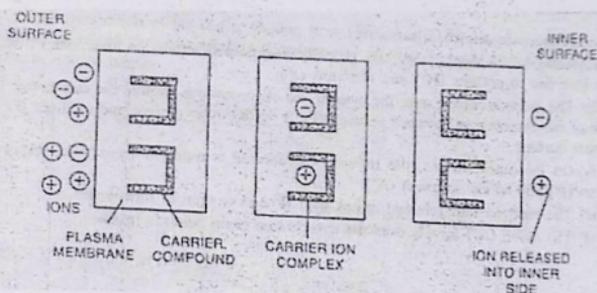
A number of theories are proposed to explain the mechanism of active absorption as follows.

1. The carrier concept
2. Cytochrome pump theory
3. Protein - lecithin theory.

### 1. The Carrier Concept

According to the carrier concept, the mineral is carried into the cell by a molecule present in the plasma membrane. The compound which carries the minerals is called *carrier molecule*.

It is formulated by *Van den Honert* in 1937, The carrier concept explains the mechanism of *active transport*. According to the theory, the-plasma membrane is impermeable to free ions. But some compound present in it, acts as a carrier. This carrier molecule combines with ions to form *carrier-ion-complex*, which can move across the membrane. On the inner surface of the membrane this complex unbounds releasing ions into the cell. On releasing the ion, the carrier molecule goes back to the outer surface again to pick up fresh ions. The *cytochrome pump theory* and the *protein-lecithin theory* are proposed based on carrier concept.

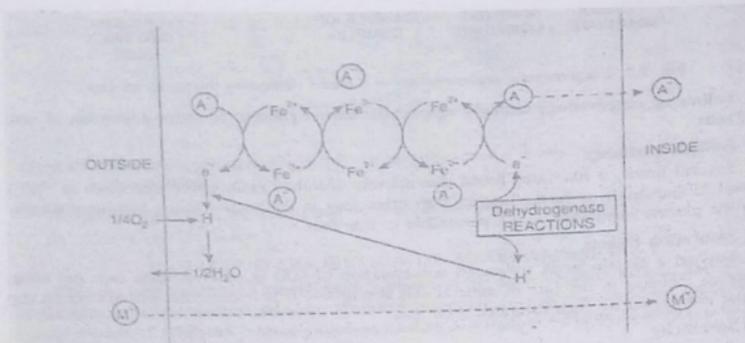


Diagrammatic representation of a model illustrating the carrier concept.

### 2. Cytochrome Pump Theory

The *cytochrome pump theory* says that the minerals are carried into the cell by a carrier molecule called *cytochrome* present in the plasma membrane. It is proposed by *Lundegardh* and *Burdstrom* (1933) as a *carrier concept* to explain the mechanism of active transport of minerals.

They believed that there was a *definite correlation between respiration and anion absorption*. When a plant is transferred from water to a salt solution, the respiration is increased. This increased respiration in salt solution is called *salt respiration* or *anion respiration*. At this time, the anion absorption is increased. *Lundegardh* (1950) proposed the *cytochrome pump theory* based on his observations on salt respiration. Salt respiration and the absorption of anions are inhibited by CO and cyanides, the inhibitors of cytochrome oxidase of electron transport chain in mitochondria.



Diagrammatic representation of the Lundegardh's cytochrome pump theory

This theory explains the absorption of minerals as follows:

1. Dehydrogenase reactions on the inner side of the membrane give rise to protons ( $H^+$ ) and electrons ( $e^-$ ).
2. The electron travels over the cytochrome chain towards outside the membrane so that the  $Fe^{3+}$  ferrous ion of the cytochrome becomes reduced  $Fe^{2+}$  on the outer surface.
3. On the outer surface, the reduced cytochrome is oxidized by oxygen releasing the electron ( $e^-$ ) and taking an anion.
4. The electron thus released unites with  $H^+$  and oxygen to form water.
5. The anion travels over the cytochrome chain towards inside.
6. On the inner surface, the oxidised cytochrome becomes reduced by taking an electron produced through the dehydrogenase reactions and the anion is released.
7. As a result of anion absorption, a cation ( $M^+$ ) moves *passively* from outside to inside to balance the anion.

#### Demerits of cytochrome pump theory

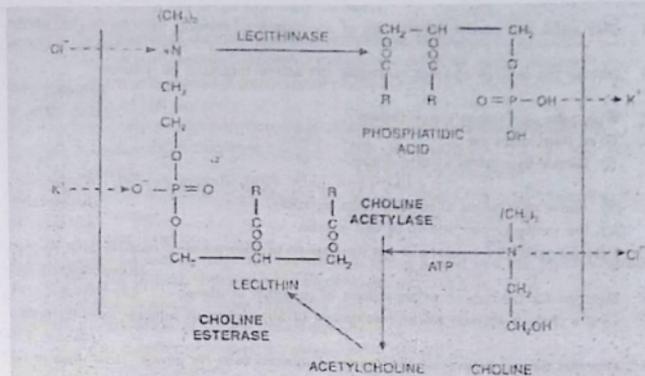
- a. It explains the active absorption of only anions
- b. It does not explain the selective uptake of ions
- c. It has been found that cations also stimulate respiration.

#### 3. Protein - Lecithin theory

The protein - lecithin theory says that the minerals are carried into the cell by a carrier molecule called lecithin present in the plasma membrane. This theory was proposed by Bennet Clark (1956). Lecithin is a phospholipid and since it is *amphoteric* in nature, it can bind with both cations and anions. In the transport of ions, lecithin is hydrolysed and synthesized in a cyclic manner.

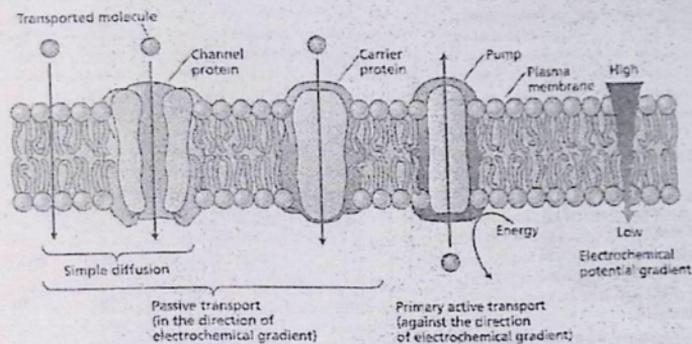
Lecithin picks up ions (both cations and anions) from the soil solution to form a *lecithin ion complex*. The lecithin ion complex moves to the inner side of plasma membrane. In the inner side of the membrane the lecithin ion complex is split into *phosphatidic acid* and *choline* by the enzyme *lecithinase*. This splitting releases the ions.

After releasing the ions, phosphatidic acid combines with choline to form *acetyl choline*. This reaction is catalysed by *choline acetylase* and this reaction utilizes ATP. The acetyl choline is converted into lecithin by the enzyme *choline esterase*.



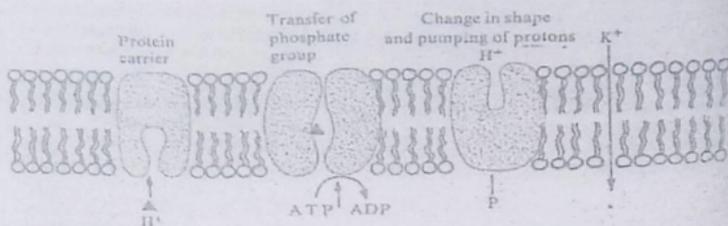
Diagrammatic representation of the Bennet-Clark's Protein-Lecithin theory

### SOME OF THE MODERN CONCEPTS OF MINERAL ABSORPTION AND UPTAKE:



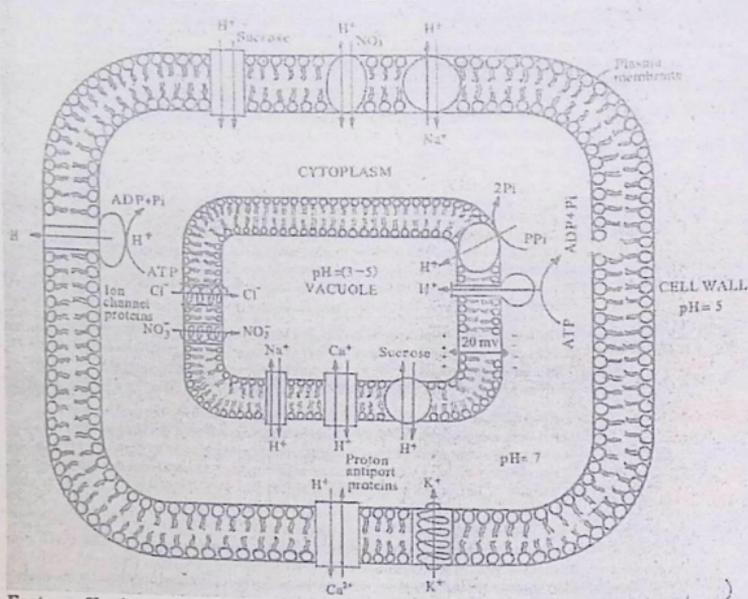
Three classes of membrane transport proteins: channels, carriers, and pumps. Channels and carriers can mediate the passive transport of solutes across membranes (by simple diffusion or facilitated diffusion), down the solute's gradient of electrochemical potential. Channel proteins act as membrane pores, and their specificity is determined primarily by the biophysical properties of the channel. Carrier proteins bind the transported molecule on one side of the membrane and release it on the other side. Primary active transport is carried out by pumps and uses energy directly, usually from ATP hydrolysis, to pump solutes against their gradient of electrochemical potential.

### Simplified diagram showing active transport:



Simplified picture of active transport. Transport of proton across the membrane is coupled with transport of other cations in opposite direction. The transport protein receives an energy boost from ATPase and thereby undergoes changes in its shape that are necessary to the transport process.

### Transport of ions across the cell and vacuole membranes:



### Factors affecting salt absorption

#### I. External factors

(i) **Temperature:** Absorption of salt is affected by change in temperature. In general, an increase in temperature results in increased absorption of salts up to certain optimum level. At higher temperature, the absorption is inhibited. The change in the temperature also affects the processes of diffusion.

(ii) **pH:** Change of pH of the soil solution affects the availability of ions to the plants. Decrease in the pH of soil solution accelerates the absorption of anions but increase in pH will

favour the absorption of cations. However, pH beyond the optimum level (higher or lower) may damage the plant tissues and inhibit the salt absorption.

(iii)Light: It has no direct effect, but indirectly affecting the transpiration and photosynthesis so that the salt absorption is affected

(iv)Oxygen: Absence of oxygen will inhibit the active salt absorption.

(v)Interaction: The absorption of one ion is influenced by the presence of other ions in the medium. Viets (1944) demonstrated that the absorption of potassium is affected by the presence of calcium, magnesium and other polyvalent cations in the soil solution. Epstein (1978) demonstrated the interaction of several ions as competitors for binding sites on carriers.

## II. Internal factors

(i)Growth: Cell division, elongation and developmental processes promote the absorption of salt.

(ii) Aging: As the root matures, it increases the surface area, which is favorable for salt absorption.

