M. A. Baset Mia, Ph.D.

Nutrition *of* Crop Plants

Plant Science Research and Practices

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PREFACE

Crop nutrition is an essential discipline of plant science of crop production. The importance of crop nutrition for the increase of yield and quality of crops is beyond to explain. In a simple manner crop nutrition is the study of uptake and utilization of elements for physiological process of response to growth and development of crop plants. It is a surprise of nature that organisms especially the phyta can acquire the elements (both mineral and non-mineral) in its organ through different biochemical processes for growth and developmen. Around sixty elements are taken up by the plants where only quarter (16) of them are essential for plants. Recently, beneficial elements are gaining eminence for special circumstances of certain plant species; they have been shown to stimulate crop growth. Crop requires balance nutrients for successful growth and development which have been discussed in the book. The main objectives of this book are to describe and discuss the essential mineral nutrients, their metabolic role and uptake mechanisms in crop plants and explain the basic processes and relationship of relevance to the scientific understanding of plant nutrition.

The book includes the classification of essential nutrients in various aspects with special emphasis on the physiological and biochemical functions, and their uptake process through membrane. Much of emphases have given on the root structure and rhizosphere in relation to nutrient uptake and their assimilation in the cellular level.

Biochemical and metabolic role of essential nutrients have been discussed comprehensively. Diagnosis of nutrient deficiencies by different techniques and the remedies of deficient plants have been discussed elaborately. Additionally, the text book has been written in simple manner with needed illustrators on the cell membrane and membrane port to where the nutrients are transported through uphill and downhill process.

The goal of this book is to establish a thorough understanding of plant nutrition, and certainly a text book for agriculturist, researchers in the field of crop science, students', academicians and for the crop cultivators as a whole. Finally, it is a consolidated book comprising different areas of plant nutrition and the stakeholders will be benefited from a single book like this.

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Chapter 1

CONCEPT AND CLASSIFICATION OF PLANT NUTRIENT ELEMENTS

1.1. GENERAL FEATURES

Plant nutrition is an important branch of plant science having close relation to biochemistry, plant anatomy and soil chemistry. It deals with the physiological aspects of plant nutrient elements. It is a wonder of universe that living organism can absorb elements and be able to utilize these elements for their body building i.e., assimilation. Elements in the earth i.e., mineral and non-mineral elements are absorbed by the plants and are incorporated into the organ through various metabolic processes. The process of elements acquisition and their utilization into the plant body is very important for agriculture, which opens up a subject, known as plant nutrition. The subject plant nutrition is very much important for ensuring sustainable agricultural system.

Plant nutrition may be defined as the physiological process of uptake and utilization of elements (both mineral and non-mineral) by the plants for growth and development process. And the elements which are utilized for the growth and development of plants are called plant nutrient elements. There are 118 elements present in the earth of which nearly sixty elements taken up by the plants. But most of them are not required or utilized by the plants, and they either remain nonfunctional or toxic to the plants whereas only 16 elements are required by the plants. The acquisition of both organic and inorganic nutrients (minerals) is a part of the plant nutrition. The nutrients are directly or indirectly involved in the metabolic as well as physiological activities in the plant body. Plant nutrition also deals with the study of the chemicals that are necessary for growth and development. It is an important branch of agriculture and has close relations with the following subjects:

- Plant Anatomy
- Plant Biochemistry
- Plant Physiology
- Soil Science
- Plant Pathology
- Entomology
- Agronomy

1.2. HISTORICAL DEVELOPMENT OF PLANT NUTRITION

The study of plant nutrition began systematically in the eighteen century before this several scientists contributed in plant productivity sporadically. There is no concrete report on the plant nutrition during this period. At first the Greek philosopher Aristotle postulated four things viz. air; water, soil and fire are required for the life. He suggested that water is essential for plants, and also reported that plants grow better in dirty water than clean water. This idea indicated that dirty water contain nutrient elements which contributed to the growth and development of plants.

In 1788, Van Helmont and Woodward proved that plant dry matter is formed largely by CO_2, which comes from air, contribution of water and plant growth is also depended on some 'peculiar terrestrial matter'.

In 1840, Justus von Liebig, a famous German chemist, recognized that plants need C, H and O which are supplied by air and water. He also concluded that plants need N, P and K in proper amount, and also suggested that plants grow better due to direct and balanced supply of those nutrients. Deficiency of any element hinders the growth (termed as his 'Law of the Minimum').

Arnon and Stout (1939) developed soilless culture of plant, a new technique for avoiding contamination from nutrient culture and reported that molybdenum (Mo) is essential for the growth of tomatoes. Essentiality of any nutrient can be proved by using soilless i.e., hydroponic culture of plant growth.

A tremendous development in plant nutrition has been achieved since 1800 to 1900, and all macro nutrients including iron were discovered as essential for plants. In nineteen century all the micronutrients were recognized as essential. The chronological development of discovery of essential nutrient elements has been shown in Table 1.1.

Table 1.1. The discovery of essential nutrient elements for higher plants (Glass, 1989; Marschner, 1995; Munson, 1998)

Element	**Symbol**	**Year of described as essential for plants**	**Discovered by Scientist**
Carbon	C	1882	J. Sachs
Hydrogen	H	1882	J. Sachs
Oxygen	O	1804	T. De Saussure
Nitrogen	N	1872	G. K. Rutherford
Phosphorus	P	1860	Ville
Potassium	K	1860	J. Sachs, Knop
Calcium	Ca	1856	F. Salm-rHorstmar
Magnesium	Mg	1906	Willstatte
Sulfur	S	1865	J. Sachs, Knop
Iron	Fe	1860	J. Sachs
Zinc	Zn	1926	A. L. Sommer and C.B. Lipman
Manganese	Mn	1922	J.S. McHague
Copper	Cu	1931	C.B. Lipman and G. MacKinney
Boron	B	1923	K. Warington
Molybdenum	Mo	1938	D. I. Arnon and P.R. Stout
Chlorine	Cl	1954	Broyer et al.

In 1972, E. Epstein introduced two criteria for an element to be considered as an essential for plant growth: (1) absence of a particular element in plant is unable to complete a normal life cycle, and (2) the element must participate in the metabolic activity of plants and it should be a constituent of the plant part of which is in accordance with the Liebig's Law of Minimum. Now the conditions have been extended from two to four, and 16 elements have been considered to be essential for plant growth and development.

The term essentiality of nutrients for plants is an important area in plant nutrition which implies certain condition of the life process of plants. The candidate of essential nutrient element must satisfy certain preconditions.

An element should be treated as essential if it satisfies the following criteria and preconditions:

i. Plant cannot complete the life cycle without it
ii. The element must be involved in the metabolic process of the plant
iii. One element cannot be replaced by other element
iv. Should satisfy the wide range of plants not a particular plant species or a particular environmental condition.

Plant nutrition can be observed as two parts namely, organic nutrition, which is mainly dealing with photosynthesis; and inorganic nutrition dealing with other nutrients i.e., inorganic elements. However, both organic and inorganic nutrition are highly corelated.

1.3. Classification of Nutrients

Classification of nutrients is very important for comprehensive study of plant nutrition; various categories of classification are put forwarded for easy understanding. Plants take elements from various sources, and among the 16 essential nutrient elements three are non-mineral, obtained from air and water and the remaining 13 elements are mineral, obtained from the soil. Essential nutrients for plants can be classified in various ways, and there are different bases of classification:

1. Classification based on the quantity of nutrient required for the plant
2. Classification based on the metabolic and biochemical functions in plant
3. Classification based on the mobility of nutrient in the soil system
4. Classification based on the mobility of nutrient within the plant system
5. Classification on the basis of the metallic properties of the element
6. Classification on the basis of functions in the plant

1.3.1. Classification on the Basis of Quantity of Nutrient Required

A. Basic Nutrients

These elements are abundant in nature and constitute around 96% of total dry matter of plant. Generally, there is no scarcity of the basic elements for plants. The following elements are considered as basic elements for plants:

Carbon (C)
Hydrogen (H)
Oxygen (O)

Among these, carbon and oxygen constitute 45% each and hydrogen is 6% of the plant body on the basis of dry matter.

B. Macro Nutrients

The nutrients which are required by plants in larger quantities (more than 0.01% on dry weight basis) are called macro or major nutrients. Their relative content is higher than that of other elements, and the following nine elements are considered as macro nutrients:

Name of Macro Nutrients

1. Carbon (C)
2. Hydrogen (H)
3. Oxygen (O)
4. Nitrogen (N)
5. Phosphorus (P)
6. Potassium (K)
7. Calcium (Ca)
8. Magnesium (Mg)
9. Sulfur (S)

Macro nutrients have again been classified into two categories:

a) *Primary (1^0) macro nutrients*

Among macro nutrients, nitrogen (N), phosphorus (P) and potassium (K) are known as primary nutrients which are required in proper ratio for successful crop production.

b) *Secondary (2^0) macro nutrients*

Next to primary nutrients, there are three elements such as calcium (Ca), magnesium (Mg) and sulfur (S) which are known as secondary nutrients.

C. Micro Nutrients

These essential nutrients are required by plants in smaller quantities (less than 0.01% on dry matter basis), and also known as minor or trace elements.

Name of micro nutrients:

1. Iron (Fe)
2. Zinc (Zn)
3. Boron (B)
4. Copper (Cu)
5. Manganese (Mn)
6. Molybdenum (Mo)
7. Chlorine (Cl)

1.3.2. Classification Based on the Biochemical and Physiological Functions

Essential plant nutrients can be classified according to the biochemical and metabolic functions (Mengel, 2001; Table 1.2).

Table 1.2. The sixteen essential elements have been categorized into four groups based on the biochemical and metabolic functions

Group	Nutrient element	Ionic form of uptake	Biochemical functions
Group A	C, H, N and S	Uptake from soil solution as HCO_3^-, NO_3^-, NH_4^+, SO_4^{2-} and from the atmosphere as CO_2.	They are mainly responsible for the building of organ and organelle in plants, and are assimilated by carboxylation and oxidation-reduction processes.
Group B	P and B	Uptake from soil solution as $H_2PO_4^-$, HPO_4^{2-}, BO_3^{2-}	They are responsible for the transfer of energy e.g. formation of ATP. The elements esterify with alcoholic groups in plants. Phosphate esters are involved in energy transfer reaction.
Group C	K, Mg, Ca, Mn, Cl	Uptake from soil solution as K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cl^-	The elements do not have any specific functions, mainly responsible for establishing osmotic potentials, the specific contributions to the structure and function of enzyme protein. Make balance with other anions in the cytosol and vacuole.
Group D	Fe, Cu, Zn, Mo	Uptake from soil solution as ionic form or as chelator like Fe-EDTA, Cu^{2+}, Zn^{2+}, MoO_4^{2-}	They are present mainly in prosthetic groups in the enzyme or protein. Contribute in electron transport through valency changes.

1.3.3. Essential Plant Nutrient Elements can also be Classified on the Basis of Metallic Properties

i. Metal elements : K, Ca, Mg, Fe, Mn, Zn, Cu
ii. Nonmetal elements : N, P, C, H, O, S, B, Mo, Cl,

Table 1.3. Essential plant nutrient element and their symbol, atomic weight and average concentration in plant parts and their available form and main source of fertilizer

Name of nutrient element	Symbol	Atomic weight	Average concentration	Concentration range	Available form	Available fertilizer
Hydrogen	H	1.00794	6%	-	H_2O	Abundant
Oxygen	O	15.9994	45%	-	O_2, CO_2	Abundant
Carbon	C	12.0107	45%	-	CO_2, HCO_3^-	Abundant
Nitrogen	N	14.0067	1.5%	1-5%	NH_4^+, NO_3^-, N_2,	Urea, KNO_3, NH_4NO_3
Phosphorus	P	30.97376	0.8%	0.1-1%	$H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}	TSP, CSP, rock phosphate
Potassium	K	39.0983	1%	1-4%	K^+	Muriate of Potash (MOP)
Calcium	Ca	40.078	0.2%	0.2-1%	Ca^{2+}	Gypsum
Magnesium	Mg	24.3050	0.2%	0.1-0.4%	Mg^{2+}	Epsom salt
Sulfur	S	32.065	0.1%	0.1-0.4	SO_4^{2-}, SO_2	Gypsum
Chlorine	Cl	35.453	100 ppm	0.1-2%	Cl^-	MOP
Iron	Fe	55.845	100 ppm	50-200ppm	Fe^{2+}, Fe^{3+}	Fe-EDTA
Zinc	Zn	65.409	20 ppm	25-150 ppm	Zn^{2+}	ZnO, $ZnSO_4$
Boron	B	10.811	20 ppm	-	BO_3^{3-}	H_3BO_3
Copper	Cu	63.546	6 ppm	5-20ppm	Cu^{2+}	$CuSO_4$ $5H_2O$
Manganese	Mn	54.938049	50 ppm	-	Mn^{2+}	$MnSO_4$
Molybdenum	Mo	95.940	0.1 ppm	-	Mo_4^{2-}	$(NH_4)_2$ Mo_4

1.3.4. Classification of Essential Plant Nutrients on the Basis of Mobility of Nutrient in the Soil

A. Mobile nutrients

The nutrients of this group are highly soluble, not adsorbed on clay complexes and are highly mobile in the soil system. There is a chance of leaching loss of these elements when applied in the soil as chemical fertilizer.

Example: NO_3^-, SO_4^{2-}, BO_3^{2-}, Cl^- and Mn^{2+}

B. Less mobile nutrients

They are soluble, but are adsorbed by clay complex so, their mobility is restricted to some extent. Leaching loss is minimum when applied in the soil as chemical fertilizer.

Example: NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}

C. Immobile nutrients

These nutrient ions are highly reactive and get fixed in the soil under both acidic and alkaline conditions.

Example: $H_2PO_4^-$, HPO_4^{2-}, Zn^{2+}

1.3.5. Classification on the Basis of Mobility within Plant System

Some nutrient elements are able to move from one organ to another organ i.e., form older leaves to younger leaves or shoot but others are fixed to a definite organ. Based on the mobility in the plant system the nutrient elements can be classified as follows:

a.	Highly mobile in xylem and phloem system:	N, P, K and Cl
b.	Moderately mobile through phloem system:	Zn
c.	Less mobile through phloem system:	S, Fe, Mn, Mo and Cu
d.	Immobile through phloem system :	Ca and B

The knowledge on nutrient mobility (mobile or immobile) is helpful in diagnosis of nutrient deficiency. Generally, deficiency is appeared in older leaves for mobile elements while if a nutrient is immobile the deficiency symptom typically appears on new leaf or organ.

1.3.6. Classification of Essential Plant Nutrients on the Basis of Functions in the Plant

a. Elements providing basic structure of plants and are abundant in the earth.
   Example: C, H and O.
b. Elements used for energy storage, transfer and bonding: these are accessory structural elements which are more active and vital for living tissues.
   Example: N, S, P and B.
c. Elements are required for charge balance, electrolytic balancing in the cytosol.
   Example: K, Ca and Mg.
d. Elements are involved in enzyme activation either as prosthetic group or as a modulator and act as electron transfer process.
   Example: Fe, Mn, Zn, Cu, Mo and Cl.

Beneficial Nutrients

These elements are not considered as essential nutrient element and also not able to satisfy the criteria of the essentiality, but their application increases the growth and yield of crop plants to some extent.

Example: Sodium (Na), Silicon (Si), Aluminium (Al), Cobalt (Co), Nickel (Ni).

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Chapter 2

ROOT AND RHIZOSPHERE IN RELATION TO NUTRIENT ABSORPTION

2.1. GENERAL FEATURES

The root is the vital part of the plant, which moves downward to the earth through the process of gravitrophism. It originates from the embryo during germination, and the first root is known as radicle. The roots are generally categorized into two types: primary roots which originate from the embryo and usually persist throughout the whole life of plant, and adventitious roots which emerge only after the damage of radicle and new roots arise from the base of plumule or stem and any other parts of the plant. Root is the descending part of the plant axis and is normally non-green in color due to lack of chloroplast. However, the aerial roots can be converted into green color due to the conversion of proplastid into chloroplast. Roots of terrestrial plants perform following functions:

- Penetrate into soil for anchorage of the plant
- Uptake of nutrients and water for growth and development of plants
- Conduction of water and nutrients through vascular bundle (xylem)
- Store food materials in parenchymatous tissue e.g., sweet potato, carrot etc.
- Produce phytohormones like auxin.

A root possesses different zones based on the specialization, where the meristem passes into different stages of differentiation with the advancement of plant age.

2.2. ROOT ZONE

Root morphology i.e., external and internal structures are the most important factors responsible for nutrient absorption and translocation. Externally a young root has four zones namely root tip, elongation, root hair and maturation zone. This division is based on the specialization of the tissue. Different root zones have diversified range of nutrient absorption and translocation capacity.

2.2.1. Root Tip or Cap Zone

This zone is located at the apex of root, thimble-shaped mass of parenchyma cells are present here. The tissues are mainly meristematic (having the capacity to divide), with dense cytoplasm having no vacuole in the cell. The main functions of root tips are:

- Protecting the root from mechanical injury and helps in penetrating into the soil
- Releasing mucilage or root exudates for making the availability of nutrients
- Performing major role in perception of gravity (i.e., geotropism or gravitropism)
- Accumulatiing of amyloplasts (also known as statoliths) at the bottom of the cells
- Providing the place of cell division for elongation:the transitional meristems arise from the root tip zone which includes the tissue systems; protoderm, ground meristem and the procambium. The root tip zone is responsible for penetration into the soil by secreting mucilage which softens the soil structure. Iron and calcium are absorbed by the root tip and elongation zone due to the lack of endodermis development.

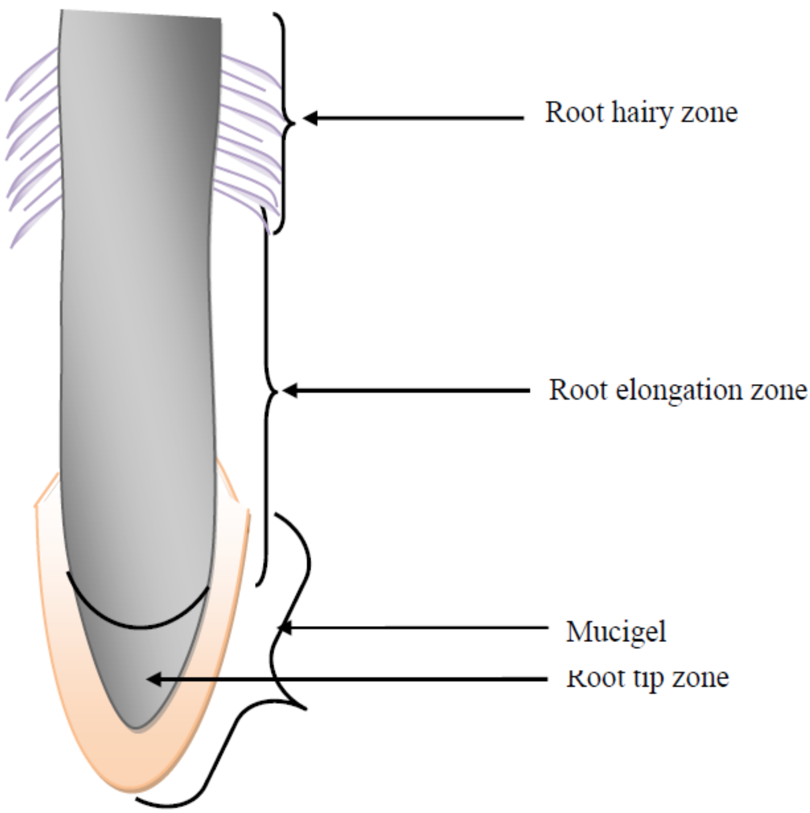


Figure 2.1. A young root showing different zones i.e., root tip, elongation and hairy zone, mucigel a gelatinous layer is also shown around the root tip (grayish colour).

2.2.2. Elongation Zone

This is an area of cell division which is located 1-2 cm behind the root tip zone. It also possesses the meristematic activity. The tissue of this region goes for quick cell division, elongation and enlargement, consequently more nutrients are absorbed by this zone.

2.2.3. Root Hairy Zone

This region lies behind the area of elongation and extends upward the epidermis with thin protrusions, root hairs that serve to increase the area of the plant-soil interface. Root hairs originate from the rhizodermis/epidermis, the outermost layer of cortex or inner layer of root cap (Hofer, 1991). Root hairs may constitute more than 60% of the surface area of the root, and act for absorption of water and nutrients. They increase the contact area of roots for the said functions. Internally, tissue of this region undergoes differentiation into various types of tissue viz. parenchyma, collenchyma and schlerenchyma. Lateral roots arise from the pericycle of this region (Charlton, 1991).

2.3. INTERNAL STRUCTURE (ANATOMY) OF ROOTS

2.3.1. Epidermis

It is the outermost layer of the roots that originates from protoderm. The epidermis consists of closely packed elongated cells which are devoid of cuticle and stomata. The outer side of the epidermal cells is elongated and forms root hair. The epidermis of root is termed as epiblema or piliferous layer or rhizodermis (Esau, 1967). The root hairs vary in width and length (Dittmer, 1949). In certain plants, the whole roots are capable of initiating and forming root hairs. The root hairs are unicellular in structure and responsible for absorption of water and nutrients. Some roots develop a specialized layer beneath the epidermis and termed as exodermis which is very much suberinized compared to epidermis and cortex.

2.3.2. Cortex

The cortex of roots may be homogenous and simple in structure, or it may have variety of structures. The cortical area occupies beneath the epidermis and extended up to endodermis, and contains normally the parenchymatous tissues. However, sclerenchymatous tissues have also been found in this area. In hydrophytic or semihydrophytic plants aerenchymatous tissues are found in the cortex. There are two types of cavities viz. lysigenous, formed due to dissolution of adjacent cells and schizogenous, formed due to the development of intercellular space, and are found in the cortex. The cortical cells are devoid of chloroplast and possess amyloplast.

2.3.3. Endodermis

It is a single layer of parenchymatous living cell, and the outermost boundary of stele. A suberin layer of strip is present in the endodermis, known as Casparian strip which acts like a gasket sealing in between the apoplast of cortex and the apoplast of stele. The Casparaian strip is a hydrophobic material which protects the movement of water and diffusion of nutrients into stele and finally acts as a barrier for passive movement of nutrients through apoplastic zone.

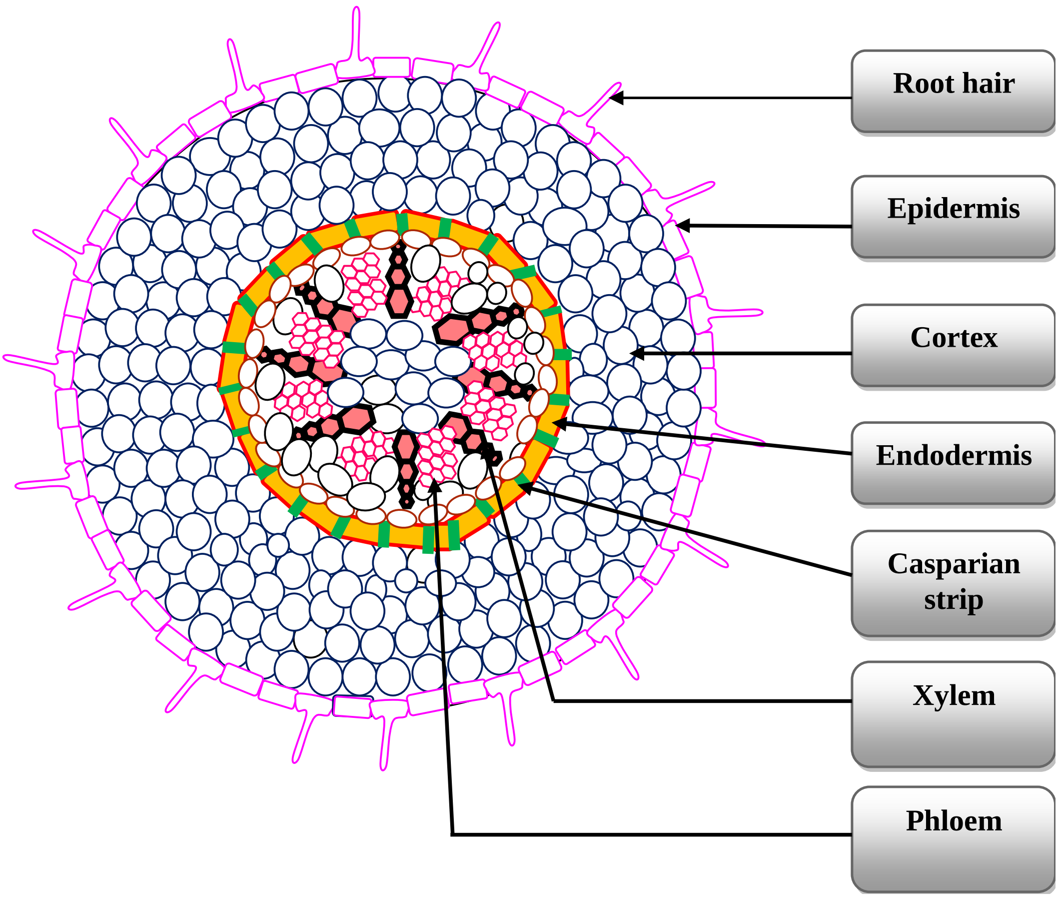


Figure 2.2. Anatomical feature of a typical root showing different types of tissue system from epidermis to pith.