### Transport of Minerals

Both xylem and phloem take part in the transport of mineral salts. Upward movement from roots to the shoot occurs through xylem. Both upward and downward movement from mature leaves occur through phloem.

### Translocation of salts in the xylem

The minerals for the most part are transported in the xylem. The transport of minerals in the xylem occurs along with the transpiration stream. Analysis of xylem sap also shows the presence of large amount of dissolved salts. These include not only inorganic salts but organic compounds of nitrogen and phosphorus. Such organic compounds are largely responsible for the movement of nitrogen and phosphorus.

A radioactive experiment was conducted by *Stout and Hoagland* (1939) to prove the upward movement of salts through xylem. The experiment was carried out on a willow plant. The xylem and phloem were carefully separated from each other along a 9 inch length of willow stem. A sheet of paraffined paper was inserted between the two tissues. Then, potassium  $K^{42}$  was fed to the plant via the nutrient solution. Practically, all the tracer elements were found in the xylem which shows that upward transport occurred in the xylem. Above and below the stripped area there were considerable amounts of  $K^{42}$  in both xylem and phloem. This indicates the lateral transfer of salts into phloem.

### Lateral transport

Some of the minerals are transferred radially from xylem to the phloem mainly through parenchymatous cells. They are also transported to other tissues where they accumulate in the living cells.

### Translocation of salts in the phloem

Minerals are transported through phloem also. The translocation in phloem mainly occurs from the leaves just before abscission. These minerals which move out of the leaf before abscission are nitrogen, potassium, phosphorus, sulphur and chlorine. The movement of ions in the phloem occurs both upward and downward into the younger leaves and to apical regions of stem and root.

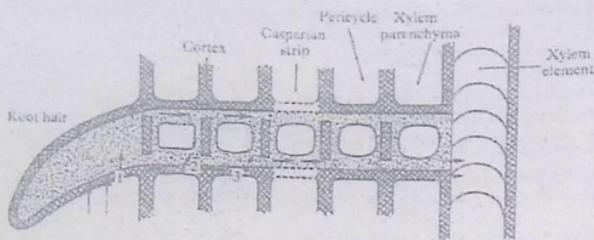
### Mechanism of Translocation

The absorbed ions diffuse freely into the root across the *epiblema*, *cortex* and finally reach the *endodermis*. Further movement is retarded by the *casparian* strip present in the

endodermis. The movement of minerals across the living tissues does not require metabolic energy. The ions are passively translocated through the wet cell walls and plasmodesmata of the cortex and to the endodermis. Then, the ions are released into the xylem from the endodermis. This is called *symplast* theory of ion uptake.

The ion movement from endodermis to xylem elements is an *active process*. It requires the involvement of metabolic energy which comes from respiration. The concentration of xylem sap remains usually greater than those of the surrounding cells. It requires extra energy because the ion movement from endodermis to xylem is against concentration gradient. Due to special casparian strips the ions are directly diffused to xylem elements. Involvement of special cells called *transfer cells* in the movement of salts into the xylem elements has been suggested by Gunning (1972).

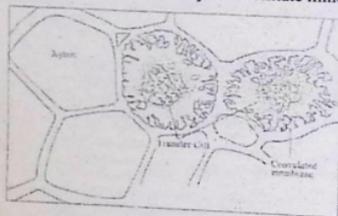
The minerals entering into xylem are translocated upward along the transpiration stream. The minerals are radially transported into phloem through the cambial cells. Some of the minerals are still transported through phloem along with the organic solutes and *some* of them accumulate in the living tissues of parenchyma.



Schematic drawing showing the apoplast and symplast in cross-section of a root. The heavily stippled dark regions constitute the apoplast while the lightly stippled regions constitute the symplast. The vacuole is not part of either system. The Casparian strip creates a discontinuity in the apoplast. Therefore, all ions absorbed by root hairs must cross the plasma membrane of a cell (1, 2 or 3) exterior to the Casparian strip, thereby entering the symplast.

#### Transfer Cells:

The transfer cells are special cells, which take part in trans-membrane flux of solutes. Transfer cells found in the xylem facilitate mineral absorption.



Xylem transfer cells.

## TRANSPORT OF WATER AND MINERAL IONS IN PLANTS

From both a physiological and a structural point of view, transport activities in the plant may be divided into two parts.

1. Transport of water and nutrients through cells. Examples of this include the movement of water from the soil solution through root cells to the vascular system and the movement of water from the vascular system through leaf cells to the intercellular spaces. Water moves for only short distances through cells. Short-distance transport, which is important in cell to cell interactions, probably takes place mostly by diffusion through plasmodesmata. In some cases, short-distance transport is an active process requiring energy expenditure by the cells.
2. Transport between roots and leaves. Long-distance movement of water and nutrients is mainly through the xylem tracheids and vessels and the phloem sieve elements of the vascular system. The xylem tracheids and vessels, which are dead cells, can be envisioned as a series of water-filled tubes. In herbaceous plants the distance of water movement through the xylem may be only centimeters while in trees the water may move through this system over 110 meters. The aqueous phase that lies outside of the cellular membrane (e.g. cell walls and the conducting cells of the xylem) are considered to represent the apoplast.

## UPWARD TRANSPORT OF WATER AND MINERAL IONS

### ASCENT OF SAP

*The upward movement of water from the root towards the top of the plant is known as ascent of sap.* The root is the absorbing end of a plant where water absorption is accomplished in land plants. The aerial parts of a plant, especially the younger ones bearing leaves, consume most of the absorbed water in transpiration. On account of this, large quantities of water pass from the root end to the top of the plant against the force of gravity. The distance is sometimes about 100-130 metres as in case of plants like *Eucalyptus*, *Sequoia*, *Pinus* and other conifers.

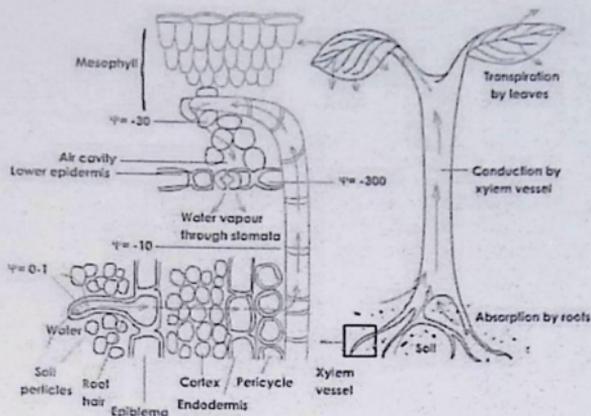
The rate of ascent of sap may be upto  $75 \text{ cm min}^{-1}$  for rapidly transpiring dicots (Huber, 1932) and upto  $10 \text{ cm min}^{-1}$  in case of gymnosperms. It cannot be accounted for by cell to cell movement. The only other possibility is the passage through sieve tubes or xylem vessels and tracheids. The fact that phloem does not take part in the conduction of water has been known since the days of girdling experiments of Malpighi (1671). By the year 1731 (Stephen Hales), it was well recognised that water passes upwards the plant through the xylem.

### MECHANISM OF ASCENT OF SAP

#### Cohesion-Tension or Cohesion-Transpiration Pull Theory

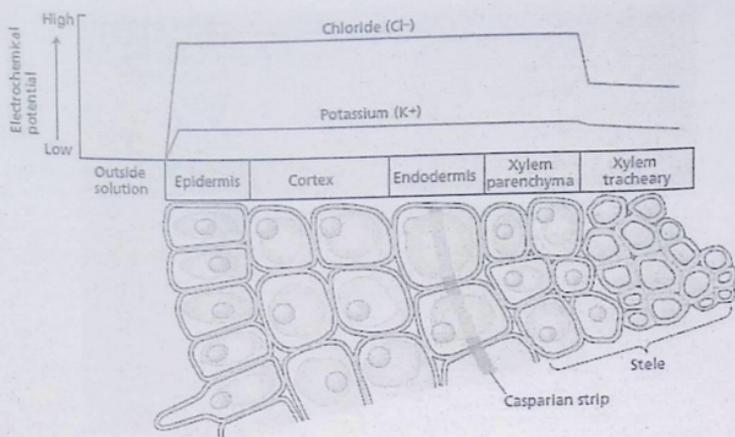
The theory was given by Dixon and Joly (1894) and further improved by Dixon (1914). It is also known as Dixon's theory of ascent of sap. The main features of the theory are:

- (i) **Continuous Water Column:** There is a continuous column of water from the root through the stem and into leaves. MacDougal (1936) has named it as hydrostatic system. It is present in tracheary elements. The latter do operate separately but from a continuous system through their unthickened areas.
- (ii) **Withdrawal of Water:** Water evaporates from the surface of mesophyll cells bordering the intercellular spaces. The water vapours pass out of the leaf through the stomata. Therefore, more water evaporates from the walls of mesophyll cells. This increases the DPD (diffusion pressure deficit) or decreases the water potential of these mesophyll cells. They withdraw water from the deeper mesophyll cells. It creates a gradient of DPD or water potential. The deeper mesophyll cells, in turn, obtain water from the parenchyma cells bordering the vascular tissues of the veins. Movement of water from xylem to mesophyll cells and then to the intercellular spaces occurs through the apoplast or water free spaces (Weatherley\*, 1963). The driving force for the movement of water is the DPD of water vapours present in the intercellular spaces which in turn is governed by DPD of the external air.
- (iii) **Development of Tension or Transpiration Pull:** Due to continuous withdrawal of water from the xylem by the transpiring leaf cells, the whole water column of the plant comes under a tension or strain -. As the tension develops due to transpiration/it is known as transpiration pull. Other terms are transpiration tension and negative hydrostatic pressure. Its value is about 20 atm.
- (iv) **Cohesion or Tensile Strength of Water:** Transpiration pull or water tension produced due to transpiration is unable to break the continuity of hydrostatic system because water molecules have a strong cohesion force. Cohesion force is the force with which water molecules cling together through their hydrogen bonding. The value of cohesion force for plant sap has been calculated to be 45-207 atm by Dixon and Joly. Due to this strong cohesion force water column does not break though it is stretched by transpiration pull. Cohesion force is, therefore, also called tensile strength. The water column does not break its connection with the xylem tube because of the adhesion force between the two. Similarly the xylem tubes are strong enough to resist collapsing when the sap contained in them is under tension.
- (v) **Ascent of Sap:** On account of the tension created by transpiration the water column of the plant is pulled up passively from below to the top of the plant like a rope. It is able to overcome both gravitational and frictional forces.



### Ions Moving through the Root Cross Both Symplastic and Apoplastic Spaces

Ion absorption by the roots is more pronounced in the root hair zone than in the meristem and elongation zones. Cells in the root hair zone have completed their elongation but have not yet begun secondary growth. The root hairs are simply extensions of specific epidermal cells that greatly increase the surface area available for ion absorption. An ion that enters a root may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may enter the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortex, an ion may either cross the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially all the way to the endodermis via the apoplast. In all cases, ions must enter the symplast before they can enter the stele, because of the presence of the Casparian strip. The apoplast forms a continuous phase from the root surface through the cortex. At the boundary between the vascular cylinder (the stele) and the cortex is a layer of specialized cells, the endodermis. As discussed in Chapters 4 and 5, a suberized cell layer in the endodermis, known as the Casparian strip, effectively blocks the entry of water and mineral ions into the stele via the apoplast. Once an ion has entered the stele through the symplastic connections across the endodermis, it continues to diffuse from cell to cell into the xylem. Finally, the ion reenters the apoplast as it diffuses into a xylem tracheid or vessel element. Again, the Casparian strip prevents the ion from diffusing back out of the root through the apoplast. The presence of the Casparian strip allows the plant to maintain a higher ionic concentration in the xylem than exists in the soil water surrounding the roots.



#### Xylem Parenchyma Cells Participate in Xylem Loading

Once ions have been taken up into the symplast of the root at the epidermis or cortex, they must be loaded into the tracheids or vessel elements of the stele to be translocated to the shoot. The stele consists of dead tracheary elements and the living xylem parenchyma. Because the xylem tracheary elements are dead cells, they lack cytoplasmic continuity with surrounding xylem parenchyma. To enter the tracheary elements, the ions must exit the symplast by crossing a plasma membrane a second time. The process whereby ions exit the symplast and enter the conducting cells of the xylem is called xylem loading. The mechanism of xylem loading has long baffled scientists. Ions could enter the tracheids and vessel elements of the xylem by simple passive diffusion. In this case, the movement of ions from the root surface to the xylem would take only a single step requiring metabolic energy. The site of this single-step, energy-dependent uptake would be the plasma membrane surfaces of the root epidermal, cortical, or endodermal cells. According to the passive-diffusion model, ions move passively into the stele via the symplast down a gradient of electrochemical potential, and then leak out of the living cells of the stele (possibly because of lower oxygen availability in the interior of the root) into the nonliving conducting cells of the xylem. Support for the passive-diffusion model was provided by use of ion-specific microelectrodes to measure the electrochemical potentials of various ions across maize roots (Dunlop and Bowling 1971). Data from this and other studies indicate that K<sup>+</sup>, Cl<sup>-</sup>, Na<sup>+</sup>, SO<sub>4</sub><sup>2-</sup>, and NO<sub>3</sub><sup>-</sup> are all taken up actively by the epidermal and cortical cells and are maintained in the xylem against a gradient of electrochemical potential when compared with the external medium (Lüttge and Higinbotham 1979). However, none of these ions is at a higher electrochemical potential in the xylem than in the cortex or living portions of the stele. Therefore, the final movement of ions into the xylem could be due to passive diffusion. However, other observations have led to the view that this final step of xylem loading may also involve active processes within the stele (Lüttge and Higinbotham 1979). Recent biochemical studies have

supported a role for the xylem parenchyma cells in xylem loading. The plasmamembranes of xylem parenchyma cells contain proton pumps, water channels, and a variety of ion channels specialized for influx or efflux (Maathuis et al. 1997). In barley xylem parenchyma, two types of cation efflux channels have been identified:  $K^+$ -specific efflux channels and nonselective cation efflux channels. These channels are regulated by both the membrane potential and the cytosolic calcium concentration (De Boer and Wegner 1997). This finding suggests that the flux of ions from the xylem parenchyma cells into the xylem tracheary elements, rather than being due to simple leakage, is under tight metabolic control through regulation of the plasma membrane  $H^+$ -ATPase and ion efflux channels.

### The Crafts-Brayer Hypothesis

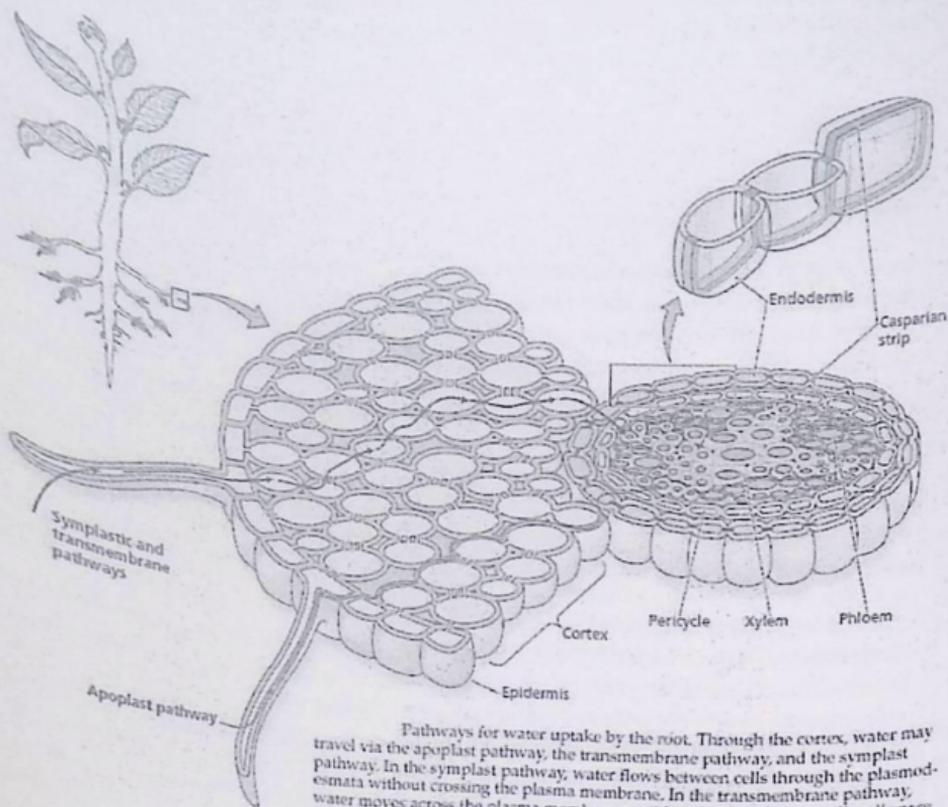
Despite lot of research on absorption and movement of mineral solutes and water in plants, the mechanisms of the processes remain obscure (Crafts and Broyer 1938). This process of secretion (into the xylem vessels), if it may be so called, is one of the least known of all processes in the realm of mineral translocation (Biddulph 1951).

Crafts and Broyer developed a hypothesis for lateral transfer of ions into the conducting elements of the xylem. Since xylem exudates commonly contain salt at a higher concentration than that of the external solution, it is obvious that an active ion transport mechanism is operating somewhere between the external solution and the non-living xylem vessels. Evidence for this comes also from experiments showing that interference with the metabolism of the root by various means inhibits transfer of ions to the vascular tissue and the shoot.

According to Crafts and Broyer (1938), absorption of ions into the cytoplasm of cortical cells is the sole active transport step responsible for the build-up of high xylem concentrations of salt. According to this hypothesis, ions are actively absorbed by the cells of the cortex. Once in the cytoplasm they diffuse within the symplast, moving from cell to cell through plasmodesmata. After reaching the stele, they leak out of the cytoplasm across the permeable plasma membranes of stelar cells into the cell walls and xylem vessels. They are kept from diffusing back into the external solution through cell wall space by the Casparian strip in the walls of the endodermis.

The cardinal feature of the hypothesis is the assumption that active transport into the cytoplasm of epidermal and cortical cells represents the sole metabolic collection step in transfer of ions into the xylem. After that, movement within the symplast is passive, following a diffusion gradient. As a result, concentrations of salt in the xylem are lower than those in the cytoplasm of the cortical cells, but often higher than those in the external solution. These conditions favour an osmotic flow of water into the xylem, resulting in the development of root pressure. The idea that the loading of nutrients into the stellar apoplast and the xylem vessels is a passive process still has adherents (Kohler and Raschke 2000).

Despite the success of the Crafts-Breyer hypothesis in accounting for various features of the lateral transfer of ions across the root into the xylem, ion transport through xylem is not fully understood.



Pathways for water uptake by the root. Through the cortex, water may travel via the apoplast pathway, the transmembrane pathway, and the symplast pathway. In the symplast pathway, water flows between cells through the plasmodesmata without crossing the plasma membrane. In the transmembrane pathway, water moves across the plasma membranes, with a short visit to the cell wall space. At the endodermis, the apoplast pathway is blocked by the Casparian strip.

## DOWN WARD TRANSPORT OF MINERAL IONS

### TRANSLOCATION OF ORGANIC SOLUTES AND MINERAL IONS

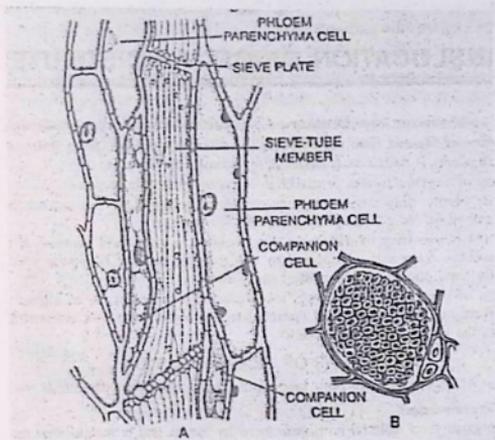
The movement of organic food materials or the solutes in soluble form, from one place to another in higher plants is called as translocation of organic solutes.

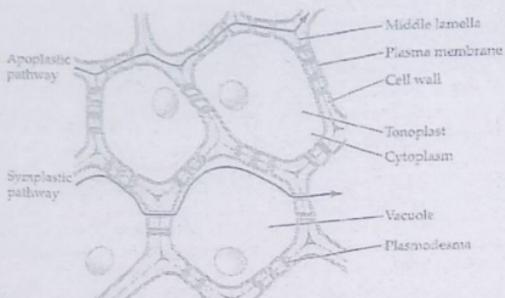
Translocation of organic solutes is essential in higher plants because:-

(i) In higher plants, only the green parts can manufacture food and it must be supplied to other non-green parts for consumption and also for storage.

(ii) During the germination of the seeds, the insoluble reserve food material of the seed is converted into soluble form and is supplied to the growing regions of young seedling till it has developed its own photosynthetic system i.e., leaves.

Translocation of organic solutes always takes place from the region of higher concentration of soluble form i.e., the supply end (source) to the region of lower concentration of its soluble form i.e., the consumption end (sink).





Part of a Traverse Section of a Root Cortex Indicating the Symplastic and Apoplastic Pathways of Water Movement After Esau (1977).

### PATH OF THE TRANSLOCATION OF ORGANIC SOLUTES

Path of Downward Translocation

Downward translocation of the organic solutes takes place through phloem.

Path of upward translocation

There has been controversy regarding the path of upward translocation of organic solutes in plants. Although translocation of organic solutes takes place through phloem, but under certain conditions it may take place through xylem.

Path of Radial Translocation

Radial translocation of organic solutes from pith to cortex takes place through medullary rays.

### MECHANISM OF TRANSLOCATION THROUGH PHLOEM

Various theories have been put forward to explain the mechanism of phloem conduction but they are not fully satisfactory. Among them Munch's (1930) hypothesis is most convincing.

#### MUNCH'S MASS FLOW OR PRESSURE FLOW HYPOTHESIS

According to this hypothesis put forward by Munch (1930) and elaborated by Craft (1938) and others, the translocation of organic solutes takes place en masse through phloem along a gradient of turgor pressure from the region of higher cone, of soluble solutes i.e., supply end to the region of lower cone. i.e., consumption end.