2.3.4. Passage Cells

They are mainly found in the endodermis and exodermis but are ubiquitous in either layer. Passage cells occur in the form of short cells in the dimorphic type of exodermis. When endodermis is well-developed, the passage cell cannot be developed. It is suggested that passage cells of the endodermis are important for the transfer of calcium and magnesium into the stele and thus into the transpiration stream. It mainly provides areas of low resistance for the movement of water and nutrient ions (Peterson and Enstone, 1996).

2.3.5. Pericycle

It is the single layer of parencymatous cells lying just beneath the endodermis and peripheral to vascular bundle. The lateral roots originate from the pericycle, some times called pericambium. Lateral roots appear at a relatively constant distance behind the tip of a growing root (Charlton, 1991).

2.3.6. Vascular Bundle

Vascular bundles (consisting of xylem and phloem), are arranged radially in root. The xylem is exarch in nature i.e., moving center to periphery. The metaxylems are larger than protoxylem and lying in the center. The xylem consists of tracheid, vessel, xylem parenchyma and xylem fiber while the phloem consists of sieve tube, companion cell, phloem fiber and phloem parenchyma. The vessel of xylem and sieve tube of phloem cells are responsible for conduction of nutrients.

2.3.7. Vessel

A vessel is a tube-like series of cells forming a pipe like structure. The wall of vessel is thick and composed of lignin. The thickening of vessel wall is performed in various ways namely annular, spiral, scalariform, reticulate or pitted. The cells are non-living but connected with each other by perforated tangential walls thus making a continuous channel for the transportation of nutrients and water.

2.3.8. Sieve tube

Sieve tube is a nonliving tube like structure, composed of elongated cells placed end to end. The transverse walls are perforated like a sieve, which is called sieve plate. The sieve cell is supported by a living cell known as companion cell. The sieve tube is responsible for the transportation of assimilates and nutrient ions from leaf to different plant parts.

2.3.9. Medulary Ray

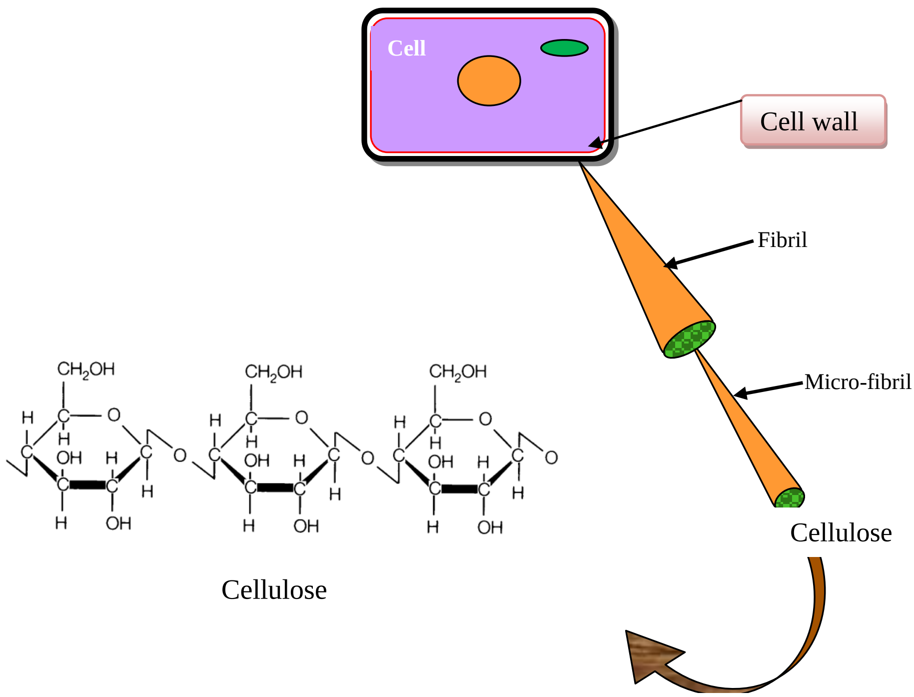
Generally, the parencymatous tissue found in between the vascular bundle is known as conjunctive tissue or medulary ray. Storing of food materials is the main function of medullary ray.

2.3.10. Pith

The parenchymatous tissues are found in the centre of root called pith. However, other types of tissues like schlerenchyma are also found in pith.

2.4. Cell Wall of Roots

The cell wall gives protection of the protoplast, and composed of cellulose, hemicelluloses and lignin. It is the part of apoplastic area and permeable to movement of water and nutrients dissolved in the water. The cell wall has two layers viz. primary and secondary wall. The primary cell wall is composed of cellulose, pectic compounds, polysaccharides and hemicelluloses (Pandey, 2001). The secondary cell wall is composed of higher amount of cellulose than pectin, and a critical component of secondary cell wall is lignin which constitutes 15-35% of dry weight of woody roots (Taiz and Zeiger, 1991). The middle layer of two adjacent cell walls is called middle lamella which is composed of Ca-pectate (Ca-salt of pectic acid), and acts as cementing material for holding two cells compactly.



2.4.1. Root Parameters in Relation to Plant Nutrition

Study of roots is essential for acquiring knowledge on plant nutrition. However, it is not easy to study the root parameters as extraction of root is very tedious job. Recently, image analysis of roots by computerized scanner is getting popular. Following root parameters are considered for root study in relation to nutrient uptake:

- Root length : length of all roots
- Root mass : weight i.e., the oven dry weight of all roots
- Root volume: all the roots volume
- Root surface area: all the root surface area
- Root length density: total root length divided by root mass
- Root:shoot ratio: generally the root and shoot weight ratio

2.5. Plasmamembrane and Biological Membrane

The membrane which encircles the protoplasm and protects it from the external environment is called plasmamembrane or plasmalemma. It is the living portion of the cell while the cell wall is nonliving. Different types of membrane like plasmalemma, tonoplast (membrane of vacuole) and organelle membrane are together called biological membrane. Various transport proteins are embeded in plasmalemma which is responsible for trafficking the transport of nutrients (Tazi and Zeiger, 1991).

Functions of Biological Membrane

- Acts as a permeability barrier which controls and coordinates the rate of diffusion and transport of nutrient elements and organic substances
- Protects the protoplasm and organelle from the external environment
- Acts as a cytoskeleton of orientation of enzymes
- Serves as a vehicle for transport of substances from one organelles to another
- Supports the synthesis of various macromolecules
- Controls the movement of nutrient elements, nutrients, solutes from one portion to another portion of cell
- Compounds with polar group such as OH, COOH, NH_2 and CHO and other inorganic salts pass through slowly

Characteristics of Biological Membrane

- It consists with phospholipid bilayer embeded with proteins
- Lipids are mainly phospholipid
- It is amphiphilic in nature
- Involves in a variety of cellular processes namely cell adhesion, ion conductivity and cell signaling

Composition of Membrane

All biological membranes consist of phospholipid bilayer sandwiched with protein. Phospholipids are the major structural component of cell membrane that constitutes phospholipid bilayers. Most of these lipids consist of diglycereide, a phosphate group and a simple organic molecule such as choline. The structure of the phospholipid molecule generally consists of hydrophilic head and hydrophobic tail.

Following types of proteins are found in the membrane:

A. Enzymatic protein
B. Carrier protein
C. Structural Protein

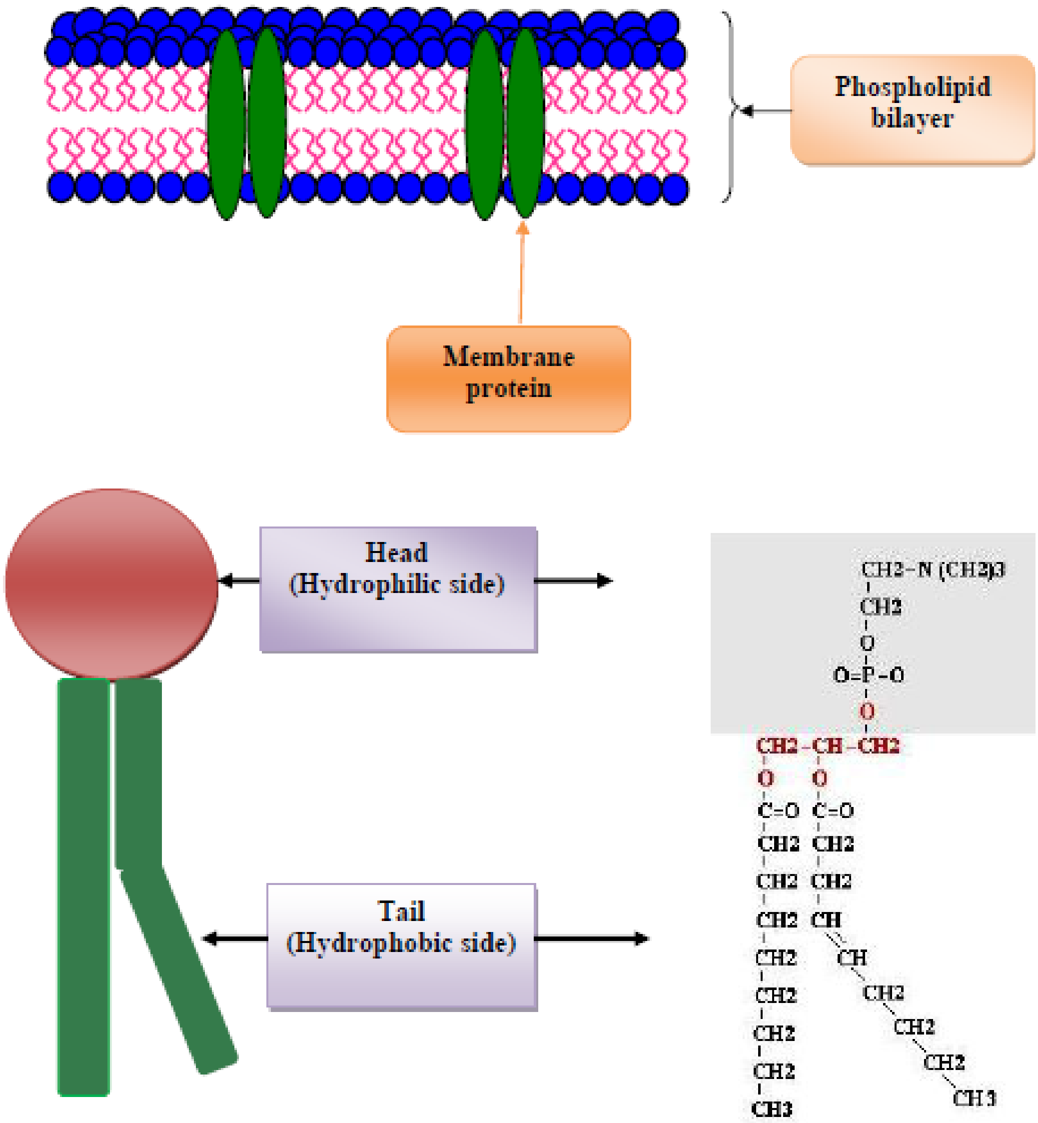


Figure 2.3. Plasmamembrane composed of phospholipid bi-layer showing hydrophilic head and hydrophobic tail.

There are several hypotheses put forward for explaining the ultrastructural features of plasmalemma, and the following hypotheses have been proposed for elucidation of the construction of membrane:

- Kavanau's lipid pillar
- Hydrophobic binding model
- Bension's model
- Lendard and Singer model
- Mosaic membrane concept
- Composite model
- Fluid mosaic model

2.6. PLASMODESMATA

Plasmodesmata (singular: plasmodesma) are thread like structure, and is the cytoplasmic connection between two adjacent plant cells. They constitute the structural and functional analogs of gap junctions found between the cells. The plasmodesmata create an intercellular continuum-the symplasm, and are microscopic channels which traverse the cell wall (Robards and Lucas, 1990). There are two types of plasmodesmata: primary plasmodesmata which are formed during cell division, and the secondary plasmodesmata which are formed between two mature cells. A typical plant cell contains 10^3 to 10^5 plasmodesmata between two adjacent cells. The size of each plasmodesma is 50-60 ηm in diameter at the mid-point and are composed of three main layers.

Plasmodesmata are composed of the following structures :

- Plasmodesmatal plasma membrane
- Cytoplasmic sleeve
- Desmotubule

2.6.1. Cytoplasmic Sleeve

This is a cytoplasmic connection, and is a fluid-filled space enclosed by the plasmalemma and a continuous extension of the cytosol. Transporting and trafficking of molecules and ions through plasmodesmata occurs through this pathway. Smaller molecules like amino acids, sugar and ions can easily pass through plasmodesmata by diffusion. Similarly protein can also easily pass through the sleeve.

2.6.2. Desmotubule

It is a tube like structure made by endoplasmic reticulum which runs between two neighboring cells. The structure of desmotuble is composed of two types of protein namely, myosin and actin. Myosin is an ATP-dependent motor protein while the actin is a conserved globular protein. An electron dense material can be seen around the desmotuble and plsmamembrane.

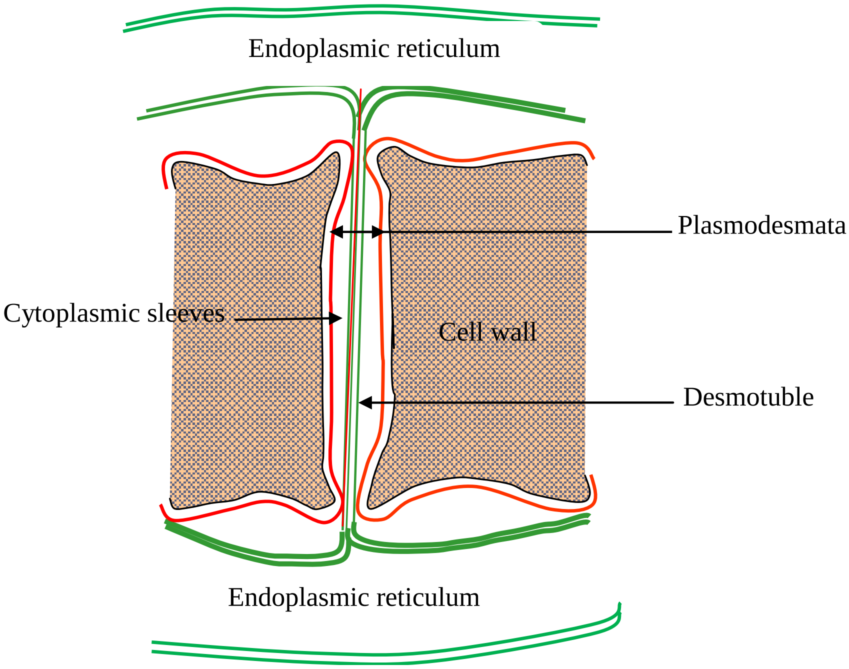


Figure 2.4. Plasmodesmata along with desmotuble and cytoplasmic sleeve (modified from Robards and Lucas, 1990)

2.6.3. Functions of Plasmodesmata

- Plasmodesmata transport nutrient elements and organic solutes from one cell to another cell. It has also been reported that plasmodesmata transports gibberellic acid in algae.
- It also communicates the information from one cell to another cell. Electric coupling between cells are associated with the density of plasmodesmata between the cells, and electrical signals play an important role in the initiation of a wound response in plants.
- Plasmodesmata have been shown to be sensitive to various levels of calcium, pH, and light, indicating that the mechanisms of plasmodesmata transport are more complex than previously it was thought.
- Plasmodesmata serve as directors of plant growth and development and may help to determine a program of cell differentiation, such as sealing off root and stem epidermal cells from the rest of the plant. This concept has been supported by a study in which RNA encoding the transcription factor KNOTTED1 (KN1) was localized to all cell layers of the developing maize meristem except the outermost, but the KN1 protein was found in all cell layers (Zambryski and Crawford, 2000).

2.7. Ectodesmata

It is the pore like connection between the hypodermal cells to outer environment, and sometimes referred to as ectoteichodes (or teichodes for short) located in plant cell wall traversing areas. They are in fact the counterpart of plasmodesmata which are well established "open" membrane connections between the cytoplasm of adjacent cells.

2.8. The Rhizosphere

The term rhizospher was first introduced by German scientist Hiltner in 1904 to describe the region of soil within the vicinity of roots and influenced by the roots (Hartmann et al., 2008). It encompasses the 1-3 millimeters of soil surrounding a plant root where complex biological and ecological processes occur. This zone is characterized by greater activity of soil microorganisms which influence on the nutrition of crop plants. The soil which is not connected with the roots is called bulk soil. In rhizosphere, higher number of microbes is found, and the population of soil microbes both beneficial and pathogenic is several times higher than bulk soil.

2.8.1. Mucigel

The root especially the root cap secretes gelatinous substances which encircle the root cap to protect it from the external environment. The mucigel make a continuum between the soil particles to the root for absorption of nutrients. The accurate function of mucigel is uncertain. However, it protects the root cap from the external environment, facilitate the uptake of nutrients and make a communication between root and rhizosphere microorganisms (Russel, 1977).

2.8.2. Root Mucilage

A variety of organic and inorganic substances released from the roots are known as root exudates. These substances nourish the soil microbes and enhance their activity thousand times higher than bulk soil. The exudates consist of sugar, amino acids, protein, fat, nucleic acids etc. (Table 2.1). The exudates serve important roles for attracting or repelling the rhizosphere microbes. The ability to release exudates is one of the remarkable metabolic activities of the roots where around 5-21% fixed CO_2 is excreted as carbohydrate (Marschner, 1995). A large body of research findings concludes that the root exudates act as a messenger that communicate biological, chemical and physical activities of roots and soil microbes (Walker et al., 2003).

Roots may regulate the soil microbial community in the rhizosphere by the exudates i.e., a variety of compounds. Rhizosphere copes with herbivores, encourage beneficial symbiosis, alter the physio-chemical properties of the soil, and inhibit the growth of competing plant species (Nardi et al., 2000). The pattern of releasing root exudates is not homogenous throughout the axis.

Table 2.1. Organic substances found in root exudates (After Mia and Shamsuddin, 2013)

Major Groups of Organic Substances	Components of Exudates
Carbohydrates	Glucose, fructose, sucrose, maltose, raffinose, galactose, ribose, xylose, arabinose, oligosaccharides etc.
N-compounds	Protein, amino acids, enzymes etc.
Organic Acids	Citric acid, malic acid, propionic acid, succinic acid, fumeric acids etc.
Fatty substances	Palmitic, stearic, oleic, linolic etc.
Growth regulators	Biotin, thiamin, niacin, choline, inositol etc.
Nucleotides	Adenine, guanine, uridine/cytidine, phosphatase etc.
Miscellaneous	Auxins, organic phosphorus, scorpoletin etc.

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Chapter 3

MECHANISMS OF NUTRIENT UPTAKE

3.1. GENERAL FEATURES

Uptake of nutrients by the plant is a very complex phenomenon as plant does not have any mouth system like animal. In plants, nutrients are absorbed mainly by the roots and must get contact with root surface. However, the structural complexity of root epiblema is the main barrier for entry of nutrients into cortex. Nutrients are not absorbed uniformly throughout roots as roots have three different regions viz. root hairy, elongation and meristematic zones, and radioactive studies showed that meristematic zone resulted in more nutrients uptake than others. This perhaps necessitates more metabolic activity of the meristematic region. Plants are also capable to absorb nutrients through aerial parts via stomata and ectodesmata. Anyway, the uptake of nutrients from soil follows two steps:

A. Movement of nutrients from soil to root surface
B. Entrance of nutrients from root surface to internal portion of roots

3.2. MOVEMENT OF NUTRIENTS TO ROOTS

Generally, roots do not have any special intension to grow towards a nutrient source. For nutrient uptake process, the individual nutrient ion must come in contact with the root surface where ionic interaction will be happened. However, the rhizosphere may be the main barrier for entry of nutrients into internal structure of roots.

3.2.1. Root Interception for Nutrient Uptake

It is the process of exchange of nutrients as exchangeable ions on root surfaces contacts with the exchangeable ions on soil surfaces. In this process root moves through the soil for searching the nutrient ions. Generally, roots go downward through the process of gravitrophic response. This mechanism is called root interception, and minimum amount of nutrients are absorbed by the root interception process. The amount of nutrients are absorbed through this process is equal to the ions that come to the contact with root, and the amount of root volume is equal to the volume of soil replaced by roots. Roots generally occupy 1% or less of the soil volume. However, root growing in fertile soil or higher nutrient concentration would contact up to maximum of 3% of the available nutrients.

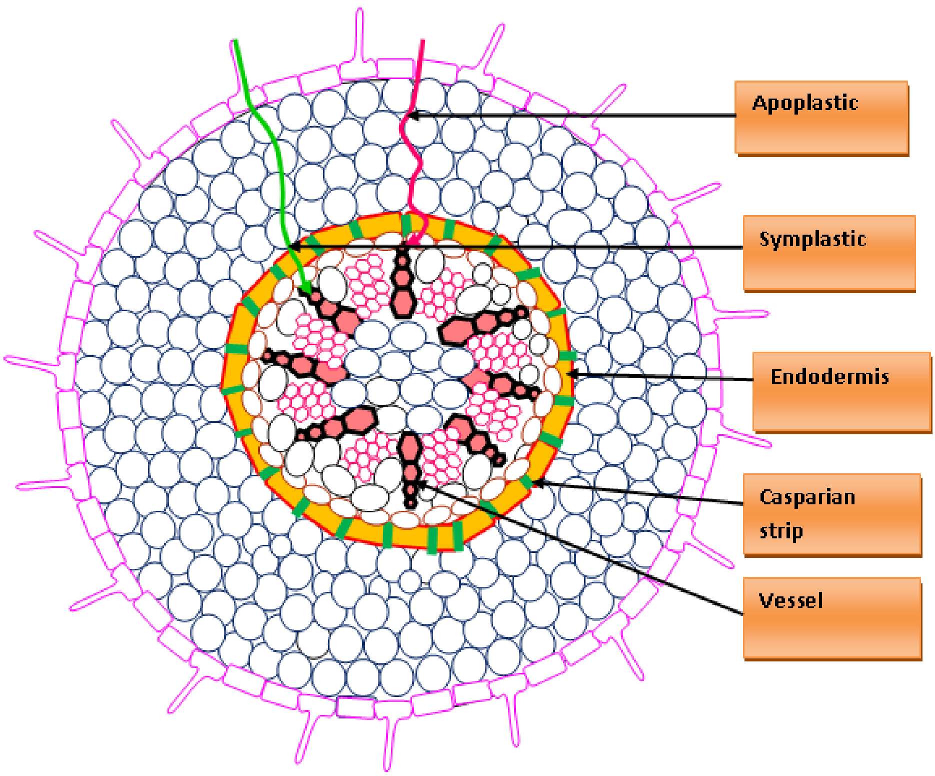


Figure 3.1. Movement of nutrient ions inside the root structure via two pathways: a) apoplastic pathway and b) symplastic pathway.