The principle involved in this hypothesis can be explained by a simple physical system as shown in the figure. Two membranes X and Y permeable only to water and dipping in water are connected by a tube T to form a closed system. Membrane X contains more concentrated sugar solution than in membrane Y. Due to higher osmotic pressure of the concentrated sugar solution in membrane X, water enters into it so that its turgor pressure is increased. The increase in the turgor pressure results in mass flow of sugar solution to membrane Y through the tube T till the concentration of sugar solution in both the membranes is equal.

If in the above system it could be possible to maintain continuous supply of sugars in membrane X and its utilization or conversion into insoluble form in membrane Y, the flow of sugar solution from X to Y will continue indefinitely.

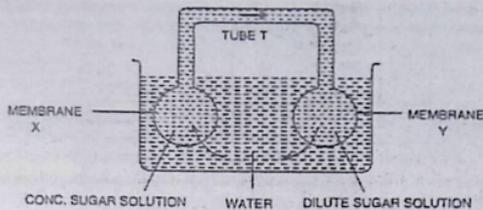
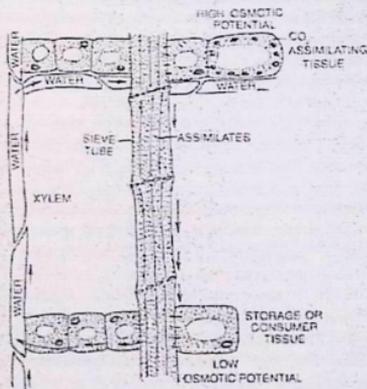


Diagram illustrating the principle of Munch's Mass Flow hypothesis.

According to Munch's hypothesis, a similar analogous system for the translocation of organic solutes exists in plants. As a result of photosynthesis, the mesophyll cells in the leaves contain higher concentration of organic food material in them in soluble form and correspond to membrane X or supply end. The cells of stem and roots where the food material is utilized or converted into insoluble form correspond to membrane Y or consumption end. While the sieve tubes in phloems which are placed end to end correspond to the tube T. Mesophyll cells draw water from the xylem of the leaf due to higher osmotic pressure and suction pressure of their sap so that their turgor pressure is increased. The turgor pressure in the cells of stem and the roots is comparatively low and hence, the soluble organic solutes begin to flow en mass, from mesophyll through phloem down to the cells of stem and the roots under the gradient of turgor pressure. In the cells of stem and the roots the organic solutes are either consumed or converted into insoluble form and the excess water is released into xylem through cambium.



#### Demerits of Munch's Hypothesis

- (1) This hypothesis accounts for the translocation in only one direction at a time, although there may be simultaneous upward and downward translocation of solutes.
- (2) There is considerable doubt regarding the magnitude of the turgor pressure at the supply end which may not be sufficient enough to overcome the resistance offered by the sieve plates in the translocation of solutes through sieve tubes.
- (3) Turgor pressure may not always be higher at the supply end.

(4) This hypothesis is based on purely physical assumptions and does not take into account the fact that whole of the translocation process is dependent upon the plant's metabolism and the metabolic energy.

#### PHLOEM LOADING AND UNLOADING

As mentioned earlier, translocation of organic solutes such as sucrose (i.e., photosynthates) takes place through sieve tube elements of phloem from supply end (or source) to consumption end (or sink). But, before this translocation of sugars could proceed, the soluble sugars must be transferred from mesophyll cells to sieve tube elements of the respective leaves. This transfer of sugars (photosynthates) from mesophyll cells to sieve tube elements in the leaf is called as phloem loading. On the other hand, the transfer of sugars (photosynthates) from sieve tube elements to the receiver cells of consumption end (i.e., sink organs) is called as phloem unloading. Both are energy requiring processes.

#### PHLOEM LOADING

As a result of photosynthesis, the sugars such as sucrose produced in mesophyll cells move to the sieve tubes of smallest veins of the leaf either directly or through only 2-3 cells depending upon the leaf anatomy. Consequently, the concentration of sugars increases in sieve tubes in comparison to the surrounding mesophyll cells.

The movement of sugars from mesophyll cells to sieve tubes of phloem may occur either through symplast (i.e., cell to cell through plasmodesmata, remaining in the cytoplasm) or the sugars may enter the apoplast (i.e., cell walls outside the protoplasts) at some point en route to phloem sieve tubes. In the latter case, the sugars are actively loaded from apoplast to sieve tubes by an energy driven transport located in the plasmamembrane of these cells. The mechanism of phloem loading in such case has been called as sucrose- $H^+$  symport or cotransport mechanism. According to this mechanism protons ( $W$ ) are pumped out through the plasmamembrane using the energy from ATP and an ATPase carrier enzyme, so that concentration of  $W$  becomes higher outside (in the apoplast) than inside the cell. Spontaneous tendency toward equilibrium causes protons to diffuse back into the cytoplasm through plasmamembrane coupled with transport of sucrose from apoplast to cytoplasm through sucrose- $H^+$  symporter located in the plasmamembrane. The mechanism of the transfer of sugars (sucrose) from mesophyll cells to apoplast is however, not known. Phloem loading is specific and selective for transport sugars.

Both symplastic and apoplastic pathways of phloem loading are used in plants but in different species. In some species however, phloem loading may occur through both the pathways in the same sieve tube element or in different sieve tube elements of the same vein or in sieve tubes in veins of different sizes.

Experimental findings have revealed certain patterns in apoplastic and symplastic loading of sugars in phloem, which appears to be related with the type of sugar transported to phloem, type of companion cells (ordinary, transfer or intermediary) and number of plasmodesmata (few or abundant) connecting the sieve tubes (including the companion cells to surrounding cells in smaller veins).

To some extent, phloem loading is also correlated with the family of plant, its habit (trees, shrubs, vines or herbs) and climate such as temperate, tropical or arid climate.

#### PHLOEM UNLOADING

It occurs in the consumption end or sink organs (such as developing roots, tubers, reproductive structures etc.) Sugars move from sieve tubes to receiver cells in the sink involving following steps:

(i) Sieve element unloading. In this process, sugars (imported from the source) leave sieve elements of sink tissues.

(ii) Short distance transport. The sugars are now transported to cells in sink by a short distance pathway which has also been called as post-sieve element transport.

(iii) Storage and metabolism. Finally, sugars are stored or metabolized in the cells of the sink. As with the phloem loading process, sucrose unloading also occurs through symplast via plasmodesmata or through apoplast at some point en route to sink cells.

Phloem unloading is typically symplastic in growing and respiring sinks such as meristems roots, and young leaves etc. in which sucrose can be rapidly metabolized. (Young leaves act as sink until their photosynthetic machinery is fully developed, at which point they become sources).

Usually, in storage organs such as fruits (grape, orange etc.), roots (sugar beet) and stems (sugarcane), sucrose unloading is known to occur through apoplast. However, according to Oparka (1986), phloem unloading in potato tubers from sieve elements to cortical cells is a symplastic passive process.

Because, there are wide varieties of sinks in plants which differ in structure and function, no one scheme of phloem unloading is available.

### ASSIMILATES PARTITIONING

The products of carbon assimilation or photosynthesis such as hexoses, sucrose, starch etc. (i.e., fixed carbon) are called as photosynthates or photoassimilates or simply as assimilates. These assimilates are produced in green leaves of higher plants which constitute the sources.

Within various compartments of photosynthesizing cells (sources), these assimilates are (i) metabolically utilized, (ii) stored or (iii) converted into transport sugars mainly sucrose for export to various sinks (through phloem) such as young leaves, roots, tubers, stems, fruits and seeds. At the sinks, assimilates are metabolically utilized and/or stored in receiver cells of sinks. Depending upon the nature and specific requirement of the sinks, the photoassimilates are differentially distributed in different sinks. This differential distribution of photoassimilates in different sinks of plant is called as assimilates partitioning.

Usually, the amount of assimilates transported to the harvest organ is much more in comparison to other organs of the plant. Therefore, transport of assimilates and their partitioning are of great research interest in agricultural plant physiology because of their roles in crop productivity.

Although attempts to increase photosynthetic activities of the leaves have met with only very little success, but the harvest index (i.e., the ratio of the harvest yield such as grains to '11e total shoot yield) or yields of many crop plants such as oats, barley, wheat, cotton, soybean, peanuts etc., has considerably been increased during recent years by sustained plant breeding efforts in selecting and developing varieties with improved transport of assimilates to edible or economically important portions of the plant.

### FACTORS CONTROLLING TRANSLOCATION AND ASSIMILATES PARTITIONING IN HIGHER PLANTS

Translocation of assimilates to different sink organs and assimilates partitioning are controlled by many factors. Some of these factors are described below:

#### 1. Competition Among Sink Tissues for Available Translocated Assimilates

Competition among various sink tissues or organs such as young leaves, stems, roots, fruits and seeds for transport sugars is an important factor in determining translocation pattern in whole plant. Experiments have shown that if a sink is removed from a plant, there is increased translocation of assimilates to other competing sinks.

Reproductive tissues such as fruits and seeds for instance, can compete with growing vegetative tissues such as young leaves and roots. On the other hand, sudden and drastic curtailing of sources (such as by shading all the leaves except one) and keeping the sinks intact in sugar beet and bean plants, resulted in increased supply of assimilates to young

leaves than to roots indicating thereby that young leaves are stronger sinks than roots in these plants.

The sink strength i.e., the ability of the sink to mobilize photosynthates or assimilates toward it depends on sink size and sink activity:

Sink strength = Sink size  $\times$  Sink activity

Sink size is the total weight of the sink tissue while sink activity is defined as the rate of uptake of assimilates per unit weight of the sink. Sink activity is in turn governed by various enzymes that are involved in metabolic utilization and storage of assimilates.

## 2. Photosynthesis and Sink Demand

Rate of photosynthesis (i.e., the net amount of carbon fixed per unit area of leaf per unit time) is strongly influenced by sink demands. A substantial increase in sink/source ratio results in increased rate of photosynthesis in the source leaves. Rate of photosynthesis declines when sink demand decreases or in other words sink/source ratio is decreased. Under such condition, rate of photosynthesis is markedly inhibited especially in those plants which usually store starch instead of sucrose during the day.

It is believed that under reduced sink demand in plant, assimilates pile up in the leaves (sources) which cause product inhibition of photosynthetic reactions.

## 3. Long Distance Signals Between Sources and Sinks

The signals between sources and sinks may be physical such as turgor pressure or chemical such as phytohormones (plant growth regulators).

### (i) Turgor Pressure

Rapid phloem unloading results in decrease of turgor pressure in sieve elements of phloem in sink tissues which is transmitted to the sources via interconnecting system of sieve elements. Consequently, rapid phloem loading occurs in sieve elements of phloem at the sources which increases their turgor and the translocation of assimilates from sources to sinks is increased.

Rate of translocation of assimilates or organic solutes would be decreased if phloem unloading at the sinks is slow.

It is believed that turgor affects transport of assimilates across the plasmamembranes by modifying the activities of proton pumping ATPase located in the membranes.