Characteristics of Root Interception

- Growth of new roots throughout the soil mass enhances root interception
- Nutrient ions are absorbed by contact exchange process (Jenny and Overstreet, 1938)
- When the oscillation volumes of two ions overlap then ion exchange takes place
- Volume of roots is equal to volume of soil displaced
- Roots occupy 1% or less of the soil volume or weight
- Absorption of nutrients can be enhanced by mycorrhizal fungi.

3.2.2. Mass Flow for Nutrient Uptake

Nutrients are transported with the connective flow of water from soil to roots. A large amount of nutrients are absorbed by roots through this process. The quantity of nutrients are transported through the process depend on the flow rate and average concentration in the water. The soluble fraction of nutrients present in soil solution (water) and not held on the soil fractions flow to the root with water is taken up by plants. Nutrients such as nitrate-N, potassium, calcium and sulfur are normally supplied by mass flow. Mass-flow of nutrients from soil to roots may enhance by transpiration when the soil solution contains lower amounts of nutrients or where the root system is weakly developed. However, this process does not play any direct role in absorbing nutrients across the plasmalemma; the greater concentration of nutrients in the rhizosphere may enhance the membrane nutrient transport (Cernusak et al., 2011).

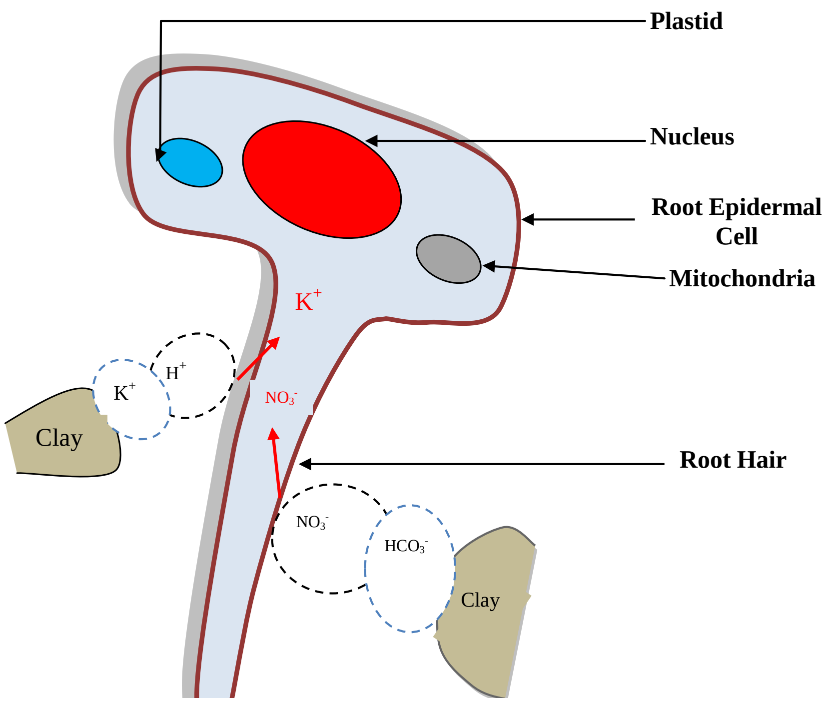


Figure 3.2. Absorption of nutrient ions through root interception.

Characteristics of Mass-Flow for Nutrient Uptake by the Roots

- Mass-flow plays an important role for nutrient present in soil solution in higher concentration, and when transpiration rate is very high.
- It occurs when plant nutrient ions and other dissolved substances are transported in the flow of water to the root.
- The amount of nutrients that move through this process is determined by the rate of water flow and nutrient concentration.
- Mass-flow depends on the soil moisture content i.e., decreases with the decreased amount of soil moisture.
- It also depends on the temperature of the atmosphere i.e., rate will be decreased with the decrease of temperature.

3.2.3. Diffusion for Nutrient Uptake

Diffusion is the process of nutrients transport from higher concentration to lower concentration through thermal kinetic of elements. It is the net passive movement of particles (atom, ion or molecule) from a region of higher concentration to lower concentration i.e., through the concentration gradient. A gradient is the change in value of quantity (concentration, pressure temperature) with the change of another variable. Nutrients such as phosphorus and potassium are absorbed strongly by soils and are only present in small quantities in the soil solution. These nutrients move to the roots by diffusion. The concentration of nutrients close to the proximity to the root decreases when the uptake process is increased. This creates a gradient for the nutrient to diffuse through the soil solution from a zone of higher concentration to the depleted solution adjacent to the root. Diffusion is responsible for majority of the P, K and Zn moving to the root for uptake. The diffusion process is of two types:

- Normal diffusion
- Facilitated diffusion

The intensity of nutrient diffusion can be measured through the following equation known as Fick's first law:

$$dC/dt = De\ Ø\ dC/dX$$

Where, dC/dt= diffusion coefficient (change of concentration with time)
dC/dX= concentration gradient (change of concentration with distance)
De=effective diffusion coefficient
Ø= cross sectional area of ion to be diffused

Again the De (effective diffusion coefficient) can be defined as following equation:

$$De = Dw\ A(1/T)(1/b)$$

Where, Dw =diffusion coefficient in water

A= volumetric soil water content
T= tortuosity factor
b=soil buffering capacity

The equation implies that the rate of diffusion coefficient is directly related to concentration gradient (dC/dX), buffering capacity and tortuosity of the soil.

Quantity of Nutrients Absorbed by the Roots

When diffusion is the major process of nutrient absorption in plant the quantity of nutrients absorbed by a certain root can be measured by the following formula:

$Q = 2\pi r \alpha c t$
Q= quantity of nutrient absorbed per centimeter root length
r= radius of the root (in centimeter)
α= nutrient absorbing power of the root in centimeter root length
c=average nutrient concentration in the media
t= time required for nutrient absorption

The absorption depends upon the concentration of nutrients in the solution and nutrient absorbing capacity of the roots.

Factors Affecting Diffusion Process in Soil

- Moisture content in the soil affects the diffusion, and decrease with the decrease of water content.
- Tortuosity of the soil strongly affects the diffusion process i.e., decrease with the complex pathway of nutrients to be diffused.
- Buffering capacity of the soil decrease the diffusion capacity of nutrients

Table 3.1. Nutrients taken up by the roots by mass-flow, diffusion and root interception

Nutrient element	Mass-flow	Diffusion	Root interception
Nitrogen	√	-	-
Phosphorus	-	√	-
Potassium	√	√	-
Calcium	√	-	√
Magnesium	√	-	√
Sulfur	√	√	-
Iron	√	√	√
Zinc	√	√	√
Copper	√	-	-
Manganese	√	-	√
Boron	√	-	-
Molybdenum	√	-	-

Factors Affecting Nutrient Uptake by Plant Roots

- Aeration of the soil
- Soil moisture content
- Soil pH
- Ion exchange capacity of the soil
- Nutrient concentration of the soil
- Role of physico-chemical properties of ions and root metabolism
- Interactions between ions
- Cation-anion relationship
- Internal concentration and nutrient status

3.3. SPACE OF INTERNAL STRUCTURE OF ROOTS

3.3.1. Apparent Free Space (AFS)

It is located outside of the cell and cell membrane, and composed of cell wall, intercellular space and middle lamella. The AFS is nonliving portion of the plant organ, and contains water free space (WFS) and Donnan free space (DFS). It is normally occupied around 10% of the root volume. In apparent free space nutrients can easily move through diffusion and mass-flow process.

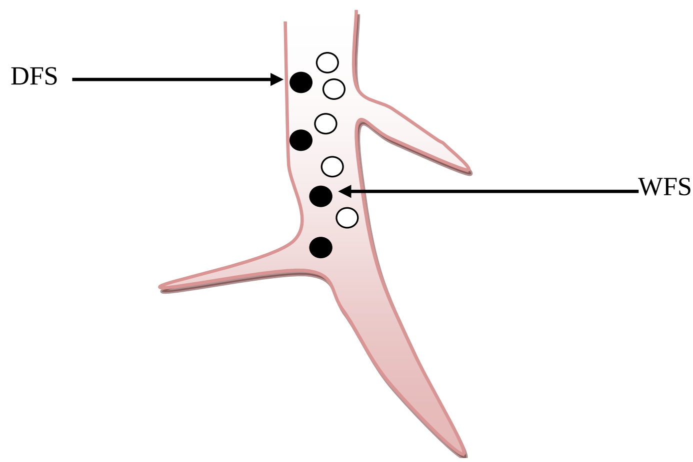
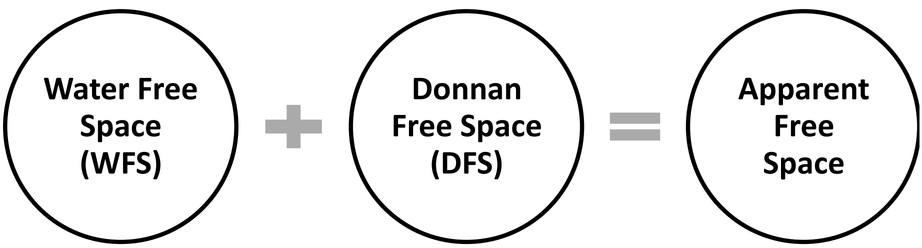


Figure 3.3. Schematic diagram showing WFS and DFS in AFS of apoplastic region of root.

3.3.2. Water Free Space (WFS)

It is the portion of root apoplasm which is occupied by water and free movement capacity of ions as well as free accessible to ions. No ionic exchange i.e., cation exchange or anion exchange occurs here. Nutrients move through diffusion and mass-flow (by transpiration pool).



In water free space, nutrients move through diffusion and mass flow (if present).

3.3.3. Donnan Free Space (DFS)

Donnan proposed that cell contains some negatively charged macromolecules at the outside of the cell i.e., in the apoplasm, and the space is called Donnan free space (DFS). In DFS, ions are exchanged by the counter ions present as adsorbed on cell wall, middle lamella. In order to neutralize the charge, more cations enter into the tissue and create further chance of ion exchange through cation exchange and anion repulsion. The adsorption site is the COO^- group of pectic or other acid present in the cell wall. The cell wall composed of cellulose microfibril which size is around 10-20 ηm in diameter. This microfibril is only a portion of the cell wall and the rest of the cell wall is composed of pectic substances (polygalactournic acid including a variable amount of neutral Ca-pectate).

Donnan effect is the behavior of charged particle near the selectively permeable membrane, the usual causes is the presence of a different charged substance which are unable to pass through plsmalemma resulting the uneven charges of both the sides. This phenomenon happened when some ions can pass easily while others cannot.

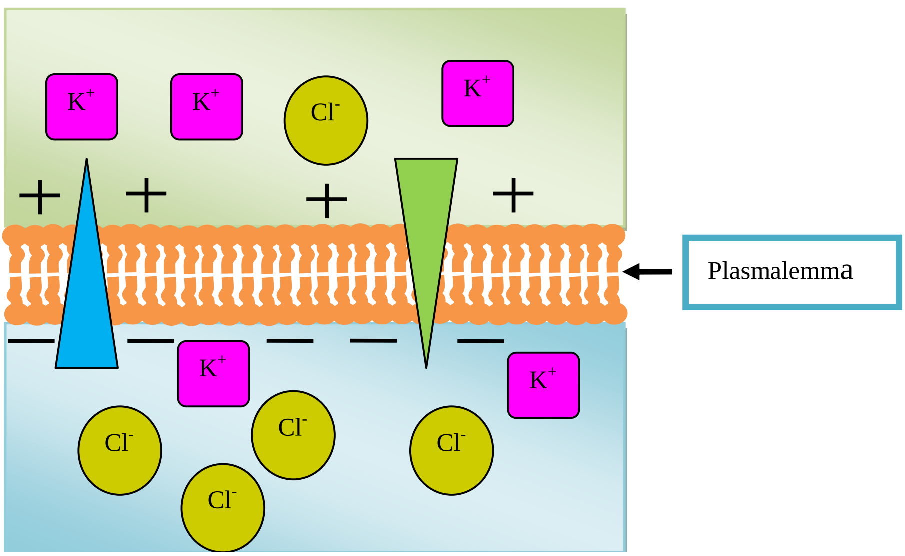


Figure 3.4. Donnan equilibrium state of ions in both side of plasmalemma.

3.4. The Mechanism of Nutrient Uptake

The uptake of nutrients by roots is very complex which may or may not require metabolic energy.

Plant nutrients are taken up by following two processes based on the requirement of energy:

- Passive uptake
- Active uptake

3.4.1. Passive Uptake Process

In this process nutrients are absorbed by roots passively without costing any metabolic energy. This is mediated by diffusion and ion exchange process, occurs outside the membrane and Casparian strip of endodermis. It is a downhill process where ions move through concentration gradient. The rate of diffusion depends upon the steepness of concentration gradient between outside and AFS (apoplastic area) of the root. In passive transport, nutrients move through the following ways:

A. Mass-flow
B. Diffusion: simple diffusion and facilitated diffusion
C. Ion exchange i.e., cation exchange and anion exchange
D. Osmosis

Characteristics of Passive Absorption

- This process does not require any energy (ATP) obtained through metabolic process.
- It is a downhill process through the concentration gradient.
- Nutrients are absorbed by diffusion, ion exchange and mass-flow process.
- Uptake process occurs quickly.
- It is not highly selective process.
- It is non-metabolic process
- Ions are in solution or adsorbed with the surface
- It is also dependent on the permeability of plasmalemma and its organizational structure.

Limitation of Passive Transport

- In actual process, the rate of nutrient uptake is too rapid to be explained the passive uptake.
- It cannot explain the uptake of nutrient against the osmosis gradient.
- There is a close relationship between nutrient uptake and metabolic activity of plant.
- Nutrient uptake has been found to be increased with the respiration rate.

Downhill Transport

The movement of nutrients from higher to lower concentration or gradients either in concentration or electrical potentialities is called downhill transport process. It does not require any energy i.e., in the process of passive transport.

3.4.2. Diagnosis of an Ion Whether It Will Be Absorbed Actively or Passively

The nutrient ion whether it will be absorbed actively or passively can be determined by following formula known as Nernst equation. This equation relates the numerical value of concentration gradient to the electrical gradient that balances it. The equation has physiological implication when used to calculate the potential of an ion across the membrane. The equation is as follows:

$\mu = \mu^0 + RT \ln a + zF\varphi$

Where, μ=electrochemical potential of an ion species

μ^0= standard chemical potential of the ion or molecule species
R= gas constant, 7.95 J/^{0}C/mol
T=absolute temperature
ln = natural logarithm
z = valancy of the ion species
F=Faraday constant
φ = electrical potential

The electrochemical potential will be equal under equilibrium condition at both sides of plasmalemma, and the equation is as follows:

$RT \ln a_o + zF\varphi_o = RT \ln a_i + zF\ \varphi i$

Where, ao= concentration outside of the cytosol

ai=concentration inside of the cytosol

The difference of electrical potential across the plasma membrane is φi- φo can be measured by voltmeter and is referred to as E.

$$E= \varphi_i - \varphi_o = \frac{RT}{zF} \ln \frac{ao}{ai}$$

The Nernst equation can be further simplified by converting natural logs to 10 base logarithm, and considering the gas constant and absolute temperature and thus gives:

$$E= \frac{58}{z} \log \frac{concentration\ outside}{concentration\ inside} \text{ mV (milli volts)}$$

Protons (hydrogen ion) can replace most of the cations easily. Therefore, plant roots also efflux (secrete) protons to make cations available for absorption. Acid rain will wash out the cations of soil solutions and colloidal surfaces by the same mechanism of cation exchange.

So as to investigate whether an ion species will move through uphill or downhill i.e., active or passive process into the cell, the concentration of that particular ion species in the apoplastic region and in the cytosol must be estimated. The electropotential (Em) between the cytosol and the outer medium can be measured by using microelectrode of a voltmeter. The electrical potential differences (Ecal) can be calculated by substituting the measured ion concentration into the Nernst equation. The difference between Em and Ecal indicates whether an ion species will move passively or actively.

Table 3.2. Measured (Em) and calculated electropotential differences (Ecal) and the resulting differences of forces (Ed) an indication whether an ion will absorve actively or paasively (Spanswick and Williams, 1964)

Ion species	E_m	E_{cal}	E_d (E_m-E_{cal})	Nature of uptake
Na^+	-138	-67	-71	passive
K^+	-138	-179	+41	active
Cl^-	-138	+99	-237	active

For better nutrient management in crop production it is necessary to acquire knowledge on root interception, mass-flow and diffusion processes. Immobile nutrients should be applied to the soil near to the root surface when buffering and ion exchange capacity of soil are high and soil moisture content is low.

3.4.3. Facilitated Diffusion

It is one type of passive transport or passive mediated transport of nutrients facilitated by integral proteins. In this process the ions are passed spontaneously through biological membrane. It may occur either across biological membrane or through aqueous compartments of an organism. This process does not require any chemical energy i.e., hydrolysis of ATP rather nutrients move down through concentration gradient by diffusion via downhill process.

3.5. Active Transport

Active transport is the energy-demanding transfer of nutrients across a cell membrane against the concentration gradient, i.e., from lower concentration to higher concentration. It is also called uphill process where the movement of an ion against its concentration or electrical gradient needs to be energized. The energy comes from the chemical source through the hydrolysis of ATP. In all cells, this is usually concerned with accumulating high concentrations of molecules that the cell needs, such as ions, glucose, and amino acids.

Characteristics of Active Transport

- It requires energy that comes from ATP
- The movement of nutrients are unidirectional
- This process requires ion pump or carrier protein

Types of Active Transport

- Primary active transport
- Secondary active transport

3.5.1. Primary Active Transport

It is also called direct active transport, directly uses energy to transport molecules across a membrane. This process requires energy (ATP) derived from metabolic activity of cells. This process requires an input of energy and does occur spontaneously, and is always unidirectional i.e., from outside to inside or vice versa, and is always mediated by transport protein. The movement of nutrients is against the concentration gradient.

3.5.2. Secondary Active Transport

In this process there is no direct involvement of ATP rather electrochemical potentialities are created by pumping ions out of the cell used. Ions are entered as cotransport or through facilitated diffusion process.

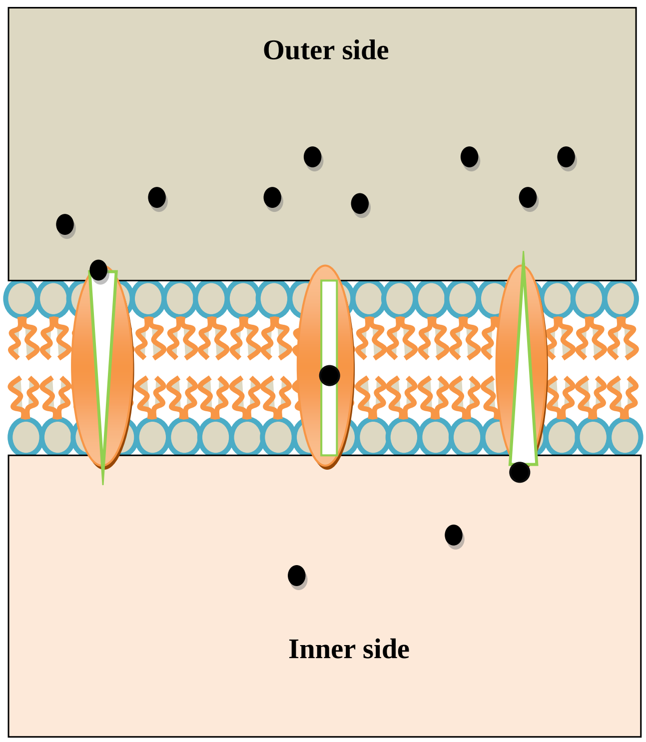


Figure 3.5. Schematic diagrame of movement of ions through ion channel in the plasmamembrane of plant cell via facilitated diffusion.

3.5.3. Theories of Active Transport

Different theories have been put forwarded to explain the active absorption of nutrients. It is an energy-costly process and is supported by metabolic energy. The following theories have been suggested to explain the active transport process of nutrient in plants.

3.5.3.1. Carrier Concept Theory

Honert (1937) proposed the carrier concept theory for nutrient transport where ion uptake process is carried out by a carrier such as protein, organic molecule or vesicle etc. It is postulated that ions undergo reversible binding with some constituents of outer space i.e., apoplastic area designated as carrier and pass through inner space as ion-carrier complex. Upon reaching the inner side they again release the ions into protoplasm. This process continues unidirectionally i.e., from apoplastic area to protoplasm or protoplasm to apoplastic area.

In carrier mechanism, activated ions combine with carrier proteins and form ion carrier complex. This complex moves across the membrane and reaches the inner space by the expenditure of energy.

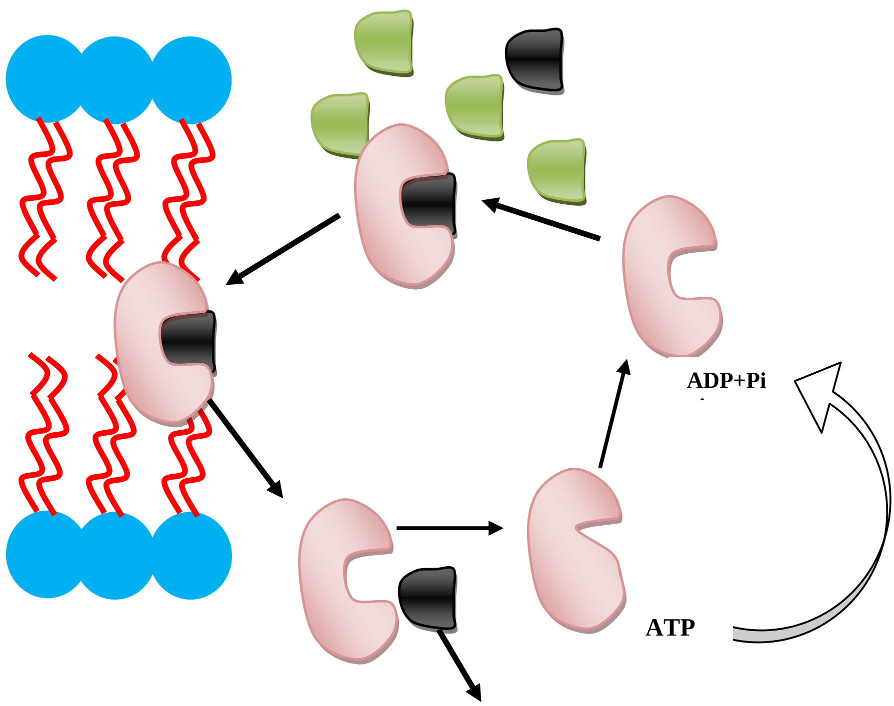


Figure 3.6. Diagrammatic presentation of carrier concept theory where ions are trapped by a carrier and form a carrier ion complex upon reaching the protoplasm the ions are released and inactivated carrier become activated by using ATP.

3.5.3.2. Cytochrome Pump or Electron Transport Theory

This theory was proposed by H. Lundegardh (1954 a, b), who suggested that anions could be transported across the membrane by cytochrome system. There is a direct relation between anion absorption and respiration, and the anion transport system takes place through cytochrome. Energy is supplied by direct oxidation of respiratory intermediates.