### (ii) Phytohormones or Plant Growth Regulators

Phytohormones such as auxin, gibberellins, cytokinins and ABA are transported throughout the plant in vascular system and evidences are now accumulating that these growth regulators might regulate source-sink relationships at least partially and affect assimilates partitioning by controlling growth of sinks, senescence of leaves and other developmental processes. The best studied cases involve remobilization of stored reserves in storage tissues such as tap roots or sugarcane stem parenchyma, from where they are directed to new, typically reproductive sinks. Formation of these new reproductive sinks is itself often under the control of growth regulators. These new sink tissues may in turn also synthesize and release growth regulators which act as strong mobilizing agents.

According to Gifford and Evans (1981), combinations of plant growth regulators may have additive, synergistic or inhibitory effects on assimilates partitioning.

## 4. Plasmodesmata

Plasmodesmata play very important role in regulating all aspects of phloem translocation including phloem loading and unloading and assimilates partitioning. Large pressure difference between the cells close plasmodesmata, the degree of closure depending upon the pressure difference. The closure of sieve pores (or sealing of plasmodesmata) by deposition of translocation, which in turn is regulated by cytoplasmic calcium level, markedly inhibits

## MINERAL ION TRANSPORTERS

### Molecular Biology of Plant-Nutrient Relations

At the beginning of the 21st century, molecular research-particularly, the characterization of DNA and protein sequences-dominates biology. Molecular biologists have identified genes coding for nutrient transport and enzymes participating in nutrient assimilation in a number of organisms, and are studying the variation in, and expression of these genes. We will now introduce the molecular biology of the major types of mineral transporters in plants, their homology to transporters in other organisms, and the expression patterns of the genes encoding them. The following discussion uses terms associated with membrane transport that were introduced in Chapter 4. To refresh the reader's memory, a membrane pump is a protein that moves a solute across a membrane against its electrochemical potential gradient by coupling this solute movement directly to the metabolic release of energy from the hydrolysis of ATP. Pumping solutes such as protons out of the cytoplasm generates an electrochemical gradient that can be coupled to the movement of other solutes. Integral membrane proteins responsible for such solute movements are called carriers and channels. Carriers and channels are classified as symports, antiports, or uniports; a symport transports a solute with another one, an antiport effects the movement of one solute in exchange for another one of the same charge, and a uniports moves a solute without an accompanying one.

The order of the following sections on transporters-pumps, carriers and channels-is cations, anions, water, and other. In the section on cations, the order roughly follows the amount of information available.

### Proton Pumps

The pumping of protons ( $H^+$ ) across biological membranes against their electrochemical potential usually requires the hydrolysis of ATP to ADP to provide energy; thus, the proteins that transport protons in such a manner are called  $H^+$ -ATPases.  $H^+$ -ATPases show similarities across a broad range of organisms. For example, the  $H^+$ -ATPases of higher plants share many characteristics with those of yeast: the vacuolar  $H^+$ -ATPases in yeast and plants have similar numbers of analogous subunits organized in comparable structures; yeast grow normally when a plasma membrane  $H^+$ -ATPase from a plant (*Nicotiana plumbaginifolia*) replaces their native forms (Morsomme and Boutry 2000). Consequently, the structure and function of vacuolar and plasma membrane  $H^+$ -ATPases from yeast serve as models for their plant equivalents.

Plants have multiple genes coding for plasma membrane and vacuolar  $H^+$ -ATPases. *Arabidopsis*, a higher plant with a very small genome, has at least five distinct genes for a subunit (subunit c) of the vacuolar  $H^+$ -ATPase (Kluge et al., 2003) and at least ten distinct genes for plasmamembrane  $H^+$ -ATPases (Arango et al. 2003). Different genes may be expressed in different tissues. In *Arabidopsis*, for example, two of the forms for subunit c of the vacuolar  $H^+$ -ATPase are constitutively expressed in all tissues, whereas one form is restricted to root and shoot apices. Also in *Arabidopsis*, one form of plasma membrane  $H^+$ -ATPase is specific to the root hairs, another to the phloem, and another to developing seeds.

Regulation of proton pumps occurs during transcription, translation, and post-translation (Portillo 2000). Transcription-of mRNAs-for these pumps responds to environmental cues; for example, salinity accelerates transcription of mRNA for both vacuolar and plasma membrane  $H^+$ -ATPases. Long leader sequences in the mRNAs for plasma membrane  $H^+$ -

ATPases seem to regulate their translation into protein. Regulation of enzyme activity (i.e., posttranslational regulation) occurs in many molecular regions of the pumps. Perhaps best characterized are the modifications to the carboxy-terminal region of plasma membrane  $H^+$ -ATPases that stimulate pumping. Examples of these modifications include the removal of amino acids from this end, the addition of the fungal phytotoxin fusicoecin, or the binding of certain acidic proteins called 14-3-3 proteins (Palmgren 2001). Nonetheless, a comprehensive framework for the regulation of proton pumps is still lacking.

### Potassium Transporters

The initial evidence for potassium and other transporters came from kinetic evidence, as discussed in Chapter 4. Molecular biology has revolutionized our knowledge of ion transport and continues to do so at a rapid rate. Potassium transporters of many types are found throughout a plant (Very and Sentenac 2003). They fall into three categories: inward-rectifying channels that provide unidirectional passage for  $K^+$  into a membrane-bound compartment, outward-rectifying channels that permit unidirectional flow  $K^+$  out of a compartment, and high-affinity carriers that may be energized by  $Na^+$  (Schachtman, 2000).

The inward-rectifying  $K^+$  channels KAT1 (Anderson et al. 1992) and AKT1 (Sentenac et al. 1992) of *Arabidopsis* were among the first membrane transport systems characterized in plants at the molecular level because of their similarity to  $K^+$  channels from prokaryotes and animals (Chapter 4). Interestingly, the homologous channels in animals are outward-rectifying, although a change in a single amino acid near the C terminus (carboxyl end of the protein) can convert them into inward-rectifying channels. In plants, domains near the N terminus (amino end of the protein) determine inward versus outward rectification. Plant inward-rectifying  $K^+$  channels are normally formed by four identical subunits, but injecting *Xenopus* oocytes (the cells that give rise to eggs in this species of frog) with RNA encoding a mix of different  $K^+$  channels produced channels composed of combinations of different subunits that were fully functional (Schachtman 2000). This further attests to the similarities among these channels. The genes for *Arabidopsis* KAT1 and its potato homologue KST1 are expressed primarily in guard cells; the channels are involved in stomatal opening and closing. Expression of the gene for *Arabidopsis* AKT1 is restricted to the roots, and this channel is responsible, together with another transport system that is more highly inhibited by  $NH_4^+$ , for root  $K^+$  absorption (Dennison et al. 2001).

Two types of outward-rectifying  $K^+$  channels have been identified in *Arabidopsis*. One type, typified by SKOR, is similar to the inward channels KAT1 and AKT1 described above. The gene for SKOR is expressed in root pericycle and parenchyma cells, and mutants without an active SKOR channel confirm that it functions in release of  $K^+$  to the xylem. The KCO1 channel typifies the other type of outward-rectifying  $K^+$  channel in having two pores with compositions similar to those from other organisms (Figure 10.3). Cellular  $Ca^{2+}$  levels modulate this channel; it closes at levels below  $0.1 \mu M$   $Ca^{2+}$  and opens completely at levels above  $0.3 \mu M$ .

Plants have several other high affinity  $K^+$  transporters (Maser, Gierth, and Schroeder 2002; Very and Sentenac 2003). Those of the HAK/KUP type may work in parallel with inward-

rectifying channels such as AKT1 in the absorption of soil  $K^+$  by roots. They are functionally distinct from inward-rectifying channels in that moderate levels of  $NH_4^+$  inhibit HAK/KUP transporters. Arabidopsis has at least six isoforms for HAK/KUP transporters, and low external  $K^+$  levels stimulate expression of the genes encoding them. Fu and Luan (1998) have characterized the transporter, AtKUP1. It effects both high-affinity and low-affinity potassium absorption by roots (mechanism 1 and mechanism 2, respectively; see Chapter 4), but the molecular switching of this entity between conformations with different affinities is unknown. We will see later in this chapter that a nitrate transporter also has such dual affinities, and in that case, the switching mechanism has been identified.

Another type of high-affinity transporter is HKT1 (Schachtman and Schroeder 1994). The gene encoding it is expressed in the root cortex and in leaf cells bordering vascular tissues under  $K^+$  starvation. Thus, HKT1 probably serves to scavenge any  $K^+$  that leaks from cortical cells, and to control xylem unloading of  $K^+$ . When the gene encoding HKT1 is expressed in yeast or in *Xenopus* oocytes, sodium ions ( $Na^+$ ) may energize HKT1 via a  $Na^+-K^+$  symport that couples  $K^+$  import against an unfavourable concentration gradient to  $Na^+$  import down a favorable concentration gradient, but such a response to  $Na^+$  has yet to be demonstrated in higher plants.

The linkage between the  $H^+$ -ATPase and high-affinity  $K^+$  transport has not been unequivocally established. Most likely there is a mechanism by which  $K^+$  crosses the plasma membrane accompanied by a  $H^+$  that was previously extruded by the plasma membrane  $H^+$ -ATPase (i.e., by a symport mechanism). In all likelihood, for every two  $H^+$  extruded by the plasma membrane  $H^+$ -ATPase, one would re-enter the cell together with one  $K^+$ . An antiport mechanism ( $H^+$  out,  $K^+$  in) cannot be ruled out. Finally, both of these mechanisms may exist. In any event, both are consistent with the finding, discussed in Chapter 4, that high-affinity  $K^+$  transport (into barley roots) is independent of the identity and rate of absorption of the counter ion.

### Calcium Channels and Pumps

As discussed in Chapter 8, calcium ions ( $Ca^{2+}$ ) within the cells of plants and animals serve as second messengers—that is,  $Ca^{2+}$  is an intermediary between the perception of a primary environmental stimulus and the organism's ultimate biological response (Rudd and Franklin-Tong 2001). To perform this role,  $Ca^{2+}$  in the cytoplasm is held at about 0.1 to 0.2  $\mu M$ , whereas its concentration in the surrounding compartments (i.e., the cell wall, vacuole, rough endoplasmic reticulum, and organelles) is about 1 mM, or 10,000 times higher.

The mode of action for  $Ca^{2+}$  as a messenger involves a signal such as abscisic acid (ABA) directly or indirectly opening a  $Ca^{2+}$  channel in a membrane such as the plasma membrane, and  $Ca^{2+}$  then entering the cytoplasm rapidly because of the high concentration gradient. Increased  $Ca^{2+}$  concentrations in the cytoplasm modulate the activities of many enzymes. Often this is mediated through calmodulin, a small protein that specifically binds up to four  $Ca^{2+}$  (see Chapter 8, and Snedden and Fromm 2001). Calmodulins are highly conserved among species; plant and animal forms differ by as few as 12 amino acids out of about 150. Once  $Ca^{2+}$  binds to calmodulin, the protein changes conformation (Figure 10.4), exposing a

hydrophobic patch that, in turn, attaches to specific binding sites on other cytoplasmic proteins (e.g., myosin V, kinesin, glutamate decarboxylase, and various kinases) and changes their activities. Calcium-calmodulin binding to  $\text{Ca}^{2+}$ -ATPases (ATP-driven calcium pumps) and to  $\text{H}^+/\text{Ca}^{2+}$  antiporters stimulates them to extrude calcium from the cytoplasm into the vacuole, rough endoplasmic reticulum, organelles, or the cell exterior, thus restoring a low calcium concentration within the cytoplasm.

Calcium channels are found in all biological membranes (White, 2000). Some are activated when a compartment becomes more negative (e.g., hyperpolarization of the cytoplasm) or more positive (e.g., depolarization of the cytoplasm) than its surroundings by a certain threshold. Others open in response to membrane stretching. Still others respond to chemical signals such as calcium-calmodulin or inositol triphosphate ( $\text{IP}_3$ ). Despite extensive characterization of plant  $\text{Ca}^{2+}$  channels in the plasma membrane, tonoplast (vacuolar membrane), and endoplasmic reticulum, the genes that code for them have yet to be identified (White and Broadley, 2003).

$\text{Ca}^{2+}$ -ATPases ( $\text{Ca}^{2+}$  pumps) universally serve to regulate low cytoplasmic  $\text{Ca}^{2+}$  levels and show structural similarities across diverse species (Geisler et al., 2000). *Arabidopsis* has at least 11 different  $\text{Ca}^{2+}$ -ATPases, which fall into two categories: One type (Figure 10.5), which is common to all cellular organisms, has a strong specificity for ATP as a substrate and is not influenced by calmodulin; the other type, which is unique to eukaryotes and of more recent origin, may use GTP or ITP in addition to ATP as substrates, and is calmodulin-regulated. In animals, the first type is restricted to the endoplasmic or sarcoplasmic reticulum, and the second type to the plasma membrane, whereas plants have both types in the endoplasmic reticulum and plasma membrane.

### Sodium Transporters

Plant biologists have studied sodium ( $\text{Na}^+$ ) transport primarily in relation to salinity tolerance (Maser, Gierth, and Schroeder 2002). Although  $\text{K}^+$  and  $\text{Na}^+$  are physically similar, transport systems in halophytes, or "salt-loving" plants, have the ability to maintain relatively low concentrations of  $\text{Na}^+$  and relatively high concentrations of  $\text{K}^+$  in the cytoplasm, despite a soil environment where the  $\text{Na}^+$  to  $\text{K}^+$  ratio is skewed in the opposite direction (see Chapter 11). These transporters include inward-rectifying  $\text{K}^+$  channels that are highly selective for  $\text{K}^+$  (see Chapter 4) and  $\text{Na}^+/\text{H}^+$  antiporters that couple  $\text{Na}^+$  efflux against a concentration gradient to proton influx along an electrogenically favorable gradient (Blumwald, Aharon, and Apse 2000). One  $\text{Na}^+/\text{H}^+$  antiporter, NHX1 from *Arabidopsis*, is a low-affinity transporter that probably serves to remove  $\text{Na}^+$  from the cytoplasm and store it in the vacuole (Apse et al. 1999). NHX1 is similar in both sequence and function to  $\text{Na}^+/\text{H}^+$  antiporters found in yeast, rice, and humans. *Arabidopsis* has at least four isoforms of this transporter. The mRNA transcripts encoding NHX1 increase manifold in plants exposed to high salinity, and transformed *Arabidopsis* or canola plants that over express this transporter are more salt-tolerant than wild-type plants (Zhang and Blumwald 2001).

### Ammonium Transporters

Ammonium ( $\text{NH}_4^+$ ) and ammonia ( $\text{NH}_3$ ) readily interconvert in an aqueous environment according to  $\text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+$ , but the reactants and products in this reaction balance at an alkaline pH ( $\text{pK}_a = 9.25$ ); therefore,  $\text{NH}_4^+$  is the predominant form at the neutral or slightly acidic pHs generally found in soils and within plants. The physical characteristics of  $\text{NH}_4^+$  are very similar to those of  $\text{K}^+$ , and  $\text{NH}_4^+$  may interfere with  $\text{K}^+$  transport, as already discussed. Root absorption of  $\text{NH}_4^+$ , however, is not subject to  $\text{K}^+$  interference (see Chapter 7), indicating that  $\text{NH}_4^+$  transporters are highly selective. One of the first  $\text{NH}_4^+$  transporters identified from any organism was the *Arabidopsis* AMT1:1, which was isolated through functional complementation of yeast mutants defective in  $\text{NH}_4^+$  transport; that is, expressing this protein in the mutants restored their ability to grow at low  $\text{NH}_4^+$  levels (von Wieren et al. 2000). Molecular biologists have found DNA sequences homologous to that encoding AMT1:1 in bacteria, yeast, rice, tomato, and animals (Sonoda et al. 2003). In fact, AMT1 transporters in plants share over 70 of their amino acid sequences.

*Arabidopsis* has at least five homologs of AMT1 (von Wieren et al. 2000). The mRNA encoding AMT1:1 is detected throughout the plant, and its levels vary with  $\text{NH}_4^+$  influx, particular under nitrogen-limited conditions (Ludewig, von Wieren, and Frommer 2002). This transporter, when expressed in yeast mutants, has a high affinity for  $\text{NH}_4^+$  with a  $K_m$  of less than  $0.5 \mu\text{M}$ . In contrast, the mRNAs for AMT1:2 and AMT1:3 are restricted to the roots, and do not vary with  $\text{NH}_4^+$  influx. These transporters, when expressed in yeast mutants, show 100 times less affinity for  $\text{NH}_4^+$ , with  $K_m$  values of around  $40 \mu\text{M}$ .

Several other  $\text{NH}_4^+$  transporters have been identified through DNA sequence homology and functional complementation of yeast mutants defective in  $\text{NH}_4^+$  transport. One of these from *Arabidopsis*, AMT2, is less than 25% identical in DNA sequence with the AMT1 class of transporters. AMT2 seems to function at relatively high levels of  $\text{NH}_4^+$  (1 mM). Another  $\text{NH}_4^+$  transporter, SAT1, which has been isolated from soybean root nodules, does not share any extended amino acid sequences with the AMT1 or AMT2 transporters. SAT1 presumably serves to facilitate movement of  $\text{NH}_4^+$  from the bacteria in a nodule to the host root cells.

### Heavy Metal Transporters

Plants face a "dilemma" with heavy metals. Certain ones ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ) are essential at low levels for survival, but even these—as do nonessential metals ( $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ )—are toxic at higher concentrations, as is the light metal  $\text{Al}^{3+}$  (see Chapter 3). Plants can absorb these elements at low levels from the soil and store large amounts of them within their tissues. Such capabilities seem ideal for cleaning up human dumpsites contaminated with heavy metals, and this potential for "phytoremediation" has stimulated research on plant heavy metal transporters (Brooks 1998; Salt, Smith, and Raskin 1998; Clemens, Palmgren, and Kramer 2002).

Heavy metal transporters in plants fall into four distinct families: CPx-type ATPases, Nramp, cation diffusion facilitators and ZIP (Williams, Pittman, and Hall 2000; Hall 2002; Kochian et al. 2002; Rogers and Guerinot 2002). The CPx-type ATPases involve a phosphorylated intermediate and are related to the  $\text{H}^+$ -ATPases and the  $\text{Ca}^{2+}$ -ATPases discussed already. The

human ailments Menke's disease and Wilson's disease result from defects in the CPx-type ATPases, ATP7A and ATP7B respectively, which are associated with  $\text{Cu}^{2+}$  transport. An *Arabidopsis* CPx-type ATPase, RAN1, has a homologous amino acid sequence to that for ATP7 A, and can restore normal function to yeast mutants deficient in  $\text{Cu}^{2+}$  transport.

Transporters of the Nramp family were first identified in mammalian macrophage where they limit the replication of engulfed bacteria by controlling divalent cation concentrations. *Arabidopsis* has at least six homologs of these transporters and rice has at least three. Expression of the *Arabidopsis* Nramp proteins in yeast increases  $\text{Cd}^{2+}$  sensitivity and accumulation, and two of these, Nramp3 and 4, functionally complement a  $\text{Fe}^{2+}$  transport mutant of yeast (Thomine et al., 2000). In *Arabidopsis*,  $\text{Fe}^{2+}$  deprivation induces the expression of mRNA encoding Nramp3 and 4, and over expression of Nramp3 increases  $\text{Cd}^{2+}$  sensitivity and  $\text{Fe}^{2+}$  accumulation.

Cation diffusion facilitators (CDFs) serve as  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Co}^{2+}$  transporters in both prokaryotes and eukaryotes, the best characterized members being mammalian ZNT1-1, -2, -3, and -4, which effect efflux of  $\text{Zn}^{2+}$ . Two genes in *Arabidopsis* have about 40 sequence identity with the DNA encoding these transporters; functional analysis of the gene products are in progress.

The ZIP family (Figure 10.6), which takes its name from zinc or iron regulated proteins, was first identified in iron-deficient-*Arabidopsis* plants (Cuerinot, 2000). *Arabidopsis* has at least 11 isoforms. One of these, IRT1 is induced by iron starvation, and when expressed in yeast, transports- $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , or  $\text{Cd}^{2+}$ . Three others-ZIP1, ZIP2, and ZIP3-can restore  $\text{Zn}^{2+}$  uptake to yeast mutants that are deficient in native  $\text{Zn}^{2+}$  transporters. Using the same yeast mutants, another  $\text{Zn}^{2+}$  transporter of this family, ZNT1, has been isolated from the  $\text{Zn}^{2+}$  hyper-accumulating species *Thalpi caerulescens*, and from its non-accumulating relative *T. arvense* (Pence et al. 2000). The ZNT1 from *T. caerulescens* shares 88% amino acid identity with ZIP proteins from *Arabidopsis*. The ZNT1 is expressed at high levels in the roots and leaves of *T. caerulescens*, but not in *T. arvense*, thus supporting the role of this transporter in  $\text{Zn}^{2+}$  hyperaccumulation.

The transport of  $\text{Fe}^{2+}$  was studied in classical work by Brown, Weber, and Caldwell (1967), who used two soybean genotypes and their progeny. Molecular biological research has now shown that  $\text{Fe}^{2+}$  transport is effected by a number of proteins belonging to the Nramp, ZIP, and other families (Curie and Briat 2003).

#### Nitrate Transporters

Plants exposed to chlorate ( $\text{ClO}_3^-$ ), a chemical analogue of  $\text{NO}_3^-$ , usually convert it to  $\text{ClO}_2^-$ , a toxic ion, and die. Resistance to  $\text{ClO}_3^-$  may derive from diminished  $\text{ClO}_3^-$  absorption and, by inference, diminished  $\text{NO}_3^-$  absorption. In the early 1970's (a long time ago in molecular years), isolation of a  $\text{ClO}_3^-$  resistant mutant of *Arabidopsis* led to the identification of a major class of  $\text{NO}_3^-$  transporters (Oostindier-Braaksma and Feenstra 1973) now known as the peptide transporter (PTR) or NRT1 family (Crawford and Glass 1998). Subsequently, another class of  $\text{NO}_3^-$  transporters, the nitrate and nitrite porter (NNP) or NRT2 family, was identified via a  $\text{ClO}_3^-$  resistant mutant of the fungus *Aspergillus nidulans* (Forde 2000).