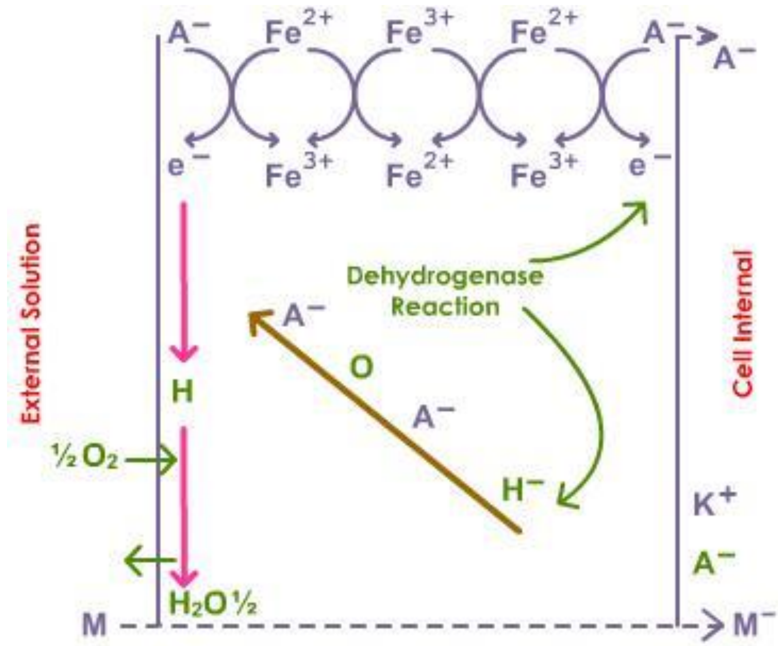


Figure 3.7. Diagrammatic representation of cytochrome pump hypothesis on salt absorption, anions (A^-) is actively absorbed via a cytochrome pump and cations (M^+) are passively absorbed.

The rate of respiration, which is mainly due to anion absorption, is called as anion respiration or salt respiration. The original rate of respiration (without anion respiration) can be observed in distilled water and is called ground respiration.

Total respiration (R_1) = ground respiration (R_g) + salt or anion respiration (R_a)

The Major Assumptions of This Theory

- The anions are absorbed actively through cytochrome chain
- The cytochrome picks up the anions from the outer surface of plsmalemma and transports to the inner surface
- The protons and electrons are produced in the inner surface of the plasmalemma
- The electrons are released by taking the anions in its place
- Absorption of cations takes place passively

Limitation of Carrier Concept Theory

- The theory does not explain the uptake or absorption of cations
- It explains the general mechanism of anions uptake not selectivity or specific ion absorption phenomenon
- It operates in aerobic respiration state however, anaerobic respiration condition is not explained

Entry of Ions into Protoplasm

The nutrient ions have to pass through plasmalemma where both hydrophilic and hydrophobic polar groups are present, and the earlier is the main active barrier of ions entry. Both active and passive processes are involved for entry of ions into the protoplasm.

3.6. SHORT DISTANCE TRANSPORT

The movement of nutrients from root epidermis to xylem vessel via cortex, endodermis, and pericycle is called short distance transport (SDT). The Casparian strip is the permeability barrier of nutrient ions consequently an obstacle of apoplastic movement in short distance transport system. The main barrier of the apoplastic movement is the Casparian strip which is made up of suberin, a lipid body present in the endodermis. Symplastic movement of nutrients is an alternative way in endodermis where large amount of ATP-ase are required to perform active transport. Short distance transport of nutrients can be performed by both apoplsatic and symplastic ways.

Uphill Process

When the movement of nutrients takes place against the concentration or electropotential gradients is called uphill process. In this process, metabolic energy in the form of ATP is required for uptake of nutrients.

3.7. ION PORTS

Following ports of ion movement in the plasmalemma are very much related to nutrient transport from one cell to another cell:

i. Uniport
ii. Symport
iii. Antiport
iv. Cotransport
v. Ion channel
vi. Carrier protein

3.7.1. Uniport

When a single ion species passes through a single channel or port is called uniport. It is an integral membrane protein. Only one ion can pass through the channel in a particular time. The uniporter may not take any energy i.e., the ion or solute can pass one side to other side of membrane through concentration gradient.

3.7.2. Symport

A cell membrane structure that transports more than one ion in the same direction is called symport. It is also an integral membrane protein as mentioned in uniport. Although two or more types of molecule are transported, there may be several molecules transported of each type. Like uniporter, the symporter does not require any energy, and the molecules or solutes are passed through concentration gradient.

3.7.3. Cotransport

It is the movement of one ion with another ion in same direction. It is an integral membrane protein that is involved in secondary active transport. It acts by binding two molecules or ions at a time and using the gradient of one solute's concentration to force the other molecule or ion against its gradient.

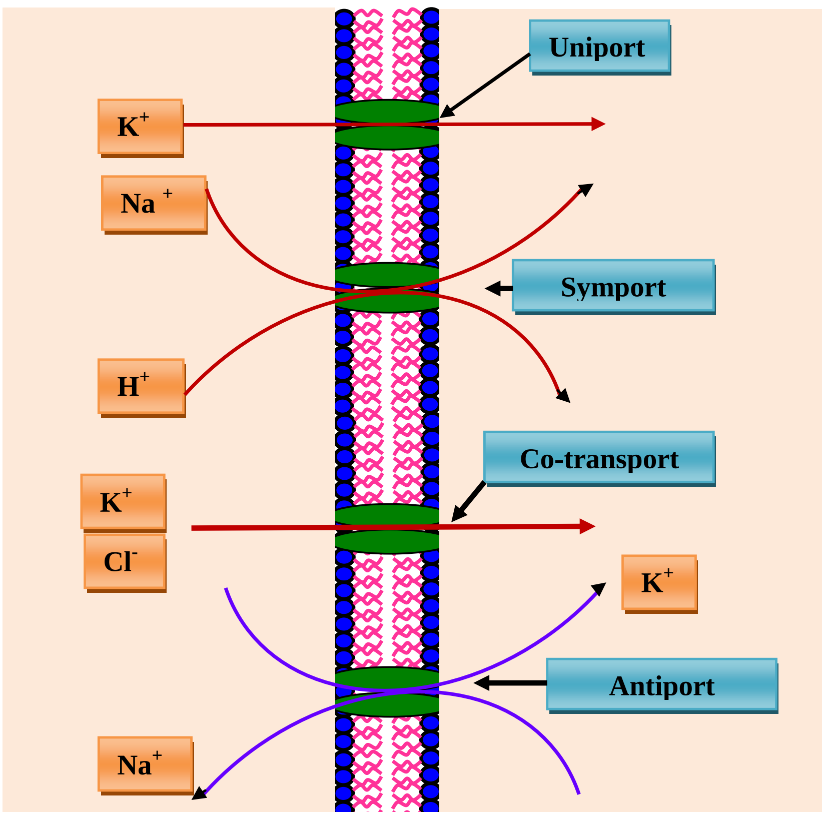


Figure 3.8. Diagrametic schetch of different types of ports in the plasmamembrane of a plant cell for ion movement.

3.7.4. Antiport

In antiport two species of ion or other solutes are passed in opposite directions across a membrane. In antiporter, ions do not require any energy, and move through concentration gradient i.e., from higher to lower or higher electrical potentiality to lower potentiality.

3.7.5. Aquaporin

It is a type of protein where water molecules can pass through, and water selective pores also known as water channel. The pore is constituted by membrane integral protein. It is very selective for the movement of water but other ions cannot pass through aquaporin.. The presence of water channels increases membrane permeability to water.

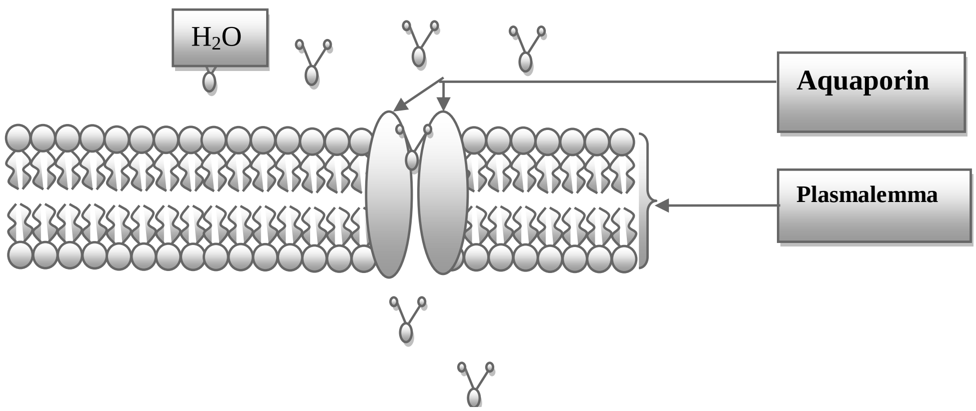


Figure 3.9. Diagrametic sketch of movement of water molecule through aquaporin in the plasmalemma of plant cell.

3.7.6. Ion Pump

Nutrient ions are transported by certain pump in the plasmamembrane. The main ion pump takes place in the plasmalemma is the plasmamembrane H^+-ATPase. Other pumps namely Na, K, Ca, Mg ATP-ase have also been documented. P-type pumps are characterized by forming a phosphorylated reaction-cycle intermediate during catalysis. In this way, plasma membrane H^+-ATPases differ from all other proton pumps in the plant cell, including the vacuolar membrane H^+-ATPase, another major proton pump which energizes the vacuolar membrane. The vacuolar membrane H^+-ATPase has more than 10 different subunits, whereas, in contrast, the functional unit of plasma membrane H^+-ATPase is a monomer, although it might be organized in the membrane as a dimer or in oligomers.

Ions are transported through pumps i.e., enzyme by the use of ATP. This is a common phenomenon in membrane transport of ions against the concentration and electrical gradient. In plant cell, most of the transport proteins get energy through electrochemical gradients of proton in the plasmalemma; the gradients are formed by the activity of plasmalemma H^+-pumps stimulated by ATP.

3.7.7. Ion Channel (Channel Protein)

The ion channel otherwise called channel protein form pore for controlling flow of ions by making channel gate. Channels are integral membrane proteins made by arranging many individual proteins together. They are present in all types of membranes, and channels are identified by the hydrated size and charge of ions that pass through the channel. Ion channels are frequently gated i.e., they have the capacity of opening and closing. There are two types of gates: one is controlled by electrochemical potential and the other is hormone, light and other stimuli mediated gate. The precise mechanism of operating of gate is not clear, however, it is assumed that it is controlled by the activity of protein. Channels are also considered to be one of the two conventional class viz. ionophoric proteins and ion transporters. It is important that the amount of ions passes through the channels are not dependent on the number of channels per cell but the capacity of individual channel i.e., number of ions passes per second. The distinctive features of ion channels which differentiate one from the other types of ion transporter protein:

- The rate of ion transport through ion channel is 10^6 ions per second
- No energy is required when the ions pass through the concentration gradient and membrane potential.

Table 3.3. Differences between active and passive transport of nutrients

Active transport of nutrients	Passive transport of nutrients
Require metabolic energy	Does not require energy
It is the uphill process i.e., absorption of nutrients against the concentration gradient.	The absorption of nutrients are performed through the concentration gradient of nutrients i.e., the downhill process.
The chemical energy i.e., ATP is required for the uptake of nutrients.	No ATP is required in absorption of nutrients or solutes.
Involves electrogenic proton pump, carrier protein etc.	Involves mass-flow, diffusion and facilitated diffusion
Ion, solutes, amino acid, proteins can be passed by active transport process	Any soluble materials can pass through passive transport process
Disrupts the equilibrium between outside and inside of the plasmalemma.	Maintains the dynamic equilibrium of nutrients, solutes and other substances between outer and inner side of the plasmalemma.

3.8. Factors Affecting Nutrients Uptake

3.8.1. Temperature

Variations in soil and canopy temperature influence on the absorption of mineral nutrients. Temperature has direct effect on the metabolic activity of cells, and increment of 10^0C temperature increases the enzyme activity by double the rate which is called Q_{10}. More nutrients will be absorbed due to increases of canopy temperature.

3.8.2. pH

The availability of plant nutrients both macro and micro are highly influenced by the pH of the soil. Most of the nutrients are absorbed in the soil pH range of 6 to 7.5 which is ideal for majority of the crop plants.

3.8.3. Light

Light has direct influence on photosynthesis and production of food materials. High light intensity increases the uptake of nutrients by increasing the mass-flow through enhanced transpiration rate. Generally, transpiration is increased under high light intensity and higher temperature condition. The active transport or absorption of nutrients is increased when supply of ATP is higher, and this phenomenon is mediated by higher photosynthetic activity under high light intensity.

3.8.4. Oxygen Tension

Oxygen is required for the respiration of plant organs. Root respiration is disturbed due to absence of oxygen. Absorption of nutrients is affected due to low oxygen tension. Supply of ATP through metabolic activity is disturbed when the oxygen tension is low.

3.8.5. Interaction with Other Ions

Interaction of ions in the soil solution influences on the absorption of nutrients. Ions compete among themselves for the ion port or ion channel. Three type of interactions namely competition, synergism and antagonisms among the ions are found in the process of absorption of nutrients.

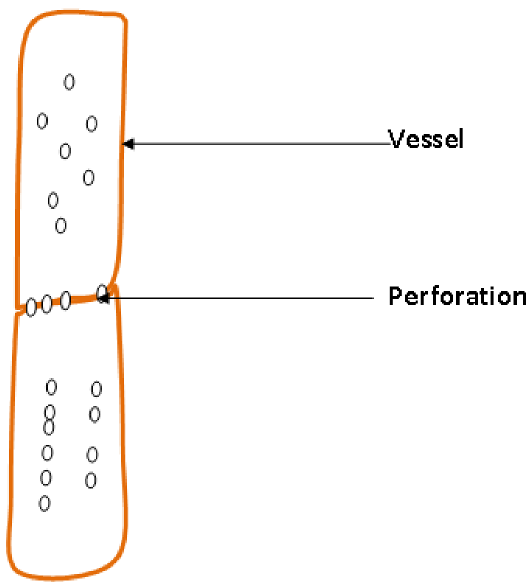
3.8.6. Growth of Plant

Plant growth significantly increases due to uptake of nutrient where higher uptake rate of nutrients are found at active growing condition of plants. The metabolic activity will be higher at higher growth rate of plants and consequently the sink strength of nutrient will be greater.

3.8.7. Internal Concentration and Nutrient Status

Concentration and nutrient status of the cytosol significantly influence the absorption of nutrients. Generally uptake rate is higher when the internal concentration of a particular nutrient is lower. The uptake rate is also influenced by the electrochemical potential of cytosol. The nutrient concentration in the xylem sap has strong implication on the uptake of nutrients.

3.9. Loading of Nutrients into Xylem Vessel



When the nutrients are absorbed by the epidermis it passes through the cortex and finally reach to the vascular tissue i.e., in the vessel. Nutrients must be released into xylem vessel for long distance transport with the transpiration stream to the shoot *vis-à-vis* leaves and get distributed to different parts of shoot. The transport of nutrients to vessels is performed via apoplastic and symplastic pathways. The Casparian strip, present in the endodermis is the main barrier for apoplastic movement, and is overcome by transmembrane transport through endodermis. The secretion of nutrients into vessel occur through the plasmamembrane as symplastic transport of the living cells adjacent to xylem namely pericycle and xylem parenchyma (Clarkson, 1993). Additionally, many transporters are present in the pericycle and xylem parenchyma. The long distance transport of nutrient elements or solutes is mediated through xylem vessel to the sink point (mesophyll tissue), and water is the main carrier of ions. The xylem vessel is nonliving tissue and perforation is present in the transverse wall. The transport is governed by the gradient of hydrostatic pressure and gradient of nutrient concentration. The process of nutrient transport through xylem vessel is mediated mainly by mass-flow accompanied with the ionic exchange between the ions present as adsorbed in the wall of vessel even adsorbed in the xylem parenchyma cell wall. The rate of nutrient transport through vessels is influenced by the rate of transpiration of plant. The divalent cation present in the xylem sap may be absorbed by the vessel wall through cation exchange process and may be reabsorbed further.

3.9.1 Xylem Unloading

Once nutrients arrive in the vessel of leaves it will be unloaded to the adjacent xylem parenchyma thereafter mesophyll tissue. The apoplastic movements of nutrients may be changed to symplastic in the mesophyll tissue, and at the end of rout the xylem parenchyma cells.

3.9.2. Phloem Protein

Phloem protein i.e., phloem specific protein is useful for transporting macromolecules in the phloem. The phloem of angiosperm contains proteinaceous structure collectively called P protein. It is deposited initially into the vessel and is ultra-structurally distinct. It influences on the growth and development of plant through long-distance transport of macromolecules in vascular tissues (Bettina et al., 1999).

3.9.3. Transfer Cell

They are special type of parenchymatous cell responsible for short distance transport of solutes through cell-to-cell transfer, and are plant cells with secondary wall growth. Some time they are found in between xylem and phloem to transport food material among them. They occur in association with veins in leaves and stems and also in many reproductive parts. These cells are ubiquitous, occurring in all plant taxonomic groups and in algae and fungi. Transfer cells are considered to play a major role in nutrient transportation by facilitating high rates of transport at bottlenecks for apo-/symplasmic solute exchange (Offler et al., 2003).

3.10. Phloem Loading and Unloading

The process in which nutrient elements and assimilates (sugar, amino acids, protein, organic acids etc.) are loaded to phloem tissue i.e., the sieve tube from the mesophyll tissue which are physiologically very much active is called phloem loading. Phloem loading commences in mesophyll cells and ends in the leaf vascular tissue. Phloem loading also occurs in storage organ during mobilization of reserve materials. The companion cells perform important function in the loading process. Assimilates and nutrients move through symplastic via plasmodesmata or through apoplastic pathway.

Phloem Unloading

When the nutrients and assimilates reach to the sink, it must be unloaded from sieve tube-companion cell complex to the phloem parenchyma subsequently to the sink tissue. The unloading process takes place either via symplastic or apoplastic pathway.

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Chapter 4

FOLIAR UPTAKE OF NUTRIENTS

4.1. GENERAL FEATURES

Foliar fertilization refers to application of fertilizer on the leaf surface and subsequently utilized by the plant. This technique provides important avenue for the growth and development of crop plants. It was first described by Tukey and Wittwer in 1950 by radio isotopic study that nutrients are absorbed by plant leaf and translocated to different parts of the plant (Tukey and Wittwer, 1956). It provides nutrients by mist onto leaves and stems of crop plants. Plants can absorb nutrients through aerial parts namely stomata, lenticels, ectodesmata. In terrestrial plants, stomata are the site of exchanging CO_2, O_2 with atmosphere. Some mineral elements like N and S are taken up as NH_3 and H_2S through stomata. Generally, plants can absorb smaller amount of nutrients by aerial parts; however, it is effective in case of micronutrients absorption in crop plants. Since the amount of such nutrients needed by plants is very small which may be supplied as foliar sprays. Plants may also uptake macronutrients through the leaves, however, sufficient amount of nutrients are not supplied in this way, and these must be taken up by the roots. The fertilizer to be sprayed as foliar must be soluble in water otherwise the nutrients will not be able to be absorbed by the mesophyll tissue. Some time foliar application may be replaced by soil application in case of leaching or washout by rainfall. All the essential nutrient elements can be applied through foliar spray but their absorption and utilization are not effective equally. Anyway, the following fertilizers are usually applied for successful crop production:

Some common foliar fertilizers used for crop production

- Iron chelate
- Zinc chelate
- Boron chelate
- Copper chelate
- Zinc and iron chelate
- Zinc+ copper+ iron chelate

4.2 Advantages of Foliar Spray

Crop yield can be increased up to 12-25% by the foliar application of nutrients when used properly. In this way short-term problems of crops like drought, flash flood etc. can be solved effectively. Nutrients applied to foliage are absorbed quickly than roots. It is very effective and useful than root uptake in complex soil where unavailability of some elements exsist. For example, iron is less available in calcareous soil and P is fixed or less available in acidic soil. Foliar application is more preferable than soil application under such conditions. It is resulted in early flowering and increased the quality of flower in Gerbera as reported by Khosa et al. (2011). The leaf anatomical feature shown in the following micrograph illustrated the tissue system of a monocotyledonous leaf indicating the absorption and translocation of nutrient elements (Figure 4.1).

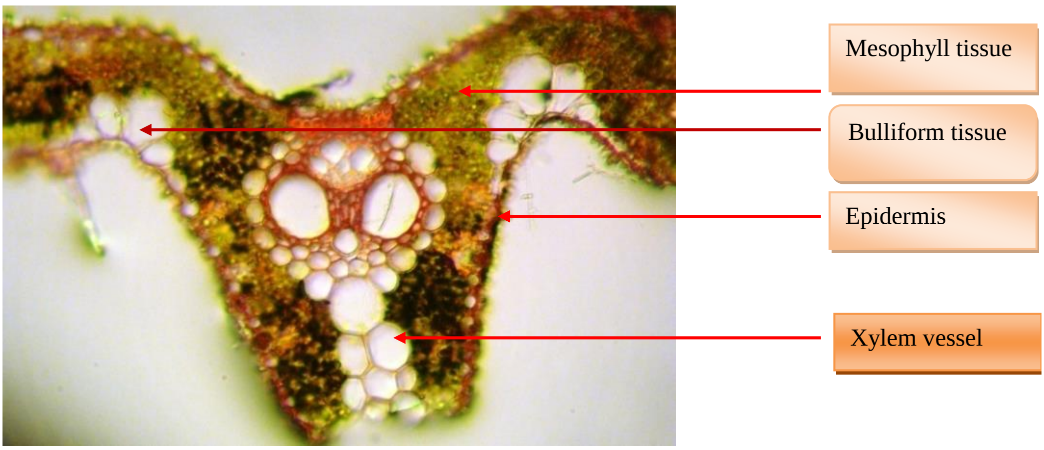

Figure 4.1. The cross section of rice (*Oryza sativa* L.) leaf showing different tissue system i.e., epidermis, mesophyll, vascular and bulliform tissue.

4.2.1. Foliar Spray Is Effective in Problem Soil

This system of fertilizer application is very much effective in case of problem soil. Most of the nutrients may be unavailable under problem soil conditions like under high and low pH conditions, light soil, saline soil or in other cases (Marschner, 1995). In sandy soil, nutrients are leached leading to severe loss of nutrients. Foliar application is very much effective than soil application.

4.2.2. Foliar Spray under Water Deficient Condition

Nutrients become unavailable to crop plants under arid and semiarid conditions where soil moisture level is low. Under drought condition roots cannot penetrate enough deeper regions for absorbing nutrients especially in the subsoil. Deficiency of nutrients created along with water deficiency. Under this circumstance foliar application of nutrients is very effective.

4.2.3. Foliar Application is Effective in Flooded Condition

Foliar application of nutrients is very much effective under flooded condition where soil application can washout the nutrients from the rhizosphere. Foliar application can ensure the sustainable crop production in hydrophytic and semihydrophytic crop plants. The nitrate-base N-fertilizer is prone to leaching loss under flooded condition.

4.2.4. Improvement of Crop Quality

Fruit quality can be improved by foliar application of nutrients especially by applying the less mobile elements. Nutrients have higher utilization rate, and can usually be applied together with pesticide. In this process dramatic increase of fruit production in tomato may be possible by spraying at flowering stage.

4.2.5. Foliar Spray Is Effective in Fruit Tree

In fruit cultivation, it is difficult to use fertilizer to the rhizosphere as the root system is very deep compared to field crops. The effective roots which are efficient in nutrient absorption are located in the deeper soil, and applied fertilizer cannot reach to that region resulting net loss of nutrients especially the fertilizer-N. In the circumstances, foliar applications of mobile elements are effective for increasing yield and quality of fruits.

4.2.6. Poor Root Growth at Flowering Stage

It is very effective to overcome the micronutrients deficiency in subsoil than soil application (Modaihsh, 1997; Torun et. al., 2001 and Grewal et al., 1997). It can guarantee the utilization of nutrients and consequently the higher yield.

It can be used to control certain physiological disorder and diseases like blossom end rot in tomatoes and other fruits, or tip burn in lettuce and cabbage which may often occur during drought condition.

4.3. Disadvantages of Foliar Application of Nutrients

Sometimes fertilizer materials are not compatible with all pesticides, and if mixed with these non-compatible materials it may cause serious damage to plants like burning. Application of fertilizers on foliage and other organ may cause burning of surface when use in clear sunshine at noon under hot climatic condition. The fertilizer remains with the water film on the leaf surface dry up due to evaporation, however, the nutrient ions are accumulated as concentrated form which causes burning the epidermal tissue. This type of problem can be avoided by applying lower concentration of nutrients and also adding surfactant for higher retention of water (Mengel et al., 2001).

The fertilizers to be used as foliar spray must be soluble in water and proper dose should be maintained. Most of these are salts and when applied at a very high' concentration the solution will cause "burning" of the plant tissues.

There is a chance of leaching and washout of nutrients when applied as foliar spray. Heavy rainfall can cause the washout of nutrients and subsequently causes the loss of nutrients.

4.4. Conditions for Effectiveness of Foliar Spray

- Leaf surface is one of the most important characteristics for effectiveness of nutrient absorption. Waxy cuticle is not good for absorption of nutrients, while non-cutinized epidermis is very much effective.
- Length of time the nutrient remains dissolved in the solution on the leaf's surface.
- Diffusion of nutrients from epidermis to mesophyll tissue through stomata and ectodesmata.
- Types of formulation i.e., water soluble nutrient materials are more effective than insoluble ones.

The frequency of foliar application depends upon the requirement of the fertilizer as judged by the deficiency symptoms of the nutrients. The foliar application should be made when first deficiency symptom appears in the plant.

Table 4.1. Common fertilizers used as foliar spray for growth and development of crop plants

Name of nutrient	Source of fertilizer	Dose (g/100L)
Nitrogen	Urea (NH_2-CO-NH_2)	1000-1200
Molybdenum	ammonium molybdate {$(NH_4)_6Mo_7O_{24}.2H_2O$}	30-60
Boron	Solubor or disodium borate ($Na_2B_8O_{13}.4H_2O$)	250-600
Copper	Copper sulphate ($CuSO_4.5H_2O$)	250-600
Zinc	Zinc sulphate ($ZnSO_4.7H_2O$)	250-600
Iron	Ferrous sulphate ($FeSO_4$)	250-350
Manganese	Manganese sulphate ($MnSO_4.H_2O$)	250-500

Source: Fertilizer Recommendation Guide (2012).

4.5. Precautions to Be Taken While Spraying

Following precautions should be made on spraying of fertilizer as foliar spray:

I. Do not spray fertilizer during mid-day as stomata remains closed due to high temperature and hot weather.
II. Do not spray when the air blows speedily.
III. The spray should contain sufficient amount of surfactant for effective absorption of nutrients.
IV. Generally foliar spray should be applied in the early morning or evening hours.
V. It is always advisable to spray dilute solution, should not be used concentrated solution.
VI. Make sure that the pH of the solution is appropriate; pH may be adjusted by adding water.
VII. For achieving best results, crops are often sprayed during critical growth stage i.e., active tillering stage, blooming time etc.

Following factors influence the foliar spray and absorption of nutrients through leaves

Plant factors

- Age of the leaf
- Nature and content of epicuticular and cuticular wax
- Presence of hair or trichomes on leaf epidermis
- Frequency, opening and closing of stomata
- Nature and properties of adaxial and abaxial side of the leaf
- Nutritional status of the leaf and plant as a whole
- Variety or cultivar of the crop plant

Environmental factor

- Temperature
- Light intensity
- Photoperiod
- Movement of air
- Humidity
- Drought
- Time of day

Nature of spray solution

- Concentration of spray
- Rate of application
- Nature of wetting agent
- pH of the spray solution
- Stickiness of the spray solution

4.6. PENETRATION OF NUTRIENTS INTO LEAF

Penetration is the entry of nutrients from the leaf surface to mesophyll tissue which is mediated by ectodesmata and stomata. Major portion of fertilizer is absorbed by the ectodesmata, the micropore connection between the outer surfaces of leaf to the mesophyll tissue. The penetration is done by passive process i.e., the downhill process. The cations are penetrated and attracted by anion located in the apoplastic area of mesophyll through ion exchange process. The translocation of spray nutrients is passively done and the rate of translocation is dependent on the concentration gradient.

Penetration is also done by stomata, which are aperture controlled for gas exchange and transpiration. It is known that these apertures i.e., guard cells size differs in plant species, their distribution, occurrence, size and shape. The ectodesmata near the guard cells seem to have different permeability characteristics (Schonherr and Bukovac, 1978).

Translocation of nutrients begins from mesophyll tissue to different plant parts soon after the penetration is completed. It is done through apoplastic and symplastic movements via passive and active transport process.

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Chapter 5

NITROGEN (N)

5.1. GENERAL FEATURES

Nitrogen is one of the most widely distributed elements in the earth. A small portion of lithosphere N is present in the soil, and a minute portion of soil-N is available to plants (Mengel et al., 2001). It is an important essential element for living organisms which is involved in the synthesis of many macro molecules namely protein, amino acids, amides, enzyme; nucleic acids etc. It is a nonmetal element having atomic weight 14, and is colorless and tasteless. Around 78% of air is composed of N_2 which is inert and plant cannot utilize this inert N_2. About 99% of the combined N in the soil is present in organic matter (Thompson and Troeh, 1993). The organic-N is complex and large which is unavailable to the plants, and become available by the activity of microbes. The microorganisms breakdown the complex organic-N and gradually make it simpler form for the uptake of plants. It is the most essential nutrient element requires for plant growth and development. This is the most frequently deficient nutrient element in crop production, and application of sufficient amount of N is required for successful crop production especially for the non-legume crops.

5.2. ISOTOPE OF NITROGEN

Nitrogen has a stable isotope namely ^{15}N, and fourteen radio isotopes having very short half life. The isotope ^{13}N possesses the longest half life of 9.965 minutes, and all the other isotopes have half life below 7.15 seconds. The stable isotope ^{15}N has been widely using in plant nutrition studies in the field of agriculture. It is extensively used to trace mineral N-compounds especially the fertilizer, and is also very important tracer for estimating the fate of nitrogenous organic pollutants. Soon after ^{15}N became available, $^{15}N_2$ was used to provide direct method for detecting biological nitrogen fixation (BNF) but has limited practical or field applications in terms of quantifying BNF (Danso, 1995; Mia et al., 2013; Mia and Shamsuddin, 2010). The isotope dilution technique was first described and applied to measure the contribution of BNF to clover grown in pots by McAuliffe et al. (1958).

5.3. Concentration of N in Plant Parts

Concentration of N varies among plant species, cultivars and habitats of plant. Generally, mesophytes possess higher concentration of N than that of hydrophytes, and leaves contain higher than stem and roots. However, in legumes, the pod contains higher concentration of N than other organ of the plant. In leaves, the mesophyll tissue contains higher concentration than xylem tissue. The magnitudes of N concentration in different types of leaves are: young leaves > mature leaves >senescent leaves. The meristematic tissue contains higher concentration of N than permanent tissue. The differences of N in organs are due to carbon allocation and composition of N in that organ. The critical concentration of N for plants has been defined as the minimum concentration where the maximum growth occurs (Gastal and Lemaire, 2002) and the critical concentration declines with the increased crop growth.

5.4. Common Fertilizer Used in Crop Production

Both organic and inorganic sources of fertilizers are found for supplying the N for crop growth and development. Here, we discussed some common sources of synthetic N-fertilizers.

5.4.1. Synthetic Inorganic-N fertilizer

A. Ammoniacal sources

i. Anhydrous ammonia: it contains the highest amount of % N (around 82%) among the chemical fertilizer which has solid, liquid and gaseous states.
ii. Aqua ammonia {NH_3 (20-25% N)} use to provide N and used as N-fertilizer. It is liquid and colorless chemicals.
iii. Ammonium nitrate (NH_4NO_3) (33-34% N), is a widely used fertilizer which liberates both NH_4^+ and NO_3^- ions. Although it has some drawbacks like, hygroscopic in nature, having risk of fire, less effective in flooded condition and greater probability of loss through de-nitrification.
iv. Ammonium sulfate {$NH_4)_2SO_4$}, (N-21%, S-24%) is a good source of both N and S.
v. Ammonium phosphate {$(NH_4)_3 PO_4$} (N-13%, P-21%) is widely used fertilizer for N and P.
vi. Ammonium chloride (NH_4Cl) used as N fertilizer having 25% N.
vii. Urea (NH_2-CO-NH_2) (N-46%) most popular fertilizer and widely used for crop production.

B. Nitrate source of N fertilizer

i. Potassium nitrate (KNO_3) (N-15%, K-36%) normally use for supplying both N and K.
ii. Sodium nitrate ($NaNO_3$) (N-16%) use for supplying N however, it also liberates Na in the soil.

iii. Calcium nitrate {Ca $(NO_3)_2$} (N-16.5%, Ca-23.5%) supply both N and Ca
iv. Ammonium nitrate (NH_4NO_3) (N-33-34%) supply both NO_3^- and NH_4^+ ions.

Table 5.1. Recommended dose of N-fertilizer for different crops at different levels of soil fertility

Name of Crops	Soil Analyses Interpretation (*level of fertility*)	Dose (kg ha^{-1})
Rice	Optimum	0-50
	Medium	51-100
	Low	101-150
	Very low	151-200
Wheat	Optimum	0-40
	Medium	41-80
	Low	81-120
	Very low	121-160
Maize	Optimum	0-85
	Medium	86-170
	Low	171-255
	Very low	256-340
Jute	Optimum	0-35
	Medium	36-70
	Low	71-110
	Very low	111-140
Cotton	Optimum	0-30
	Medium	31-60
	Low	61-90
	Very low	91-120

Source: Fertilizer Recommendation Guide (2012).

5.5. Methods of N-fertilizer Application

In crop production, N-fertilizer should be applied as broadcasting into several splits based on the nature of crops. Foliar spray may also be done when the plant cannot take it through roots. Recently, fertigation i.e., application of N-fertilizer along with irrigation water is being practiced for better crop production. Urea super granule (USG) and urea mega granule (UMG) can be used as deep placement in between the seedlings of irrigated crop plants especially the wetland rice cultivation.

5.6. Forms of N Uptake by the Crop Plants

Nitrogen is generally taken up by the plants as NO_3^- and NH_4^+ form. However, in legume-*Rhizobium* symbiosis system N is taken up as N_2 and sometimes urea is also intake by foliage through stomata and ectodesmata. The enzyme urease is induced in the cytoplasm for the breakdown of urea into ammonium in case of urea feeding plants. Urea is a metabolite in the plant system and its utilization is enhanced by the presence of urea in the medium and suppressed by the presence of NO_3^- (Merigout et al. 2008) in the soil. In upland condition, NO_3^- is predominantly preferred whereas under submerge condition NH_4^+ is taken up by the plants. There is specific protein for ammonium transport in the membrane called Amt proteins (ammonium transporter). There are some advantages and disadvantages of uptake of NO_3^- or NH_4^+ which are summarized in the Table 5.2.

Table 5.2. Comparative statements of ammonium and nitrate nutrition of crop plants

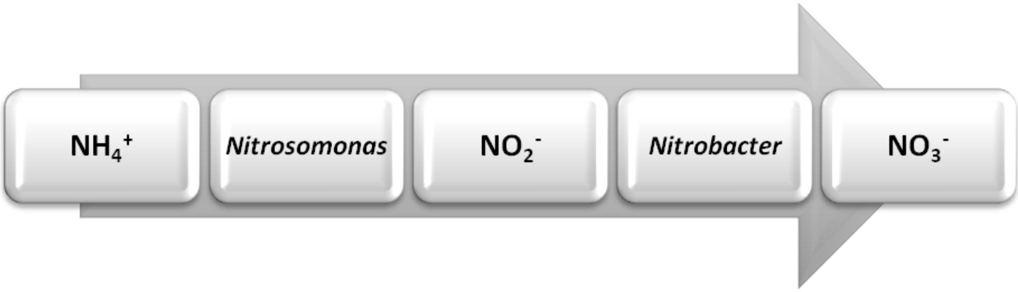
Parameters	Nitrate (NO_3^-) uptake	Ammonium (NH_4^+) uptake
Favorite crop plants	Generally upland crops, plants of the Chenopodiaceae family.	Predominantly the lowland crops of water logged condition.
Influence on other ion uptake	Enhances the uptake of K^+, Ca^{2+}, Mg^{2+} and other cations.	Enhances the uptake of anion namely phosphate ($H_2PO_4^-$) and decline the pH of the soil. Reduce the uptake of K^+, Ca^{2+}, Mg^{2+} etc.
Under saline condition	Crop productivity can be improved by the application of nitrate based fertilizer.	Crop productivity is not influenced due to ammonium based fertilizer application.
Leaching loss	Leaching loss is predominant in nitrate application.	Leaching loss of ammonium is minimum due to absorption by clay minerals.
Requirement of energy	Its absorption is an active process; require energy through the hydrolysis of ATP.	It has double absorption system namely energy dependent (active transport) and energy independent (passive transport).
Induction of deficiency of other elements	Iron and certain trace elements deficiencies can be induced by nitrate nutrition	No such deficiencies have been observed.
Adapted plant species	Member of Euphorbiaceae plants.	Member of Ericaceae family plants.

5.7. Losses of N from Soil

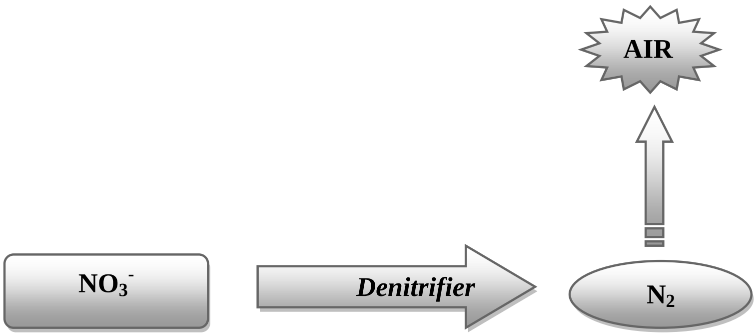
Available soil-N can be lost through various processes namely volatilization, leaching, de-nitrification, fixation by clay minerals.

5.7.1. Ammonification, Nitrification and De-nitrification

The process of conversion of complex N-compounds to simple inorganic form is a common phenomenon in the soil. A variety of soil organisms convert the organic nitrogen into ammonium is known as ammonification. Some of the bacteria like *Nitrosomonas* spp. or *Nitrococcus* spp. convert ammonium to nitrite (NO_2^-). This nitrite is further oxidized to form NO_3^- through the activity of bacteria *Nitrobacter* spp. These two groups of bacteria are together called nitrifying bacteria and the process is called nitrification.



De-nitrification is the process where NO_3^- is converted to gaseous nitrogen (N_2) consequently returned to the atmosphere. Denitrifies comprises several bacteria, responsible for the conversion of NO_3^- to NO_2^- and finally N_2. The potential bacteria of this group are *Thiobacillus denitrificans, Micrococcus denitrificans, Paracoccus denitrificans and Pseudomonas* spp.



5.8. TRANSLOCATION OF N-COMPOUNDS

Transportation of nitrate, ammonium and amino acids from root to shoot are mediated through xylem vessel. Nitrate is assimilated as ammonium in shoot in cytosol and chloroplast. However, very few cases this process is also mediated in roots where proplastid is present. Cytosolic GS1;2 and plastidic NADH-GOGAT1 are responsible for the primary assimilation of ammonium ions in the roots. The translocation of nutrients in the plant body is done by the following processes:

- Short distance transport
- Long distance transport
- Remobilization of N

5.9. THE ASSIMILATION OF NITRATE

The process of reduction of nitrate to ammonia, and then to amino acid is called nitrate assimilation. The process comprises two steps viz. nitrate reduction and ammonia assimilation.

The reduction of nitrate to ammonia is mediated by two enzymes namely nitrate reductase (NR) and nitrite reductase (NiR). The reduction requires a great deal of energy and the overall reaction is given below:

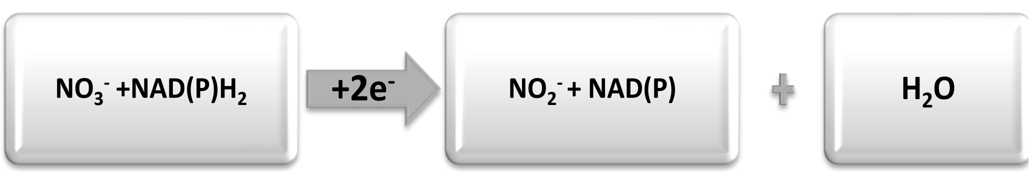
5.9.1. Nitrate Reductase (NR) Enzyme

It is a complex enzyme, present in the cytoplasm and requires NADH and NADPH as an electron donor. In higher plants, the molecular weight of NR is up to 200000 Dalton, and it contains several prosthetic groups comprising FAD, cytochrome and molybdenum. The NR contains three internal cofactors (FAD, heme, and MPT) and two metal ions (Fe and Mo) in each subunit (11, 12, 18, 77, 81, and 94). During catalytic turnover, the FAD, Fe, and Mo cyclically are reduced and oxidized. Thus, NR exists in oxidized and reduced forms, with the 12 to 18 possible oxidized and reduced forms (3 states for FAD, 2 states for Fe, and either 2 or 3 states for Mo) having only transient existence *in vivo.*

5.9.1.1. Characteristics of Enzyme Nitrate Reductase (NR)

- Nitrate reductase is a complex enzyme with a molecular weight of about 200000 Dalton, in higher plants and it may be up to 500000 Dalton
- Contains several prosthetic groups, including FAD, cytochrome, and molybdenum
- It is localised in cytoplasm in higher plants cell which requires more NADH and NADPH as an electron donor
- Half life of this enzyme is few hours
- It is an inducible enzyme i.e., its activity of which increases with the increase of nitrate.

The main site of nitrate reduction is the leaf and root. The NR is located in the outer membrane of chloroplast and proplastid of roots. It is a substrate inducible enzyme i.e., in the absence of substrate the level of enzyme is minimum in shoot and roots. Generally, lower concentration of NO_3^- is reduced in the root. When the external supply is higher the majority of NO_3^- is reduced in the leaves and the concentration of NR increased. The overall reaction of the nitrate reduction to nitrite is given below:



5.9.1.2. Factors Affecting the Activity of NR

The activity of NR is influenced by the following factors

- External supply of nitrate
- Plant/leaf age
- Intensity of light

5.9.2. Reduction of Nitrite to Ammonia

The nitrite produced by the nitrate reduction system is reduced to ammonia by the enzyme nitrite reductase (NiR) as nitrite is rarely found in the cytoplasm. This enzyme has an iron porphyrin group (shirohemo) with the molecular weight of 60000-70000 Dalton, and it has been established that it also possesses an iron-sulphur group. The enzyme is located in the chloroplast, probably attached with the thylakoid membrane. In root, the NiR is present in the plastid (proplastid). The overall reaction of nitrite reduction is given below:

$$NO_2^- + 6\ \text{ferredoxin}_{red} + 8H^+ \xrightarrow{6e-} NH_4^+ + 6\ \text{ferredoxin}_{ox} + 2H_2O$$

5.9.2.1. Characteristics of Nitrite Reductase (NiR)

It is an iron porphyrin (siroheme) containing protein with a molecular weight of 60000 to 70000 Daltons. The enzyme also contain iron-sulfur centre (4F-4S) as has already been found in sulfite reductase (Lewis, 1986).

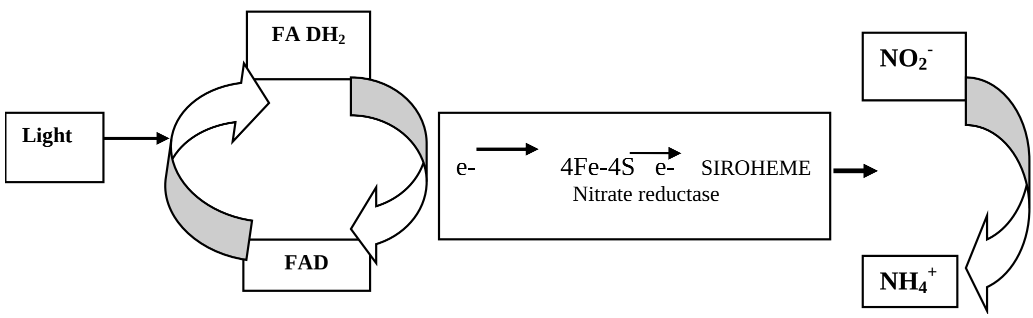


Figure 5.1. Schematic diagram of functioning of nitrate reductase enzyme.

5.9.3. The Glutamate Dehydrogenase (GDH) Pathway

In this pathway, NH_3 attaches with the carbon skeleton of α-ketoglutaric acid, an intermediate product of TCA cycle and form glutamic acid. The reaction is catalyzed by enzyme glutamate dehydrogenase (GDH) where NADH acts as hydrogen donor. The enzyme located in mitochondrion however, NADPH dependent enzyme which is also present in chloroplast. The pathway involves the reductive amination of α-ketoglutaric acid to form glutamate shown in the following equation:

$$\alpha\text{-ketoglutaric acid} + NADH + NH_3 \longrightarrow \text{Glutamic acid} + NAD^+ + H_2O$$

The affinity of GDH to NH_3 is comparatively lower than those of GS and GOGAT.

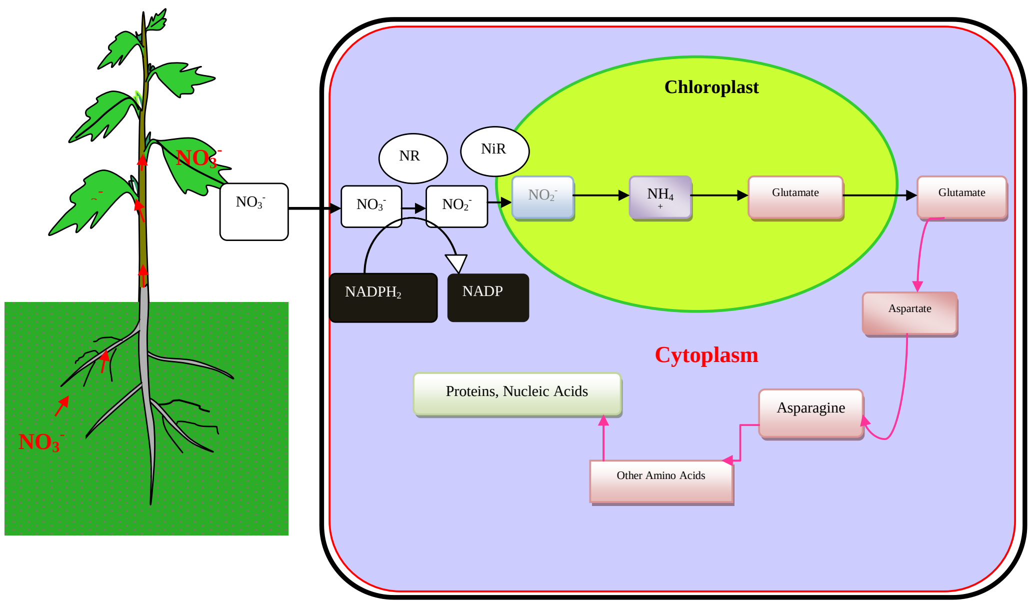
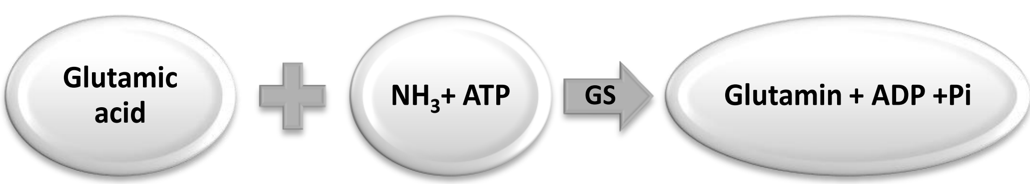


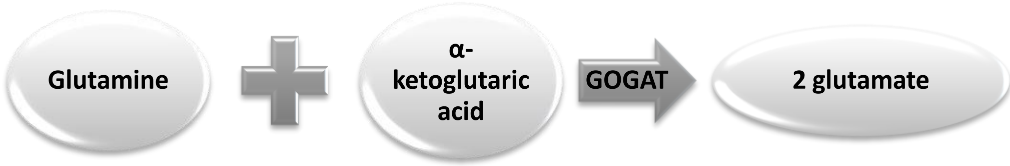
Figure 5.2. Schematic presentation of NO_3^- uptake, reduction and assimilation into amino acid, protein and other N compounds in plant cell.