Although PTR transporters generally shuttle peptides across the plasma membrane in both prokaryotes and eukaryotes, a few closely related members of this family serve in plant  $\text{NO}_3^-$  transport. These transporters couple unfavourable  $\text{NO}_3^-$  transport in plants,  $\text{NO}_3^-$  must move against both a charge and a concentration gradient to highly favorable proton transport (see Chapter 4). In plants most of the members of this family appear to be low-affinity  $\text{NO}_3^-$  transporters, but one, NRT1:2 from *Brassica napus*, may also transport amino acids such as histidine. Another, NRT1:1 from *Arabidopsis*, may show dual affinities for  $\text{NO}_3^-$  (K<sub>m</sub> values of 50  $\mu\text{M}$  and 4 mM in *Xenopus* oocytes). Phosphorylation at a threonine residue switches NRT1:1 from a low-affinity  $\text{NO}_3^-$  transporter to a high-affinity one (Liu and Tsav 2003).

The NRT2 transporters from the alga *Chlamydomonas reinhardtii* function as high-affinity  $\text{NO}_3^-/\text{H}^+$  cotransporters ( $x = 2$  or 3) when expressed in *Xenopus* oocytes. One of these, NRT2:1, has equal affinity for  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ; another NRT2:2 is specific to  $\text{NO}_3^-$ ; and a third NRT2:3 is specific to  $\text{NO}_2^-$ . A large number of genes encoding members of the NRT2 family have been identified in higher plants through DNA sequence homology. Barley may have up to ten closely related genes, whereas *Arabidopsis* has at least four.

Regulation of  $\text{NO}_3^-$  transport is an active area of research (see Chapter 7, and Crawford and Forde 2002). Exposure of roots to  $\text{NO}_3^-$  strongly and rapidly induces the expression of mRNAs encoding most of the NRT1 and all the NRT2 transporters (Wang et al. 2000). By contrast, mRNAs for at least two NRT1 transporters, NRT1:2 from *Arabidopsis* and NRT1:1 from tomato, are constitutively expressed. Downstream products of nitrogen metabolism such as glutamine can repress the expression of genes encoding NRT2 transporters, but have a lesser influence upon those encoding NRT1 transporters. The mRNAs for both NRT1 and NRT2 transporters are more prevalent in roots than in other parts of a plant.

As we have discussed in Chapter 7,  $\text{NO}_3^-$  or  $\text{NH}_4^+$  ions, once absorbed, are assimilated into nitrogenous compounds. Transporters for these compounds and genes coding for them are being described (Williams and Miller 2001).

### Phosphate Transporters

We have already noted in Chapter 9 that the concentration of phosphate in soil solutions is very low and that morphological adaptations have evolved that enlarge the root-soil interface and promote its absorption. In Chapter 11 we will explore symbiotic relationships between plant roots and fungi (mycorrhizae) effective in phosphate absorption.

Turning now to the membrane transport of phosphate, it has to operate against both electrochemical and concentration gradients. Phosphate is present at external pH values below 7.0 mainly as  $\text{H}_2\text{PO}_4^-$ , or at higher pHs, as  $\text{HPO}_4^{2-}$ , so that its import into the (negatively charged) cell interior is counter to the electrochemical gradient. As for concentrations, external phosphate concentrations are in the low micromolar values, but in the cytosol, at millimolar concentrations. That being the case, the acquisition of phosphate is exceptionally demanding energetically.

As for nearly all nutrients, plants possess both high-affinity and low-affinity mechanisms for uptake of phosphate (Ragnoffiama 1999, Kochian 2000, and Rausch and Bucher 2002). A high-affinity P transporter, PH084 has been identified in yeast, and similar transporters have

been found in *Arapidopsis*. The mode of transport is probably  $H^+ / Pi$  cotransport (Smith, 2002). Rausch and Bucher (2002) have enumerated and discussed the phosphate transporters and their phylogenetic relationships.

#### Sulfate Transporters

High-affinity sulfate ( $SO_4^{2-}$ ) transporters were first described by Smith et al. (1995) in *Stylosanthes hamata*, a tropical legume. They isolated genes coding for three such transporters, SHST1, SHST2, and SHST3. A fourth such gene, HVST1, was found in barley (Smith et al. 1997). SHST1, SHST2, and HVST1 represent a subfamily of genes coding for  $H^+ / SO_4^{2-}$  cotransporters in roots. These transporters have a high affinity for  $SO_4^{2-}$ , the  $K_m$  being 10  $\mu M$ , almost precisely the value (9.5  $\mu M$ ) determined for high-affinity  $SO_4^{2-}$  absorption by barley roots forty years earlier (Leggett and Epstein 1956). Smith and colleagues (1997) concluded that HVST1 is likely to be the transporter effecting the  $SO_4^{2-}$  absorption by barley roots delineated in kinetic terms by the earlier authors (Leggett and Epstein). SHST3 is expressed not only in roots but also in leaves. It codes for a lower-affinity  $SO_4^{2-}$  transporter with a  $K_m$  of 100  $\mu M$ . Thus the high- and low-affinity transporters are distinct polypeptides (Smith et al. 1995). Additional members of these gene families have been isolated in *Arabidopsis* (Takahashi et al. 1997).

#### Water Transporters

Water molecules are small and uncharged and thus were earlier thought to move across biological membranes via diffusion through the lipid bilayer (see Chapter 4). The membrane permeability of water, however, is several times higher than expected via diffusion and changes more rapidly than could be achieved through altering lipid composition (Tyreman et al., 1999). Preston et al. (1992) first demonstrated that a protein from human red blood cells serves as an aquaporin, a membrane channel specific to water transport (Figure 10.7). Molecular biologists have now identified more than 150 genes—over 35 in *Arabidopsis* alone (Javot and Maurel, 2002) encoding membrane intrinsic proteins (MIPs) that are similar in sequence to this aquaporin (Maurel and Chrispeels, 2001). These MIPs account for 5 to 10 of the total protein in membranes and appear to be responsible for the high, rapidly changing permeability of membranes to water (Johansson et al. 2000).

MIPs in plants fall into three families. Members of one family are specific to the plasma membrane (PIPs), members of another family are specific to the vacuolar membrane, or tonoplast (TIPs), and members of a third family are similar to an MIP found in soybean nodules, but have yet to be localized in non-nodulated species (nodule-like MIPs or NLMs). Given the diversity of MIPs, it is not surprising that the gene expression differs dramatically among various tissues. For reasons not always obvious, the highest levels of mRNA encoding MIPs are found in vascular tissue, meristems, and tissues that experience rapid metabolite or water flux.

A number of MIPs from plants have properties consistent with the functions of an aquaporin. Seven PIPs and three TIPs from *Arabidopsis* enhance water uptake when expressed in *Xenopus* oocytes. Genetically engineered *Arabidopsis* plants with diminished levels of PIPs

have only 30 of the membrane water permeability found in control (wild-type) plants. De-phosphorylation of a spinach PIP under water stress appears to decrease its membrane permeability to water.

#### Other Transporters

Using functional complementation of yeast mutants or DNA sequence homology with microbial transporters, molecular biologists have identified a large number of putative transporters for inorganic and organic nutrients in plants. These include transporters for other cations (Maser et al., 2001), other anions (Barbier-Brygoo et al., 2000; Smith, Rac and Hawkesford, 2000), amino acids and peptides (Delrot, Atanassova, and Maurousset 2000; Ortiz-Lopez, Chang, and Bush 2000), and carbohydrates (Ward et al. 1998; Buttner and Sauer 2000; Delrot, Atanassova, and Maurousset 2000, Lemoine 2000). Expression of these proteins in yeast mutants or *Xenopus* oocytes has been the principal tool for characterizing their transport properties. Such information is just beginning to be exploited for crop improvement. The promise to enhance yields, fertilizer efficiency, stress tolerance, bioremediation, and food value has been the proverbial-although genetically modified-carrot to lure researchers on.

### FACTORS AFFECTING MINERAL NUTRITION/ MINERAL UPTAKE IN PLANTS

#### I. External factors

- (i) **Temperature:** Absorption of mineral salt is affected by change in temperature. In general, an increase in temperature results increase in the absorption of salts up to a certain optimum level. At very high temperature the absorption is considerably inhibited. The inhibition might be due to denaturation of proteins which are directly or indirectly involved in mineral salt absorption. The change in temperature also affects the process of diffusion. The rate of diffusion depends upon the kinetic energy of diffusing molecules or ions which, in turn, dependent upon temperature.
- (ii) **pH:** Change of pH of the soil solution affects the availability of ions to the plants. Decrease in the pH of soil solution accelerates the absorption of anions but increase in pH will favour the absorption of cations. However, pH beyond the optimum level (higher or lower) may damage the plant tissues and inhibit the salt absorption.
- (iii) **Light:** It has no direct effect, but indirectly affecting the transpiration and photosynthesis so that the salt absorption is affected
- (iv) **Oxygen:** Absence of oxygen will inhibit the active salt absorption.
- (v) **Interaction:** The absorption of one ion is influenced by the presence of other ions in the medium. Viets (1944) demonstrated that the absorption of potassium is affected by the presence of calcium, magnesium and other polyvalent cations in the soil solution. Epstein (1978) demonstrated the interaction of several ions (K, Cs, Li, Rb and Na) as competitive for binding sites on carriers. For example, K, Rb and Cs compete with one another for the same binding sites. Li and Na, on the other hand, are not competitive because they have different binding sites.

#### II. Internal factors

- (i) **Growth:** Cell division, elongation and developmental processes promote the absorption of salt.

- (ii) Aging: As the root matures, it increases the surface area, which is favorable for salt absorption.

## MINERAL ION TOXICITY IN PLANTS

### Iron (Fe)

Iron is considered a micro-nutrient because only small amounts are required to aid in normal plant growth. It plays an important role in respiration, photosynthesis and the production of healthy green leaves. The micronutrient's availability to plant roots depends on the pH level of the soil with iron more readily available in soil with a low pH. Iron and manganese both play an important role in plant growth and development, but often compete for absorption, as an abundance of one of these micronutrients makes the other less available to plant roots. Fertilizers should contain an equal ratio of manganese and iron so both are readily available to plants.

Iron toxicity is not common, but some plants do secrete acids from the roots, which lowers soil pH. These plants can take up too much iron, leading to toxicity. Iron toxicity also can occur when chelated iron is added to soil. Chelates help increase nutrient uptake and solubility of metal micronutrients, which in turn makes over-absorption possible. The symptoms of iron toxicity include bronzing and stippling of leaves. The leaf discoloration is caused by the plant creating enzymes to control free radicals that are present in high iron levels. Some plants that are prone to iron toxicity include tomatoes, basil, phlox and impatiens. Iron toxicity in sensitive plants can be avoided by checking pH balance of soil and maintaining a pH level of 5.8 or higher and by using a fertilizer with a balanced ratio of manganese and iron and by using iron chelates carefully.

### Manganese (Mn)

Mn is an essential micronutrient that plays a pivotal part in many metabolic and growth processes in plants including photosynthesis, respiration, and the biosynthesis of enzymes such as malic enzyme, isocitrate dehydrogenase, and nitrate reductase. It is also a cofactor required for multiple plant enzymes, for example, Mn dependent superoxide dismutase (MnSOD). Further more, manganese is involved in carbohydrate and nitrogen metabolism, synthesis of fatty acid, acyl lipids, and carotenoid as well as hormonal activation. The contribution of manganese to the functionality of photosystem II (PSII) especially during the course of splitting of water molecules into oxygen and its role in the protection of PSII from photo damage are of significant importance.  $Mn^{2+}$  is the most stable and soluble form of manganese in the soil environment. However, lower soil pH, less soil organic matter, and decreased redox potential increase the availability or toxicity of  $Mn^{2+}$  to plants. Contrary to some elements such as aluminum or copper, there is a tendency for manganese to easily translocate from roots to the upper parts of plants. This mobility is the reason why symptoms of Mn toxicity are first visible in aerial organs of plants. The appearance of visual features in plants affected by Mn toxicity varies with the type of plant species, plant age, temperature, and light level. The symptoms may include crinkled leaves, darkening of leaf veins on older foliage, chlorosis and brown spots on aged leaves, and black specks on the stems. Mn toxicity has been associated with a decreased  $CO_2$  assimilation but unaffected chlorophyll (Chl) level in *Citrus grandis* seedlings and depleted Chl content in pea (*Pisum sativum* L.) and soybean

(*Glycine max* L.), indicating diversity among plant species in response to Mn excess. Combined effects of excessive manganese and light on plants have received particular attention due to interactive effects of light intensity and Mn excess on plants. In maple trees (*Acer platanoides*) leaves exposed to intense sun light had more concentrations of Mn than shade leaves. Mn toxicity depressed shoot and root growth of rice (*Oryza sativa* L.) and sunflower. However, light intensity did not play any part in exacerbating adverse effects of Mn toxicity in cowpea (*Vigna unguiculata* Walp). It seems that the negative impacts of Mn toxicity are alleviated or accentuated by different light levels, depending on plant variation and tolerance.

#### Nickel (Ni)

Ni is a micronutrient that is required by both higher and lower plants in very small amounts but its phytotoxicity is deemed to be more important than its shortage. Ni has various oxidative states but its divalent state ( $Ni^{2+}$ ) is the most stable type in the environment and biological systems. Although the role of Ni in metabolic processes of plants has not been identified as extensively as other elements such as Mn or Cu, it is a key factor in the activation of enzyme urease, which is needed for nitrogen metabolism. Moreover, it plays a part in seed germination and iron uptake. The concentration level representing nickel toxicity in plants varies greatly from 25 to 246  $\mu\text{g}\cdot\text{g}^{-1}$  dryweight (DW) of plant tissue, depending on the plant species and cultivars. Ni at excess competes with several cations, in particular,  $Fe^{2+}$  and  $Zn^{2+}$ , preventing them from being absorbed by plants, which ultimately causes deficiency of Fe or Zn and results in chlorosis expression in plants. Excess nickel adversely affects germination process and seedling growth traits of plants by hampering the activity of the enzymes such as amylase and protease as well as disrupting the hydrolyzation of food storage in germinating seeds. Plant growth parameters and attributes are also affected by Ni toxicity. M. R. Khan and M. M. Khan investigating the toxic effect of nickel and cobalt on chickpea (*Cicer arietinum* L.) showed that toxicity of Ni on the biomass production was more pronounced than Co and both metals led to poor nodulation, resulting in the reduced yield. Al-Qurainy also demonstrated that Ni at the concentration 150  $\mu\text{g}\cdot\text{g}^{-1}$  of soil severely reduced plant height and leaf area in bean. Ni, especially at high concentrations, can readily move through phloem and xylem vessels, thereby translocating smoothly from the root to the upper parts of plants. This ease of movement towards shoots is due to the pattern by which Ni is distributed within the tissue of roots, which differs from some other HMs such as Pb and Cd so that it can pass through the endodermal barrier and amass in the pericycle cells. Several studies in plants including maize and cowpea have confirmed this phenomenon and indicated that Ni toxicity can result in inhibited lateral root formation and development. Moreover, the agglomeration of Ni in root apex greatly hampers mitotic cell division in this organ, which ultimately results in growth reduction. The induction of ROS, due to Ni toxicity, is observed in both agronomic and non-agronomic plants such as *Jatropha curcas* L. or wheat, which results in a wide range of physiological and biological disorders including the impairment of cell membrane and enzymatic imbalance. The adverse impact of toxic levels of Ni on the photosynthetic apparatus and performance is conspicuous. Srekanth et al. reported that Ni toxicity can lead to reduced Chl content and interruption of electron transport. Excess Ni perniciously influenced photosynthetic protein complexes and the rate of Hill reaction in maize (*Zea mays* L.) dwindled by increasing Ni concentration.

## Copper (Cu)

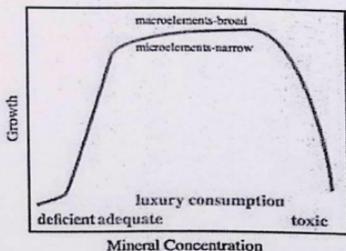
Cu is an essential micronutrient that participates in many vital physiological functions of plants including acting as a catalyzer of redox reaction in mitochondria, chloroplasts, and cytoplasm of cells or as an electron carrier during plant respiration. However, Cu becomes toxic when its concentration in the tissue of plants rises above optimal levels. Cu exists in many states in soils but is mainly taken up by plants in the form of  $\text{Cu}^{2+}$ . The concentration of copper in soil is typically between 2 and 250  $\mu\text{g}\cdot\text{g}^{-1}$  and healthy plants can absorb 20–30  $\mu\text{g}\cdot\text{g}^{-1}\text{DW}$ . But copper availability depends greatly on soil pH and its phytoavailability increases with declining pH. In addition, uptake of Cu by plants and its toxicity are contingent on nutritional condition of plant,  $\text{Cu}^{2+}$  concentration in soil, length of exposure, and genotype of a species. A plethora of research studies such as in Rhodes grass (*Chloris gayana* Knuth), in clove (*Syzygium aromaticum* L.), in cucumber (*Cucumis sativus*), and in some *Eucalyptus* species indicate that copper has a propensity for the accumulation in the root tissues with little upward movement towards shoots. Therefore, the initial characterization of Cu toxicity is the hindrance of root elongation and growth. The subsequent symptoms include chlorosis, necrosis, and leaf discoloration. Excess Cu can become attached to the sulfhydryl groups of cell membrane or induce lipid peroxidation, which results in the damaged membrane and the production of free radicals in different plant organelles and parts. Of these pernicious effects, damage to the permeability of root cells and structural disturbance of thylakoid membranes can be mentioned. Cu at toxic levels through redox process cycling between  $\text{Cu}^+$  and  $\text{Cu}^{2+}$  triggers the formation of reactive oxygen species such as singlet oxygen ( $\text{O}^2$ ) and hydroxyl radical ( $\text{HO}\cdot$ ), leading to injuries to macromolecules, for example, DNA, RNA, lipids, carbohydrates, and proteins. Decreased photosynthetic competence, low quantum efficiency of PSII, and reduced cell elongation are also associated with Cu toxicity. These trends have been observed in various levels of copper applied to different plants. In an *in vivo* study of bean (*Phaseolus vulgaris* L. cv. Dufrix), it was shown that toxic concentration of Cu (15  $\mu\text{M}$ ) depleted PSII action centers and led to photo-inhibition and disruption of its repair cycle. Moreover, the results obtained with rapeseed (*Brassica napus* L.) indicated that content of chlorophylls (Chl a and Chl b) as well as carotenoid was markedly dropped when this plant was exposed to 6  $\mu\text{mol}\cdot\text{dm}^{-3}$  concentration of Cu. Seedling growth characteristics are shown to be adversely affected by Cu toxicity. There is a significant negative correlation between the root and shoot elongation with increasing Cu levels, Cu toxicity causes a noticeable depression in seedling fresh weight in spinach (*Spinacia oleracea* L.). Cadmium and Copper toxicity significantly decreased biomass production in tomato seedling (*Lycopersicon esculentum* Ibiza F1). Also, Cu toxicity was found to be more pronounced and resulted in more induction of lipid peroxidation in the young seedlings, especially at high concentrations, than that of Cd.

## Zinc (Zn)

Zn is an essential trace metal that despite having no redox activity is particularly involved in many vital physiological events in plants. Zinc is an indispensable component of

special proteins known as zinc fingers that bind to DNA and RNA and contribute to their regulation and stabilization. Moreover, it is a constituent of various enzymes, for example, oxidoreductases, transferases, and hydrolases, as well as ribosomes, and plays a role in the formation of carbohydrates and chlorophyll and root growth. Zinc, in its divalent state ( $Zn^{2+}$ ), is the most pervasive form found in soil and acquired by plants. Zn bioavailability/phytoavailability is dependent on various variables including the total Zn concentration in soil, lime content and organic matter of soil, clay type, and presence of other HMs, soil's pH, and the amount of salt in the substrate. Of these, pH is the most important factor influencing Zn availability and higher pH is generally associated with the decreased absorption of Zn by plants. Zn at high soil concentrations ( $150$  to  $300 \mu\text{g}\cdot\text{g}^{-1}$ ) is strongly toxic and its phototoxicity, in addition to the bioavailability factors, depends on plant type and plant development stage. Visual signs of trouble in plants as a result of Zn toxicity are reported to be chlorosis in young leaves due to iron or manganese deficiency and appearance of purplish-red color in leaves due to phosphorus deficiency, which indicate that  $Zn^{2+}$  in excess can easily supersede other metals, especially those with similar ionic radii in the active sites of enzymes or transporters. Moreover, necrotic spotting between the veins in the blade of mature leaves and inward rolling at leaf margins are attributed to Zn toxicity. Excess  $Zn^{2+}$  in cells can produce ROS and adversely influence integration and permeability of membrane. Zn toxicity, akin to other HMs, hampers the functionality and efficiency of the photosynthetic system in different plant species. Excessive concentration of  $Zn^{2+}$  reduced the content of accessory photosynthetic pigments including Chl *a* and Chl *b* by disturbing the absorption and translocation of Fe and Mg into chloroplast. The elevated level of  $Zn^{2+}$  is reported to cause a decline in initial and maximum Chl fluorescence, resulting in the repression of PSII activity. Zinc in excess is found to have genotoxic effects on plants, resulting in genetic-related disorders and damages to plants. High levels of Zn ( $100\text{mg}\cdot\text{L}^{-1}$ ) in cells resulted in abnormal chromosomes, which was followed by a sticky metaphase and premature separation of chromosomes in Bambara groundnut (*Vigna subterranean*). Growth parameters and structure of plant parts are shown to be negatively affected by Zn toxicity. Zn toxicity was found to decrease the length of root and shoot as well as area of leaves in tomato (*Solanum lycopersicum* L.).

Concentration of any particular mineral impacts overall plant growth



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