5.9.4. Glutamine Synthetase-Glutamate Synthase (GS-GOGAT) Pathway

The enzyme glutamine synthetase (GS) is a large and complex molecule. In the plant system two isoform namely GS_1 present in cytosol and the other one GS_2 present in plastid. The enzyme glutamate synthase is also known as glutamin oxoglutarate aminotransferase (GOGAT) which produces glutamate from glutamine and α-ketoglutaric acid. The enzyme plays central role in the regulation of nitrogen assimilation in both eukaryotics and prokaryotes.



The reaction is catalyzed by glutamine synthetase (GS) enzyme which requires energy as ATP. The glutamine can be converted by the following reaction and is catalyzed by the enzyme GOGAT.



The enzyme is found in chloroplast of leaves and plastid of roots (Miflin and Lea, 1980; Emes and Fowler, 1979). The higher affinity of GS to $NH4^+$ resulted in the maintenance of $NH4^+$ to a normal state for escaping the toxic level.

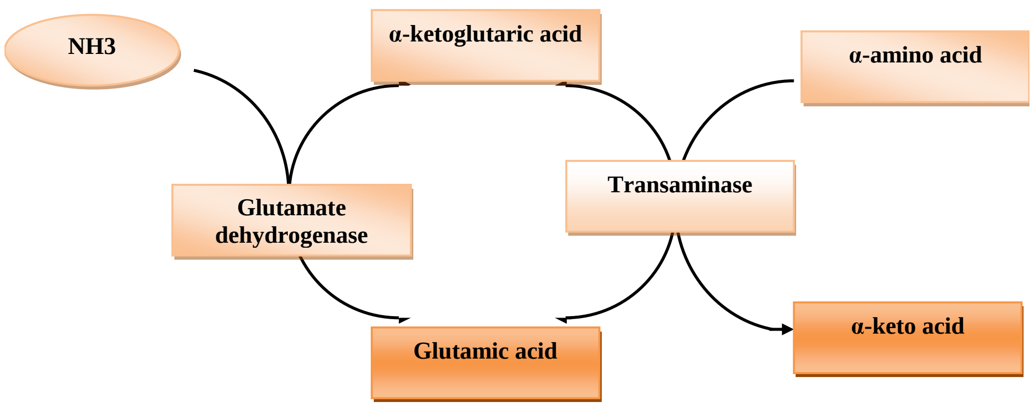
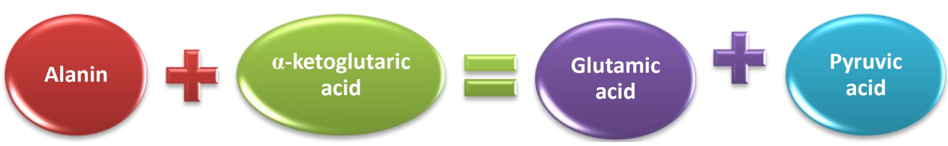


Figure 5.3. Schematic presentation of amino acid synthesis via transamination.

5.9.5. Biosynthesis of Other Amino Acids

Transamination

A transamination reaction is the transfer of amide group from amino acid to the carboxyl group of a keto acid. This reaction is catalyzed by a class of enzyme known as aminotransferase. Actually it is the replacement of amine group of an amino acid to a ketone group of another organic acid. The most common and major keto acid is the α-keto-glutaric acid, an intermediate product of TCA cycle.



Sources of Carbon

The carbon for the skeleton of amino acids is obtained from the TCA cycle, and is shown in the diagrammatic scheme (Figure 5.4).

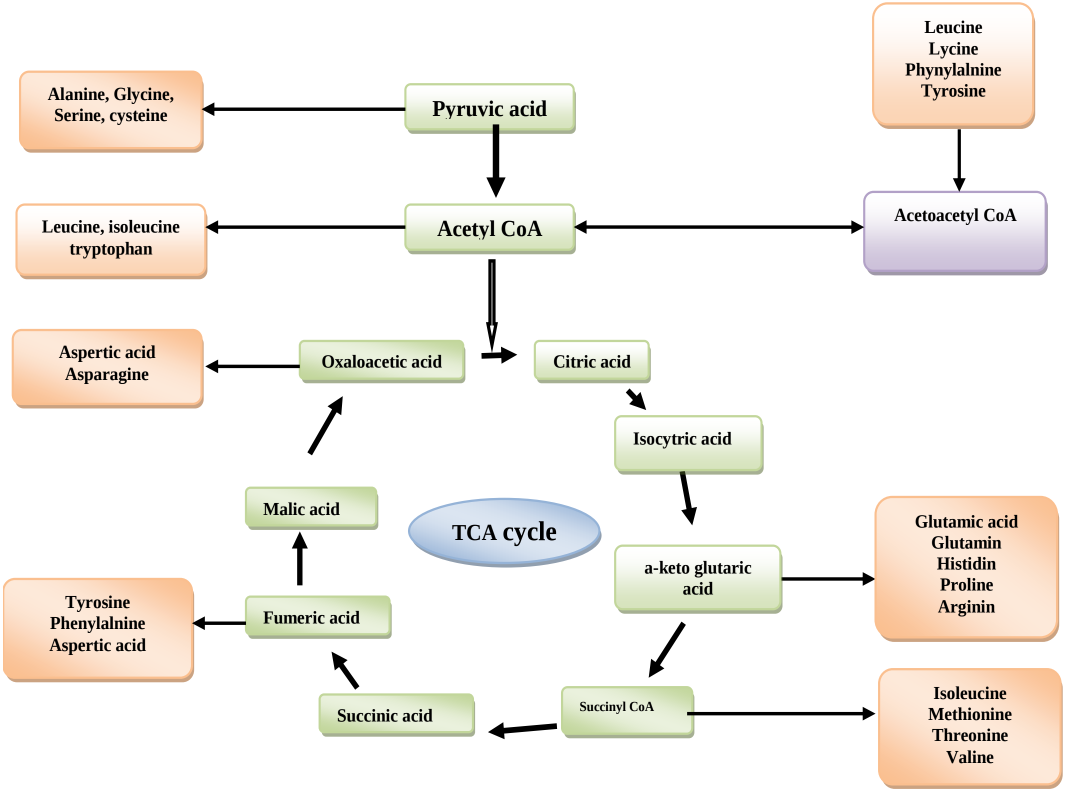


Figure 5.4. Simplified scheme of source of carbon for the skeleton of amino acids building.

5.10. Nutritional Efficiency of Nitrogen

5.10.1. Agronomic Efficiency of Nitrogen (AEN)

It is referred to the increase in crop yield per unit of applied nitrogen which can be calculated by the following formula:

$$AEN = \frac{\text{GYF} - \text{GYC}}{\text{FNA}}\ \text{kg/kg}$$

Where,
AEN: Agronomic efficiency of fertilizer-N
GYF: Grain yield of fertilizer-N applied crop
GYC: Grain yield of control crop
FNA: Amount of fertilizer-N applied to the crop

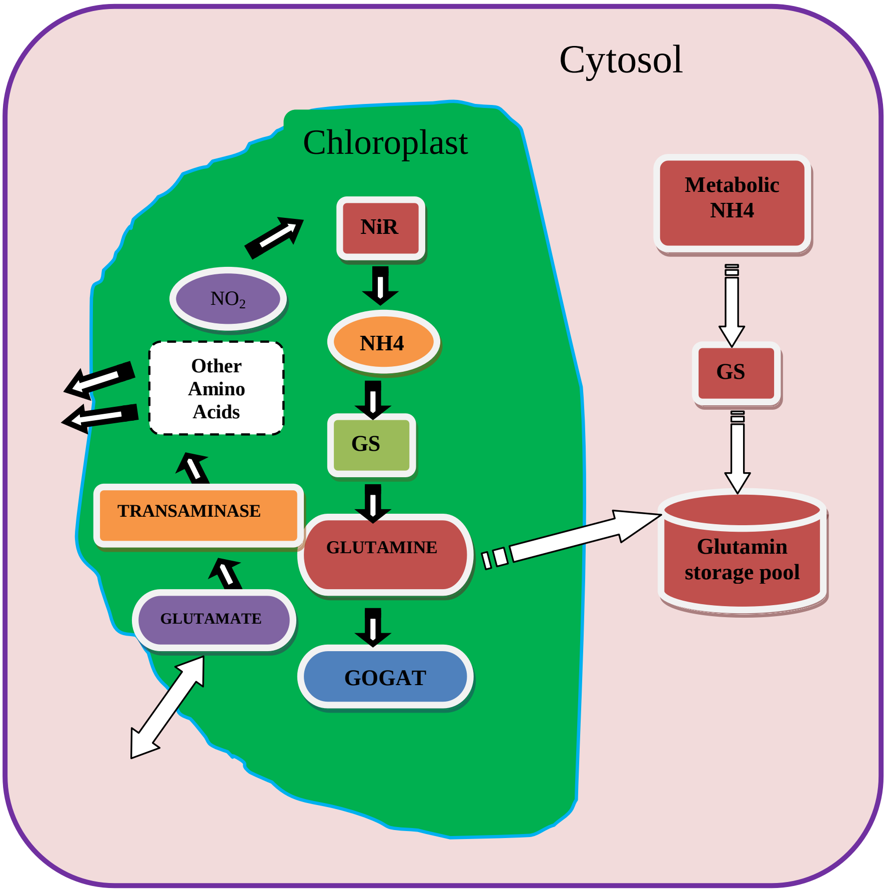


Figure 5.5. Simplified diagrammatic scheme for the synthesis of amino acids, protein and other N-compounds from nitrite and ammonium.

5.10.2. Apparent Nitrogen Recovery (ANR)

It is referred to the increase in nitrogen recovery per unit of applied nitrogen which can be calculated by the following formula:

$$ANR = \frac{\text{NUF} - \text{NUC}}{\text{FNA}}\ \text{kg/kg}$$

Where,

ANR: Apparent nitrogen recovery
NUF: Nitrogen uptake in fertilized crop
NUC: Nitrogen uptake in control crop
FNA: Fertilizer-N applied crop

5.10.3. Physiological Efficiency of Nitrogen (PEN)

It refers to the ability of a crop to transform a given amount of an acquired nutrient into grain yield. The PE can be calculated by the following formula:

$$PEN = \frac{\text{GYF} - \text{GYC}}{\text{NUF} - \text{NUC}} \text{ kg/kg}$$

Where,

PEN: Physiological efficiency of nitrogen
GYF: Grain yield of fertilizer-N applied crop
GYC: Grain yield of control crop
NUF: Nitrogen uptake by fertilized crop
NUC: Nitrogen uptake by control crop

5.10.4. Nitrogen Harvest Index (NHI)

It is the ratio of grain N to total N of the whole plant body which can be calculated by the following formula:

$$\text{NHI} = \frac{N\ uptake\ by\ grain\ or\ economic\ part}{N\ uptake\ by\ whole\ plant}$$

5.11. Physiological and Metabolic Functions of Nitrogen

5.11.1. Biosynthesis of Amino Acids, Amide, Protein, Enzyme, Nucleic Acids etc.

Nitrogen is the basic nutrient element for producing protein, amide, DNA, RNA and other macro-molecules.

It is the components of many essential compounds viz. nucleic acid (10% of total N), protein (16-18% N), enzyme, lipid, chlorophyll (45-60%), phytochrome, plant hormone (IAA, cytokinin), vitamin (B_1, B_2, B_6, PP). High energy phosphate (ATP, UTP, GTP, ADP, AMP), coenzyme (CoA, CoQ, NAD (P), FAD, FMN etc.) and iron porphyrin etc.

5.11.2. Formation of Chlorophyll

Chlorophyll is a pigment which traps the solar energy and converts it into chemical energy. Among the four species of chlorophyll namely, chlorophyll a, chlorophyll b, chlorophyll c and chlorophyll d, chlorophyll a and chlorophyll b are the most common in higher plants. Chlorophyll molecule consists of two parts viz. a porphyrin head and a phytol tail. The porphyrin head contains four atom of N as a tetra-pyrrole ring with a Mg atom in the centre and a long hydrophobic hydrocarbon tail that anchor them in photosynthetic membrane. Chlorophyll a has a methyl group in the position III and chlorophyll b has aldehyde group instead of methyl group (Figure 5.7). The mesophyll tissue of leaves is the reservoir of chlorophyll pigment. The Mg atom is attached with four N as chelated form, and the loss of Mg from the porphyrin converted into pheophytin, a non-green compound.

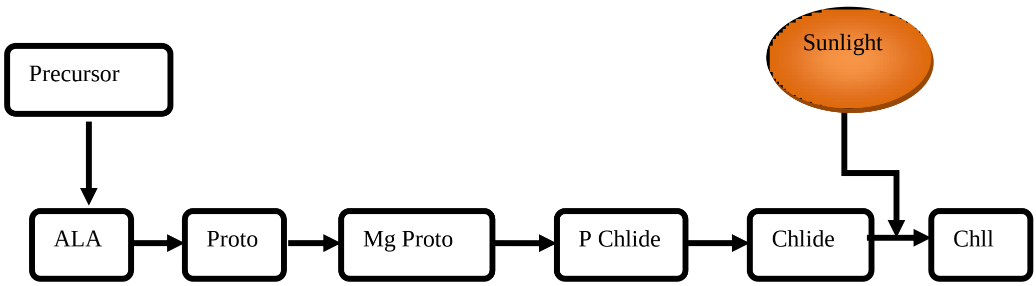


Figure 5.6. Schematic presentation of chlorophyll synthesis in higher plants, ALA; 5-aminolevulinic acid; Proto: protoporphyrin IX; P chlide: protochlorophyllide; Chlide: chlorophyllide; Chll: chlorophyll (adopted from Wettstein et al., 1995).

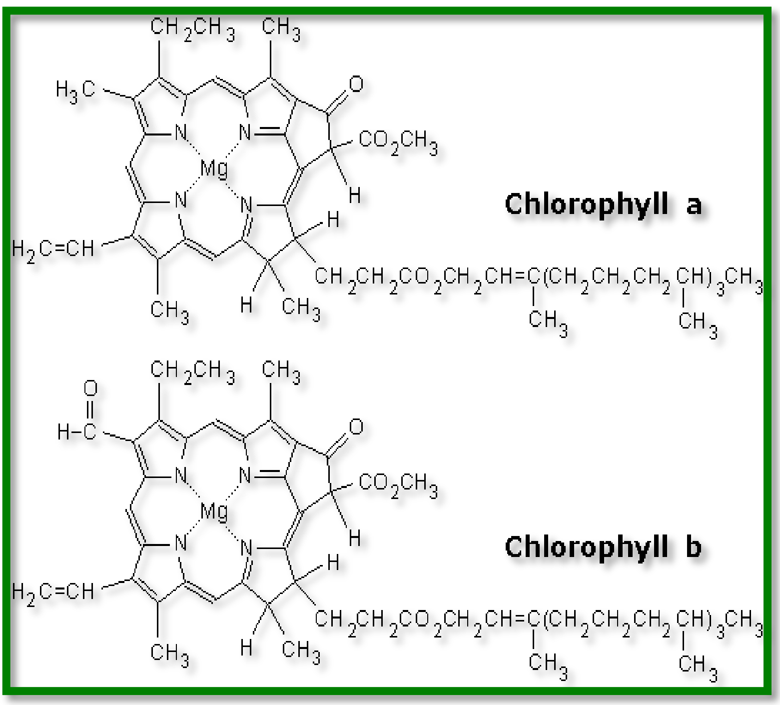


Figure 5.7. Molecular structure of chlorophyll a and chlorophyll b.

Photosynthesis is related to nitrogen content in leaves as N is the component of Calvin cycle and thylakoid of chloroplast. The thylakoid N is proportional to chlorophyll content of leaf of C_3 plants (50 mol thylakoid mol^{-1} chlorophyll) (Evans, 1989). The capacity of photosynthesis and subsequently the dry matter production is proportional to supply of N (Lahav and Turner, 1983).

Determination of Leaf Chlorophyll Content

Chlorophyll can be determined following the method of Coombs et al. (1985). After collecting the fresh leaf of plants, 0.05g leaf to be weighed and the samples are taken in small glass vial covered by aluminum foil with 10 mL 80% acetone. The samples to be kept in dark condition until the leaf color changes. Thereafter, spectrophotometer readings of the extracts prepared by extracting chlorophyll with acetone are taken for the determination of chlorophyll a and chlorophyll b. The amount of chlorophyll a, chlorophyll b and total chlorophyll are computed according to Witham et al. (1986) on fresh weight basis:

$$\text{Chlorophyll a (mg g}^{-1}\text{ leaf tissue)} = [(12.7\,(D_{663}) - 2.69\,(D_{645})\,] \times \frac{V}{1000xW}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ leaf tissue)} = [(22.9\,(D_{645}) - 4.68\,(D_{663})\,] \times \frac{V}{1000xW}$$

Total chlorophyll content = chlorophyll a + chlorophyll b

Where, D645 = Optical density of the chlorophyll extract at 645 ηm wave length
D663 = Optical density of the chlorophyll extract at 663 ηm wave length
12.7, 2.69, 22.9 and 4.68 are absorbance co-efficient
V = Final volume (mL) of the 80% acetone with chlorophyll extract = 10 mL
W = Weight of fresh sample in gram (g) = 0 .05 g

5.11.3. Non-protein N-compounds of the Plant

Many non-proteinous compounds like purine (adenine, guanine) and pyrimidine bases (thyamin, cytosine, uracil), nucleosides, nucleotide, nucleic acids (DNA, RNA), alkaloids, the ornithin alkaloids, cytochromes, phytochrome and phytohormone commonly found in plants.

5.11.4. Agronomic Importance of N

5.11.4.1. Application of N-fertilizer enhances the production of leaf area index (LAI) resulting the increases of photosynthetic activity and subsequently enhances the biomass production. However, higher level of LAI does not give the guarantee of increasing biomass rather it creates the mutual shading of leaves consequently decreases the photosynthetic activity.

5.11.4.2. Higher level of N-fertilizer application increases the shoot growth but not the root growth proportionately i.e., root: shoot ratio decreases due to application of N-fertilizer.

5.11.4.3. Deficiency of N results in the decrease of harvest index consequently number and size of grain are also reduced.

5.11.4.4. Imbalance N concentration in the plants i.e., under high N level and severe deficient condition, plants may suffer from disease infestation.

5.11.4.5. Higher level of N application may create plant to lodge. However, under optimum level of N it produces the maximum biomass and yield (Sinclair and Horie, 1989).

5.12. DEFICIENCY SYMPTOMS OF NITROGEN IN CROP PLANTS

5.12.1. Plants grow slowly due to N-deficiency, proteins and other N-compounds cannot be formed because of limited N-supply. Stunted growth accompanied with spindle-shaped and pale green leaves of crop plants are characteristics of N-deficiency symptoms. The pale green leaves are the results of chlorophyll breakdown.

Figure 5.8. Nitrogen deficiency showing stunted growth and delay in flowering of rice.

5.12.2. Generally, N-deficient plants produce more wood due to nonproduction of amino acid, protein in the organ. In special cases, carbohydrate, the backbone structure of protein is not used in the metabolism process rather produce the anthocyanin.

5.12.3. The most common deficiency symptom of N in the plants is the chlorosis of older leaves i.e., de-greening of leaves. Under severe nitrogen deficiency these leaves become completely yellow and then fall off the plant.

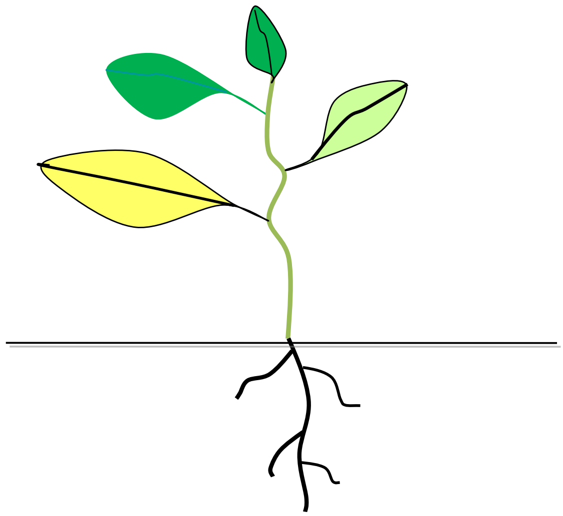


Figure 5.9. Showing chlorosis of older leaves turning into yellow indicating the N deficiency.

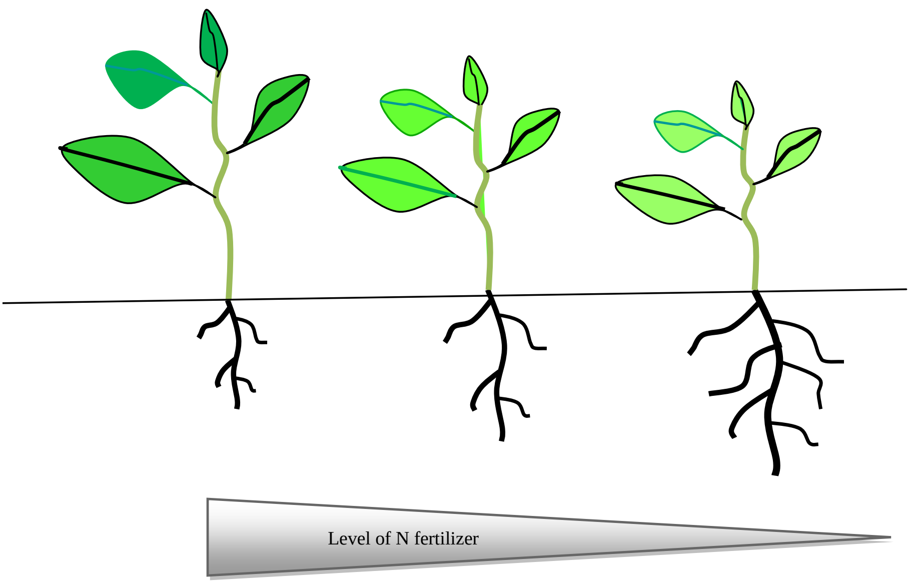


Figure 5.10. Figure showing increasing root growth with the declining the rate of N-fertilizer application (adapted from Marschner, 1995).

5.12.4. Nitrogen deficiency is characterized by poor growth rate, the plants remain small and leaves become spindle shaped.

5.12.5. Under N-deficient condition root growth increases than shoot growth, consequently it increases the root shoot ratio.

5.12.6. Plants suffering from N-deficiency mature earlier leading to shortening of vegetative growth of the plant.

5.12.7. Nitrogen deficiency in cereals results in poor tillering, lower number of grain per panicle and smaller grain size. However, sometimes it increases protein content because of low carbohydrate translocation to the grain.

5.13. DIAGNOSIS OF N-DEFICIENCY IN CROP PLANTS

5.13.1. Based on the above discussion following points to be considered for quick diagnosis of plants suffering from N deficiency. In initial deficiency condition, the oldest leaf becomes yellow and falls from the mother plant. Yellowing of more than one leaf is the best indication of N-deficiency.

New leaves become pale in color as compared to older ones and seem to be smaller than normal. The tiny leaves fail to increase in normal size. The plants cannot mature and fail to produce new leaves and branches. Ventral side of leaves may gain red or purple color. Plants suffering from N-deficiency wilt quickly at first sign of stress. Additionally, the N-deficient plants lost the capacity to withstand biotic and abiotic stresses.

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Chapter 6

PHOSPHORUS (P)

6.1. GENERAL FEATURES

Phosphorus is one of the most important macronutrients required for normal growth and development of crop plants. Its atomic weight is 31, and has an isotope ^{32}P. It does not occur as abundantly as other nutrients do. The concentration of P in surface soil varies from 0.02 to 0.1%. The quantity of P in the soil does not reflect the availability to the plants. Phosphorus is absorbed by the plant largely as orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}), present in the soil solution largely depends upon soil pH and quantity present in the soil. The ion $H_2PO_4^-$ is absorbed faster than HPO_4^{2-}, and the main site of P absorption is root tip and elongation zones of roots. The greater accumulation of P is performed through root tip area. Plants may also absorb organic P as nucleic acid and phytin in special cases. Phosphorus moves from soil to root surface through diffusion and mass flow, and the earlier is greater than the later.

6.2. ORGANIC P

It represents about 50% of the total P and varies with soil depth. The organic P is mineralized and converted to available form through the activity of enzyme phosphase. This enzyme present in the microorganisms which helps in the mineralization process. Many of the organic P has been characterized as inositol phosphates (10-50%), phospholipids (1-5%) and nucleic acid (0.2-2.5%). Generally, P mineralization and immobilization take place simultaneously as follows:

Uptake of P by the Plants

It is mainly taken up by the plant as $H_2PO_4^-$ and HPO_4^{2-} forms which are present in the soil solution.

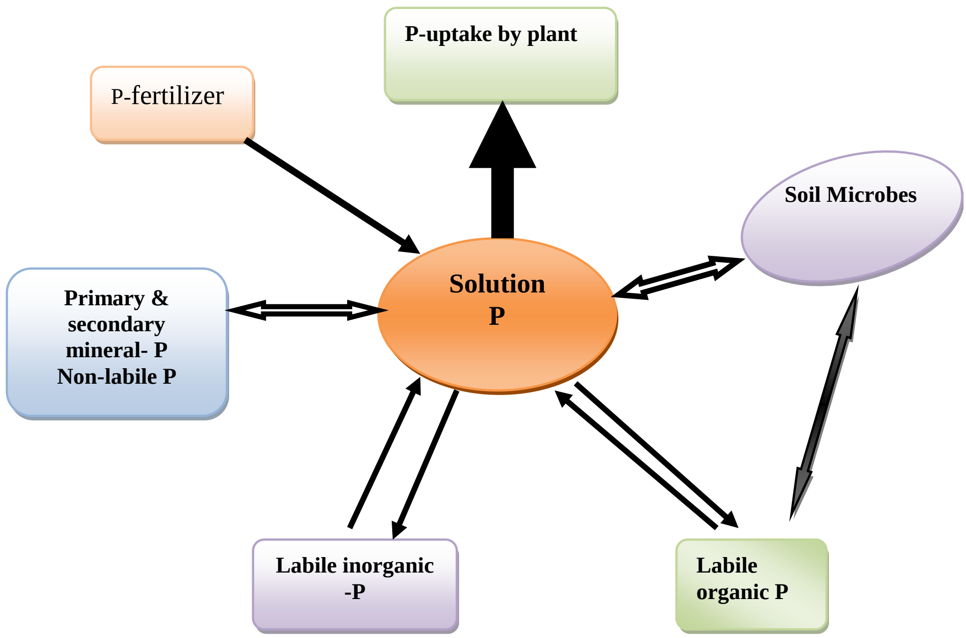


Figure 6.1. Simplified schematic representation of P cycle in soil.

Table 6.1. Phosphorus containing fertilizers with their chemical formulae and approximate P concentration

Name of fertilizer	Chemical formula	Concentration (appox. % P)
Rock phosphate	$[Ca_3(PO4)_2]_3$. $CaFx.(CaCO_3)x.(Ca(OH)_2)_2$	6.16-28.6
Single super phosphate (SSP)	$Ca(H_2PO_4)_2$	7-9.5
Triple super phosphate (TSP)/ CSP	$Ca(H_2PO_4)_2$	19-23.5
Diammonium phosphate (DAP)	$(NH_4)_2HPO_4$	20-23
Monopotassium phosphate	KH_2PO_4	22.44
Dipotassium phosphate	K_2HPO_4	18
Bone meal		9

Conversion Factor of P

% P= $P_2O_5 \times 0.44$
% $P_2O_5 = P \times 2.27$

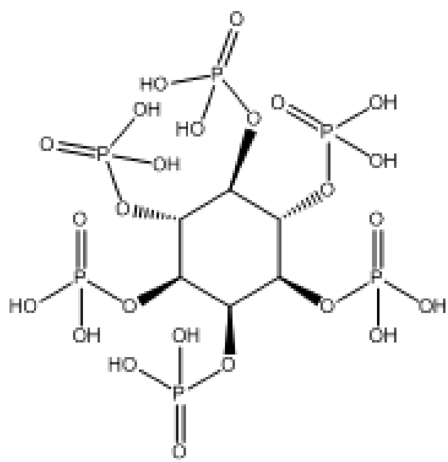
6.3. Assimilation of Phosphorus

The main step of assimilation of P in the cell is the formation of ATP (adenosine triphosphate). The overall reaction of the process is the addition of inorganic phosphate with ADP (adenosine diphosphate) to form a phosphate ester bond.

Table 6.2. Recommended dose of P-fertilizer for different crops

Name of Crops	Soil Analyses Interpretation *(Soil fertility level)*	Dose (kg ha^{-1})
Rice	Optimum	0-8
	Medium	9-16
	Low	17-24
	Very low	25-32
Wheat	Optimum	0-10
	Medium	11-20
	Low	21-30
	Very low	31-40
Maize	Optimum	0-25
	Medium	26-50
	Low	51-75
	Very low	76-100
Jute	Optimum	0-4
	Medium	5-8
	Low	9-12
	Very low	13-16
Cotton	Optimum	0-17
	Medium	14-34
	Low	35-51
	Very low	52-68

Source: Fertilizer Recommendation Guide (2012).



Phytic acid

Phytic Acid

i. Phosphate in the soil solution is readily taken up by plant roots and incorporate into a variety of organic compounds including sugar phosphates, phospholipids and nucleotides
ii. P is found about 0.2 to 0.8% of the total dry matter.
iii. P remains in oxidized form in the cell i.e., no reduction is required like N and S assimilation.

iv. It remains as esterified through a hydroxyl group to a carbon chain C-O-P simple phosphate ester (sugar phosphate) or remain as energy phosphate bond P-P (e.g., ATP)
v. Phosphate along with protein/fat is incorporated into biological membrane as phospholipid and phosphoprotein.

Phytic acid or phytate is the principal storage form of phosphorus. It is a saturated cyclic acid found in many plant tissues especially in bran and seeds. Phytic acid has a strong affinity in binding different minerals namely Ca, Zn, Fe etc.

6.4. Physiological and Biochemical Functions of Phosphorus

Phosphorus has profound functions on growth and development of crop plants. The main functions have been summarized here under different sub-headings:

6.4.1. Energy Transfer

The most important function of P is the transfer or storage of energy as adenosine triphosphate (ATP), adenosine di-phosphate (ADP), adenosine monophosphate (AMP), guanosine triphosphate (GTP), uridine triphosphate (UTP), cytidine triphosphate (CTP) etc. When the terminal phosphate molecules split off from ATP or ADP a large amount of energy (12000 cal mol^{-1}) is liberated. Energy obtained through photosynthesis and metabolism of carbohydrate and lipid is stored in phosphate compounds for further utilization by the plant. ATP is the storehouse of energy of all kind of biological reactions. The ATP is the energy rich phosphate bond which is required for the synthesis of starch. Similarly other phosphate bond compounds like ADP, AMP, GTP, UTP are required for the synthesis of simple sugar viz. sucrose and cellulose.

6.4.2. Structural Components of Several Compounds

Phosphorus acts as a structural element of DNA, RNA, coenzyme, phosphoprotein, phopspholipid, glycolipid etc. Amine choline is often the dominant partner forming phosphotidal choline (lechithin).

6.4.3. Participation of P in Oxidation-reduction Reaction

Phosphorus plays an important role in the oxidation reduction reaction such as NAD and NADP are in oxidation reduction reaction in which hydrogen transfer take place.

6.4.4. Regulatory Role of Organic Phosphate

i. Inorganic P controls some regulatory role of some key enzymes.
ii. It can stimulate the phosphofructokinase enzyme which enhances the ripening of some fruits like tomato.
iii. Large quantities of P are found in seed and fruit; hence it is regarded as essential for seed formation, and is also associated with the maturity of crop.
iv. Phosphorus is required for better root growth, and increased root growth in different crop plants have been found by the application of P-fertilizer.
v. Crop quality has been found to be improved by the balanced application of P-fertilizer. Similarly resistance to diseases has also been found by the balanced application of P.

6.5. Phosphorus Deficiencies in Crop Plants

6.5.1. Deficiency of P resulted in the lowering of cell and leaf size but unaffecting the chlorophyll content. However, the photosynthetic activity of chlorophyll is reduced due to deficiency of P.

6.5.2. Generally P deficiency occurs in crop plants where soils are acidic, leached and calcareous. Cold weather can cause a temporary deficiency.

6.5.3. All plants may be affected due to deficiency of P, although this is an uncommon disorder. The ventral site of tomato leaves and the stem may turn into purple color due to P-deficiency..

6.5.4. Generally, the plants become dark green or purple color due to anthocyanin pigmentation under P deficiency condition.

Figure 6.2. Purple colored leaf in maize (*Zea mays* L.) due to deficiency of phosphorus.

6.5.5. Plant growth may be stunted due to P deficiency, and sometimes the deficiency symptoms are confusing with the symptoms of nitrogen deficiency. Symptoms include poor growth, and leaves may turn blue/green but not yellow—oldest leaves may be affected first. Fruits may become small in size and acidic in taste.

6.5.6. The P-deficiency in tomato may result in necrosis of thin walled central pith cells of stem and thereby may produce large intercellular space (Lyon and Garcia, 1944).

6.5.7. Phosphorus deficiency delays the flower initiation and decreases the number of flower in crop plants (Bould and Parfitt, 1973; Rossiter, 1978).

6.5.8. The common susceptible plants are carrots, lettuce, spinach, apples, gooseberries etc.

6.5.9. Phosphorus deficiency increases the secretion of phytase from roots of various plant species which may provide an efficient mechanism for certain plants to utilize inositol hexaphosphate in soil (Bilyeu et al., 2008; Li et al., 1997).

6.5.10. Deficiency of P decreases the synthesis of DNA and RNA which cause restriction of nuclear division or might cause defects in parts of chromosome or gene suppression.

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Chapter 7

POTASSIUM (K)

7.1. GENERAL FEATURES

Potassium is a macro nutrient element having the atomic weight of 39. There are three isotopes of potassium of which ^{40}K is radioactive. As an essential nutrient element it performs numerous physiological functions in plants. In plants, K is the most abundant cation present in cytoplasm, although it is not a constituent of any organic molecule or plant structure, and is necessary for the functions of all living cells. The requirement of K for optimum plant growth and development is 20~50 mg g^{-1} dry weight of the plant (Marschner, 1995). The average K content in the earth crust is around 23 g kg^{-1}, and the greatest part of the K is bound in primary minerals and secondary clay minerals (Mengel et al., 2001).

7.2. POTASSIUM CONTAINING FERTILIZER

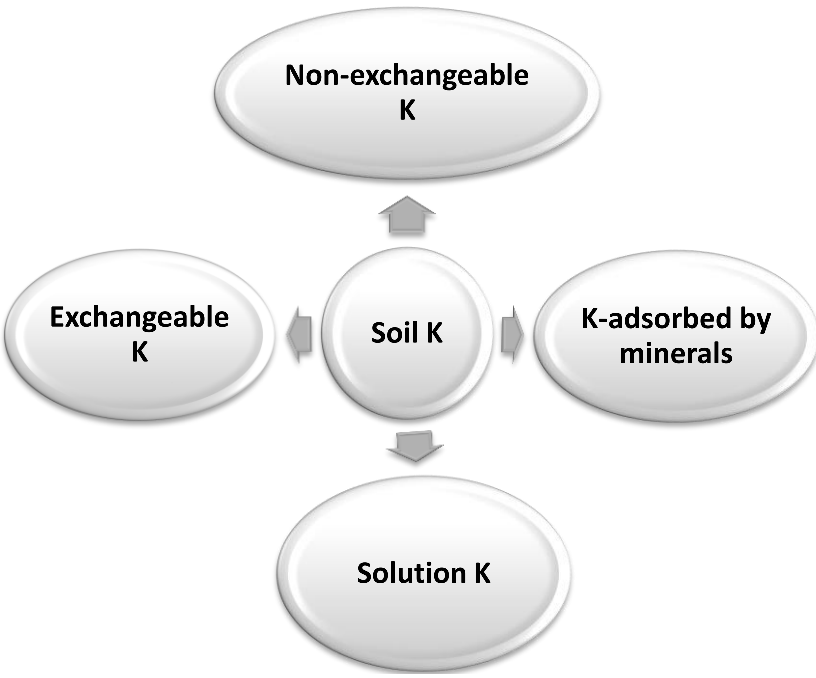
Potassium is predominantly applied as muriate of potash (MOP). However, there are several fertilizers are also used for supplying K to the plants. Following fertilizers are widely used for growth and development of crop plants (Table 7.1).

Table 7.1. Potassium containing fertilizers with their concentration of K

Name of Fertilizer	Chemical Formula	Concentration (% Appox.)
Muriate of potash (potassium chloride)	KCl	52.0
Potassium sulfate	K_2SO_4	43.0
Potassium nitrate	KNO_3	36.5
Potassium hydroxide	KOH	69.0
Potassium orthophosphate	K_2HPO_4	41.6
Potassium metaphosphate	KH_2PO_4	31.6
Potassium magnesium sulfate	K_2SO_4, $MgSO_4$	18.33
Manure salt	-	22.5

7.3. METHOD OF APPLICATION OF K-FERTILIZER

Potassium fertilizers are applied as broadcast or banded before seed sowing or transplanting of seedling. Fertigation and foliar application can also be done under special circumstances. It is present in the soil in various forms (Tisdale et al., 1985) as shown by following scheme:



7.4. FACTORS AFFECTING K AVAILABILITY IN SOIL

- Type of clay minerals present in the soil
- Content of exchangeable K in the soil
- Cation exchange capacity of soil
- Soil moisture and temperature
- Soil reaction i.e., acidity and alkalinity condition
- Interaction with other ions
- Soil management practices
- Crop factors i.e., growth and development of crop plants

7.5. UPTAKE AND MOVEMENT OF K IN THE PLANT BODY

Potassium is taken up by plant as K^+ ion, and it is highly mobile in the plant body. It is not assimilated in organic compounds, and cytosolic concentration of K is ranged from 40 to 200mM depending upon apoplastic K concentration (Szczerba et al., 2006). The uptake of K is influenced by genetic characteristics and growth stage of plants. Luxury uptake is found under adequate supply of K-fertilizer and in healthy plants. The uptake of K is predominantly performed by mass-flow which is influenced by the rate of transpiration.

Table 7.2. Recommended dose of K-fertilizer for different crops under Bangladesh soil condition

Name of Crops	Soil Analyses Interpretation *(fertility level)*	Dose (kg ha^{-1})
Rice	Optimum	0-33
	Medium	34-66
	Low	67-99
	Very low	100-132
Wheat	Optimum	0-30
	Medium	31-60
	Low	61-90
	Very low	91-120
Maize	Optimum	0-40
	Medium	41-80
	Low	81-120
	Very low	121-160
Jute	Optimum	0-13
	Medium	14-26
	Low	27-39
	Very low	40-52
Cotton	Optimum	0-25
	Medium	26-50
	Low	51-75
	Very low	76-100

Source: Fertilizer Recommendation Guide (2012).

7.6. METABOLIC AND PHYSIOLOGICAL FUNCTIONS OF POTASSIUM IN CROP PLANTS

Potassium has both biophysical and biochemical functions in plant cell (Leigh and Wyn, 1984). Most of the K present in the vacuole of the cell as simple salt. The metabolic and biochemical functions of K are not specific.

7.6.1. Activation of Enzymes

Enzymes are biological middleman for performing chemical reactions in living cell, which bring together other molecules in such a way that the chemical reaction can take place smoothly. Potassium activates at least sixty enzymes in the plant body by changing the conformational shape especially the active site. However, it does not involve in the enzyme activation as metal activator. It also neutralizes several organic anions and other compounds to stabilize pH between 7 and 8.

7.6.2. Potassium Influences on Synthesis of Protein

Potassium is not a part of protein however; it is required in major steps of protein synthesis. Under K-deficient condition, N-compounds namely amino acids, amides and nitrates are predominantly accumulated. It is suggested that K is involved in the translocation process and binding of tRNA to ribosome.

7.6.3. Increased Photosynthesis Is Influenced by Potassium

Potassium influences the photosynthesis by H^+ flux across the thylakoid membrane by establishing the transmembrane pH needed for ATP synthesis. When plants are K deficient, the rate of photosynthesis and the rate of ATP production are reduced, and all the processes dependent on ATP are slowed down. Conversely, plant respiration increases which contributes to slower growth and development.

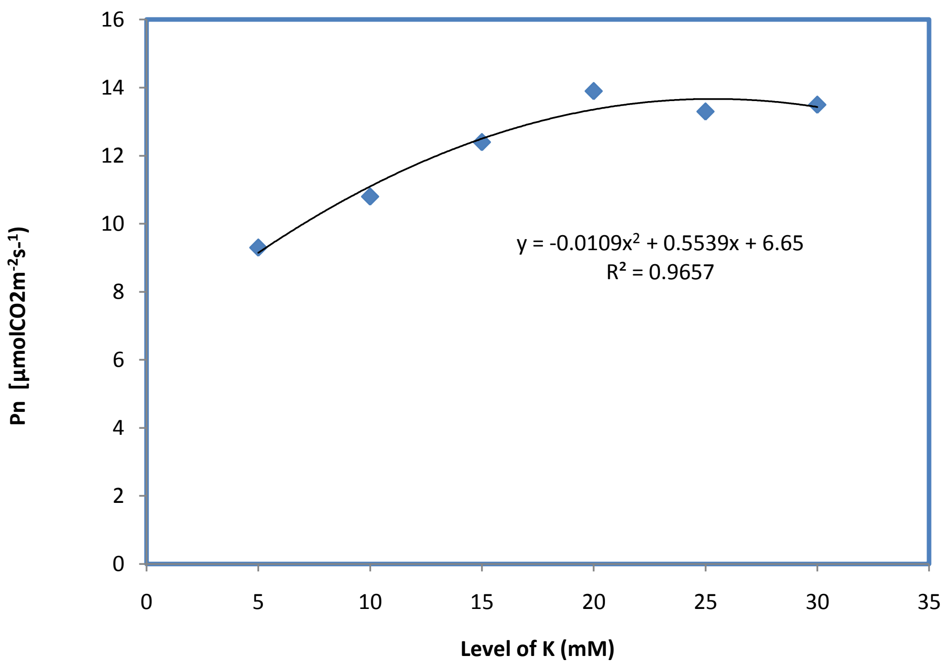


Figure 7.1. Influence K on the net photosynthesis (Pn) of mustard (adapted from Zhang et al., 2010).

7.6.4. Role of K on Maintenance of Osmoregulation

Potassium influences on the regulation of stomatal opening and closing. The opening and closing of stomata of epidermal cells are caused by an increase in turgor pressure of the guard cells as a result of the accumulation of potassium salts. Movements of K into the guard cell help in accumulating water and consequently swelling of guard cells which influence the opening of stomata. Although the osmotic adjustment is largely the result of a greater inhibition of volume expansion than solute deposition, the contrasting behavior of hexose and K deposition indicates that the adjustment is greatly a regulatory process (Sharp et al., 1990).

7.6.5. Influence of K Supply on Nastic Movement

Nastic movement is a nondirectional movement of plants due to change of turgor. Leaves of certain plants especially the Fabaceae family plants close under various stimuli like touching, warming, blowing, or shaking. Different types of nastic movements like nyctinastic and seismonastic in different legumes are influenced by K in the motor cells present in pulvini. Nastic movements can be observed in opening and closing of flowers and sleeping of leaves. In seismonastic movement, response is made by mechanical shocks such as blows, shaking or pressure. The possible mechanism of seismonastic movement is the stimulation of pulvinus. When stimulus reaches pulvinus, the motor cells become flaccid by the action of K losses from the motor cell. The release of K from motor cell is resulted in the water loss through the process of osmosis.

7.6.6. Mitigation of Stress by the Application of K

Balanced application of K can alleviate the damage caused by abiotic stress namely drought, salt, chilling, high light intensity, and heat (Cakmak 2005). Adequate supply of K can reduce the drought stress and increases the root longevity in cotton (*Hibiscus rosa-sinensis* L.) (Egilla et al., 2001). Foliar application of KNO_3 alleviates the salinity stress in strawberry.

7.6.7. Role of Potassium on other Physiological Functions

i. *Phloem transport:* the high K^+ concentration in the sieve tubes are probably related to the mechanism of phloem loading of sucrose.
ii. *Cation-anion balance:* K influences on the counter balancing of immobile anions in the cytoplasm, and quite often mobile anions in vacuoles as well as in the xylem and phloem.
iii. Potassium serves essential role on osmoticum and charge carrier of ions in cells.
iv. It influences on the electrical neutralization of anions present in cytoplasm and vacuole.
v. It also influences on the long- and short-term control of membrane polarization.
vi. Application of K influences on the synthesis of starch, stimulate the enzyme activity which is responsible for the synthesis of starch. Deficiency of K shows the availability of soluble carbohydrate content in the cell. Under higher level of K availability in the cell result in the efficient translocation of sugar to the storage organ. Potassium influences on the conversion of sugar of sink tissue leading to higher translocation of sugar (Terrance and Geiger, 1982).
vii. Potassium influences on the improvement of crop quality by reducing disease infestation and enhance lodging resistance.

7.7. Deficiency of K in Crop Plants

Generally eroded leached sandy soil is the condition of K deficiency for growing crops.

i. K is an essential macronutrient required for plant growth and development. Its deficiency affects several physiological and metabolic processes, and results in reduction of plants growth rate followed by chlorosis and necrosis.
ii. The symptom generally found in older leaves by characteristic tip burning.
iii. K-deficient plants are susceptible to water stress by decreasing turgor and become flaccid. They also susceptible to diseases and pest infestation, lodging and frost damage.
iv. Deficiency of K induces ripening disorder of some fruits and reduces tuber yield.
v. K-Deficiency leads to build-up of sugars, down-regulation of nitrate uptake and synthesis of nitrogen-rich amino acids.

Figure 7.2. Deficiency symptom of K in tomato leaf showing burning of the margin and tip.

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Chapter 8

SULFUR (S)

8.1. GENERAL FEATURES

Sulfur is a secondary macronutrient element with the symbol of S having atomic weight of 32. It is multivalent non-metal element, and abundant in the earth. Both organic and inorganic forms of S are found in the earth, and the major reservoir is the organic form. The organic S decreases with the increases in soil depth. It has 25 isotopes ranging from 26 to 49 of which four are stable isotopes e.g., ^{32}S, ^{33}S, ^{34}S and ^{36}S. The radioisotope ^{35}S is very common and widely used in plant nutritional study.

Sulfur is essential for crop production, and is considered as a secondary element along with Mg and Ca, but it is sometimes called "the 4th major nutrient". It is an essential macro nutrient element for growth, development and physiological functions of crop plants. Some crops can take up as much S as P. It has become more important as a limiting nutrient in crop production in recent years for several reasons. Higher crop yields can be obtained through optimum use of S fertilizer.

8.2. SULFUR-CONTAINING FERTILIZER

Table 8.1. The common S containing fertilizers with their chemical formulae and approximate concentration (%)

Name of Fertilizer	Chemical Formula	Concentration of S (% approx.)
Gypsum	$CaSO_4.2H_2O$	18.6
Potassium sulfate	K_2SO_4	17.6
Epsom salt/ Magnesium sulfate heptahydrate	$Mg\ SO_4\ 7H_2O$	13.0
Sulfur coated urea	$(NH_2)_2CO + S$	10.0-20.0
Zinc sulfate	$ZnSO_4.H_2O$	17.8
Ammonium sulfate	$(NH_4)_2SO_4$	24.2
Pyrite	FeS_2	53.5
Ammonium bisulfite	NH_4HSO_3	32.3
Single superphosphate	$Ca(H_2PO_4)2+CaSO_4.2H_2O$	13.9

8.3. Uptake of S in Plants

Sulfur is mostly taken up by the plant as sulfate ion (SO_4^{2-}), small quantities of SO_2 can be absorbed through leaves.

8.3.1. Concentration of S in Plants

Generally, S concentration in plants ranges between 0.1-0.4 percent. Crops of Brassicaceae family require greater amount of S than Poacaeae family, and the requirements among the families are in the order of Poacea< Fabaceae <Aliaceae < Brassicaceae. The transport of SO_4^{2-} through plasmamembrane is mediated by co-transport with H^+, and at least three H^+ is required per mole of SO_4^{2-} (Clarkson et al., 1993). Sulfate transport in the vacuole across the tonoplast is performed by electrochemical potential gradient.

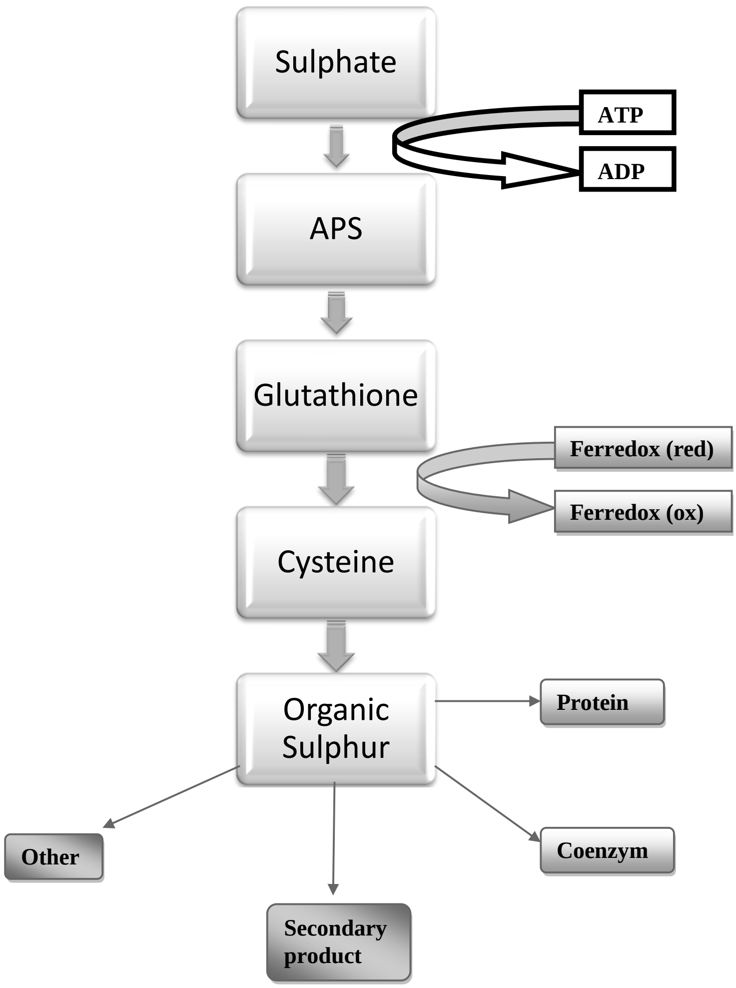


Figure 8.1. Pathway 1 of sulfur assimilation i.e., the reduction of S in crop plants, where, APS-adenosine phosphosulfate.

8.3.2. Assimilation of Sulfur

Sulfur is secondary essential element required for plant growth and development for its presence in organic compound such as amino acids (methionine and cysteine), glutathione, proteins and sulpholipids (Abdallah et al., 2010). Sulfur must be reduced to form amino acids. The biochemical pathway of assimilation of sulfur is the reduction of sulfate (SO_4^{2-}) to sulphide to form S-containing amino acids. Two pathways have been postulated for the reduction of S. In pathway 1, assimilation of S is mediated by activation of sulfur ion by ATP, and the reaction is catalyzed by ATPsulfurylase enzyme. The APS (Adenosine phosphosulphate pyrophosphate) is converted into glutathione thereafter form cysteine, and subsequently other S-containing organic compounds according to the need of the crop plant. In pathway 2, the activation of SO_4^{2-} ion by ATP, and the reaction is catalyzed by the enzyme ATP-sulfurylase leading to the formation of adenosine phosphosulfate (APS) and pyrophosphate. The APS is further phosphorylated and form phosphoadenosine phosphosulfate (PAPS).

In assimilatory SO_4^{2-} reduction system, the required enzyme system is found in chloroplast. However, a smaller amount is also found in plastid of root cells. Therefore, S can also be assimilated in roots.

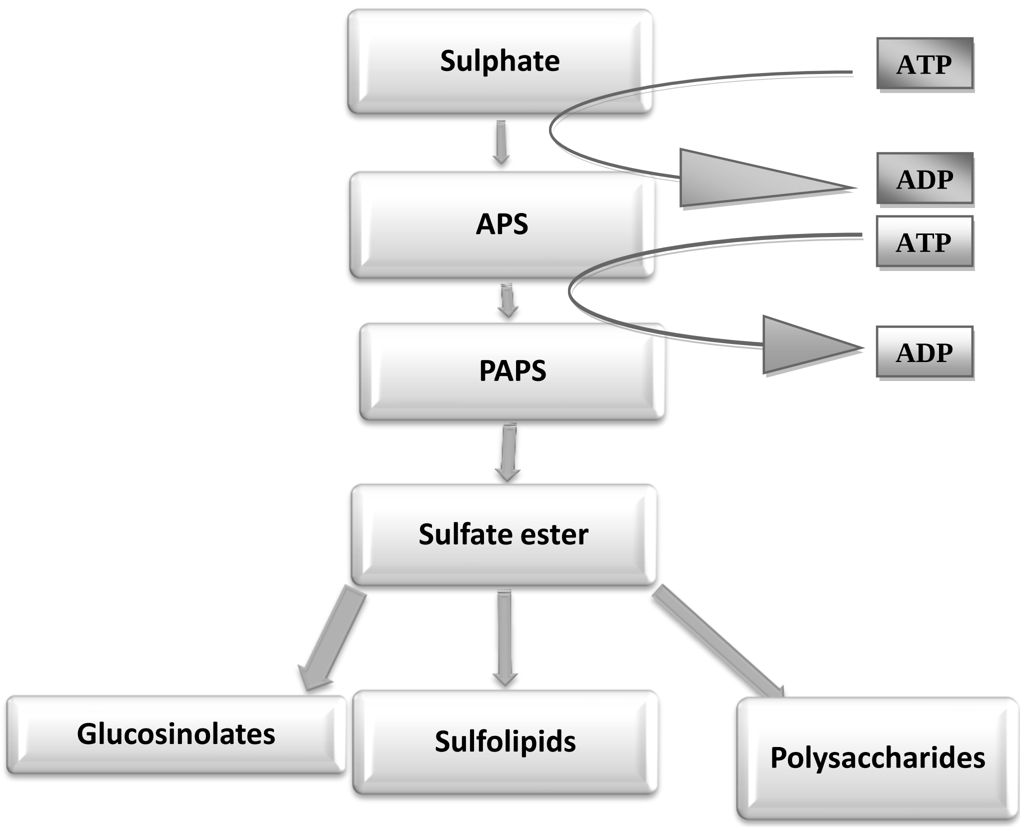


Figure 8.2. Pathway 2 of sulfur assimilation in crop plants in synthesis of sulfate ester, where, APS-adenosine phsphosulfate, PAPS-phoshoadenosine phosphosulfate.

8.4. Metabolic Functions of Sulfur

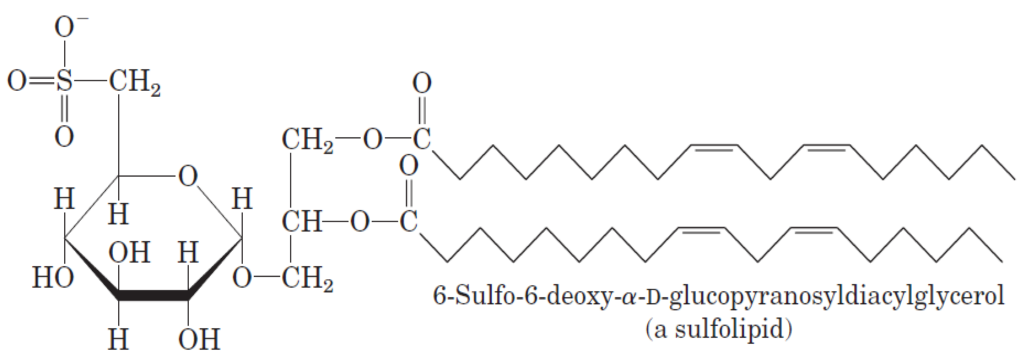
8.4.1. Sulfur is the constituent of amino acid cysteine and methionine and thus protein and enzymes. Other S-containing compounds are formed from the S-containing amino acids. Those amino acids are the precursor of S-containing enzyme and secondary plant product.

8.4.2. It is the constituent of many coenzymes and prosthetic groups namely ferredoxin, biotin (vitamin H) and thyamin pyrophosphate (vitamin B_1).

8.4.3. It is reported that SO_4^{2-} is generally stored into vacuoles of mesophyll cells which is released at prolonged S-deficiency, and very small amount is translocated which is not sufficient to support new growth (Clarkson et al., 1983; Bell et al.,1995). Therefore, S-deficiency is appeared in the younger part of the plants. Under S-deficient condition, the major fraction of S is in protein, and remobilization does not occur if N deficiency exists. Under low N-condition SO_4^{2-} is exported from mature leaves (Sunarpi and Anderson, 1997).

8.4.4. The concentration of insoluble S is almost similar in all types (older and younger) of leaves. However, the concentrations of glutathione and glucosinolates increased from the oldest to the youngest leaves, and the opposite may occur for SO_4^{2-} (Mechteld et al., 1998).

8.4.5. Sulfur acts as a constituent of biological membrane via sulfolipid especially abundant in thylakoid membrane of chloroplast. Sulfolipid of plant is present in plastid which offers negative charge in the thylakoid membrane leading to stabilization of photosynthetic complex. It is a non-phosphorus glycolipid which synthesis is not affected by the unavailability of phosphorus (Benning, 2008).



6-Sulfo-6-deoxy-α-D-glucopyranosyldiacylglycerol
(a sulfolipid)

8.4.6. Isothiocyanate, a volatile compound containing sulfur as a structural constituent is considered responsible for the characteristic odor of plant species like onion, garlic and mustard. The pungent smell is responsible for the presence of this organic compound. Several isothiocyanates are produced by enzymatic (myrosinase) conversion of metabolites called glucosinolate. The isothiocyanate is harmful to the plant itself and also acts as defense mechanism against animal.

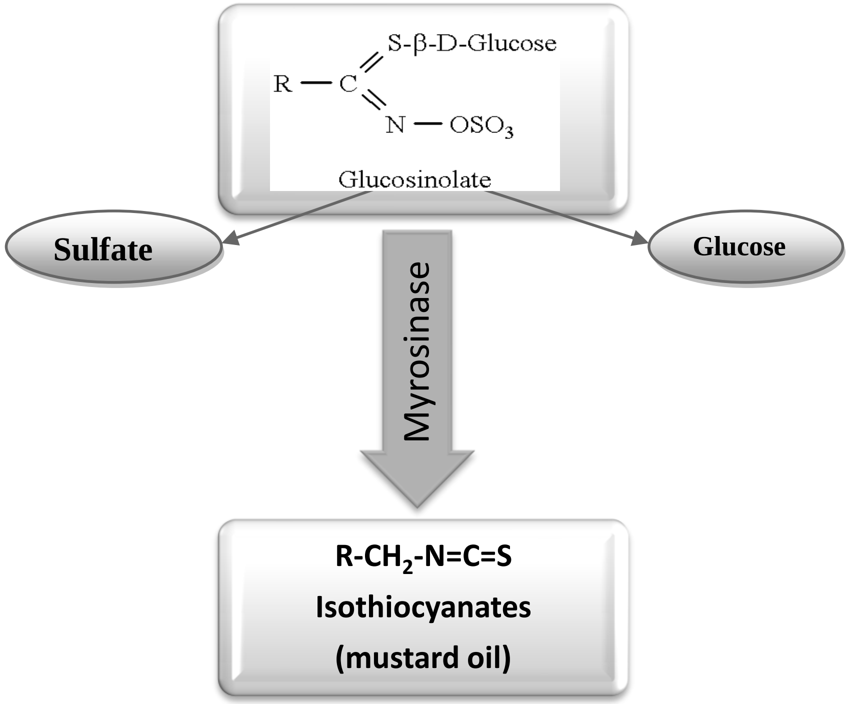


Figure 8.3. Schematic flow of formation of isothiocyanate (found in mustard oil) from glucosinolate.

The relationship between S and N is not surprising since both are components of protein and are involved in chlorophyll formation. They are also linked by the role of S in the conversion of nitrate to amino acids. Crops having high N demand will usually also have high S needs.

8.5. Deficiency Symptoms of Sulfur in Crop Plants

8.5.1. Low organic matter, light soil and heavy rainfall are the precondition of sulfur deficiency in crop plants. Sulfate and anions are mobile in the soil and are subject to leach in the soil. Sulfur is an immobile element in the plant system especially in the phloem transport; hence its deficiency occurs in meristematic area i.e., in young leaves, twig of plants. Its deficiency is characterized by stunted growth, and general yellowing of tender leaves (Figure 8.4). In some cases an interveinal chlorosis appears i.e the veins remaining green but the middle portion becomes yellow in color. Under acute deficiency condition the entire plants turn into yellow, and deficiency may result in delaying maturity of certain crops like groundnut. Under condition of sulfur deficiency protein synthesis will impair leading to the symptoms of chlorosis.

8.5.2. Sulfur requirement for optimal growth varies between 0.2 and 0.5 % in the dry weight basis. Its requirement among the crop families is in the order of Brassicaceae>Fabaceae> Poaceae.

Figure 8.4. Deficiency of sulfur in okra showing yellow color of tender leaves.

8.5.3. The decrease in the protein content of sulfur-deficient plant is correlated with the preferential synthesis of protein with lower proportion of methionine and cysteine.

8.5.4. In legumes, nodulation is restricted due to S-deficiency in plants. On the other hand, deficiency of S delays in fruit ripening and fruits are not duly matured.

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Chapter 9

CALCIUM (CA)

9.1. GENERAL FEATURES

Calcium is a seconadry macro nutrient element for plants of which molecular weight is 40. It is the fifth most abundant element in the earth crust, and is relatively larger divalent cation. Calcium is nontoxic macronutrient element and greater uptake does not create any adverse effect in the plant system. It is present in the soil as mineral namely calcite ($CaCO_3$), gypsum ($CaSO_4.2H_2O$), apatite, plagioclase, hornblende etc. (Troeh and Thompson, 1993). Calcium concentration in the earth crust is around 36.4 g kg^{-1} which is higher than that of other nutrients. Soils differ widely in Ca concentration and pH. Based on the Ca affinity plants are classified as calcicoles which are found in calcarious soil (high in Ca) and calcifuges which are found to be grown in acidic soil (low in Ca).

Table 9.1. Differences between calcicole and calcifuges plants with special references to Ca utilization

Calcicole plant	Calcifuges plant
Higher level of intercellular Ca present	Lower concentration of intercellular Ca present
Higher concentration of malate is present	Lower amount of malate present in the cell
Ca-oxalate crystal is found in the vacuole	Ca-oxalate crystal is not found in the vacuole

9.2. UPTAKE AND CONCENTRATION IN THE PLANT TISSUE

Calcium is taken up by the plant as divalent cation (Ca^{2+}), and average concentration is ranged from 5 to 30 mg Ca g^{-1} dry matter of plant. Heavy uptake of K^+ reduces the absorption of Ca^{2+} in the plant. The concentration of Ca in plant tissue is genetically controlled; however also affected by supply and availability of calcium in the soil. Generally, Ca content in the soil is relatively higher than K content; but uptake is very minimal. It is highly immobile in the plant system especially in the phloem where translocation is restricted. Calcium occurs in the cell as Ca^{2+}, and adsorbed by various non-diffusible groups such as carboxylic and phenolic compounds, Ca is found in apoplastic region of cell. It enters into plant cell via Ca^{2+}

ion channels in their plasmalemma, and high level of Ca^{2+} is toxic for the cytoplasm (White, 2000). The optimum level of Ca^{2+} in cytosol is maintained by Ca^{2+} efflux by Ca^{2+} ATPase and H^+/Ca^{2+} antiporters. It is translocated through xylem; and may traverse the roots either through apoplastic or rarely symplastic pathway (White and Broadley, 2003). Calcium reaches xylem via apoplastic region where Casparian strip is not well developed or absent.

9.3. Interaction of Ca^{2+} with Other Cations

Calcium availability is related to the soil cation exchange capacity (CEC) and other cations present in the soil. It competes with major cations namely K^+, Mg^{2+}, NH_4^+, Fe^{3+} and Al^{3+}. Higher levels of soil Na^+ will displace Ca^{2+} and lead to Ca^{2+} leaching which can result in poor soil structure and may causes Na^+ toxicity in soil. However, application of Ca^{2+} ameliorates the salinity in soil through the enhance uptake of K^+. Any free Ca^{2+} reacts with P to form insoluble (or very slowly soluble) Ca-P compounds that are not readily available to plants. When the pH of a soil decreases, more of Fe^{2+} and Al^{3+} elements become soluble and combine with Ca^{2+} for essentially insoluble compounds. Higher level of Ca^{2+} in soil or plant can inhibit the uptake and assimilation of boron.

9.4. Commonly Used Ca-Containing Fertilizer

Table 9.2. Calcium containing fertilizer with their chemical formula and approximate concentration of Ca (%)

Name of Fertilizer	Chemical Formula	Concentration (% Appox.)
Dolomite	$CaCO_3$, $CaMgCO_3$	36
Calcium nitrate	$Ca(NO_3)_2$	19
Zypsum	$CaSO_4.2H_20$	20
Triple super phosphate (TSP)	$Ca(H_2PO_4)_2$	14
Single super phosphate (SSP)	$Ca(H_2PO_4)_2$	18-21
Synthetic chelator	Ca-EDTA	3-5
Rock phophate	$[Ca_3(PO_4)_2]_3.CaF_x.(CaCO_3)_{x.}[Ca(OH)_2]_x$	35

9.5. Application of Ca-Fertilizer

Generally, Ca-fertilizer is applied as broadcast during land preparation; foliar spray can also be performed in case of severe deficiency. For example, synthetic chelator viz. Ca-EDTA or other Ca-containing fertilizer like calcium nitrate, calcium-amonium nitrate can be applied as foliar spray. Following factors are considered to determine the availability of Ca^{2+} in the soil:

- Total supply of Ca in the soil
- pH of the soil

- Type of soil colloid
- Cation exchange capacity of the soil
- Ratio of Ca^{2+} to other cations

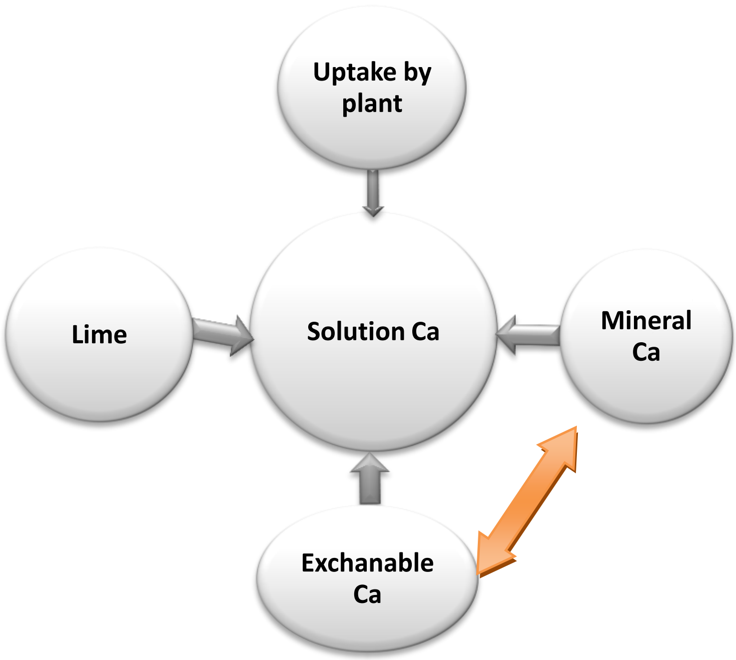

Figure 9.1. Simplified scheme of Ca equilibrium and transformations in soil.

9.6. Physiological and Metabolic Functions of Calcium

Calcium is a structural component of cell wall, and is therefore important in the formation of new cells. It is the nutrient which works outside the protoplasm i.e., in the apoplastic region. It is a nontoxic element which also detoxifies other toxic elements i.e.. ameliorate the adverse effect of salinity. The important physiological and biochemical functions are summarized below:

9.6.1. Stabilization of Cell Wall

Calcium helps in stabilizing the cell wall by acting as cementing material by forming calcium pectate. In dicotyledons, the binding of Ca^{2+} with $R.COO^{-}$ is higher than that of monocotyledons. This binding is more pronounced in storage tissue of certain crops (Sugawara et al., 1981). It has the role of regulation of permeability of membrane and structural integrity of cell. Prevention of leaking of ions through cell membrane can be performed by the application of calcium. In Ca-deficient cells, enhanced respiration and leakage of ions through cell membrane are common phenomenon.

9.6.2. Enlargement of Cell

Increase of root growth is mediated by the application of Ca which enhances cell division and cell enlargement. The extension of cell by the application of Ca is done through incorporation of cellular materials in the cell wall.

9.6.3. Modulation of Enzyme

Calcium enhances the activity of many enzymes like α-amylase, phospholipase, ATP-ase etc. by changing the modulation (Wyn Jones and Lunt, 1967). It also stimulates the membrane bound ATP-ase activity. The inhibition of enzyme activity is done by Ca^{2+} especially in the cytoplasm.

9.6.4. Osmoregulationary Function and Balancing of Cation and Anion

Calcium stored in the vacuole is helpful for balancing the cation and anion. The calcium oxalate is found in many hydrophytes and halophytes which help in the regulation of osmosis. It also helps in mitigation of heat stress by improving stomatal functions and other processes. It is also thought to have an influence on the development of heat shock proteins that aid in tolerate the stress of prolonged heat.

9.6.5. Signal Molecules As Second Messenger

Calcium ions are important signaling molecules, as once they enter the cytoplasm they exert allosteric regulatory effects on many enzymes and proteins. Calcium can act in signal transduction resulting from activation of ion channels or as a second messenger caused by indirect signal transduction pathways such as G-protein-coupled receptors. It involves in chromatin or mitotic spindle organization.

9.6.6. Calmodulin

Calmodulin (CaM) (calcium modulated protein) is a calcium binding protein which is a multifunctional messenger protein. It is the most prominent Ca^{2+} transducer in eukaryotic cells having diverse cellular functions. Plants have a unique set of CaM-related and several unique CaM target proteins.

9.7. Deficiency Symptoms of Calcium in Crop Plants

9.7.1. Calcium deficiency is rare in nature and vague because it sometime accompanied with high acidity. However, acidic, alkaline and sodic situations are the soil conditions for creating deficiencies of Ca. The visible deficiency symptoms in agronomic crop plants are seldom observed.

9.7.2. Deficiency can be observed in meristematic area viz. shoot tip by reduction of growth. It appears in the young leaves and near the point of growing parts of roots and stem. Stunted growth is the consequent of calcium deficiency. The shoot tip may be deformed, chlorotic and finally become necrotic especially in the leaf margin.

A

B

Figure 9.2. Showing Ca deficiency symptoms A: Blossom end of tomato rotten and B: young leaves are deformed shaped in banana.

9.7.3. The young leaves may be severely distorted with the tips hooked back and margins curled backward or forward or rolled.

9.7.4. Deficiency of Ca prevents the unfolding of new leaves and tip become colorless and is covered with gelatinous materials which help in adhering with one another in maize.

9.7.5. The most common symptoms are the blossom end in tomato and bitter pit of apple. A similar Ca-deficiency disorder occurs in water melon. Internal browning of Brussels sprout, blossom end rot of peeper and cavity spot of carrots has been described by Maynard (1979). White heart and hollow heart in cucurbit are the typical symptoms of Ca deficiency. Fruit analysis for Ca is prerequisite for identifying its deficiency in fruit setting despite of leaf analysis. It is necessary to ensure the supply of Ca during fruit setting for achieving quality fruit.

9.7.6. Shoot may die back, eventually plants may die. Plant growth may become stunted with numerous tillers production.

9.7.7. Two diseases i.e., rosette and die back are commonly found due to Ca deficiency in crop plants. Colors may be dull or bluish green.

9.7.8. The low calcium containing fruits lose their storage capacity and shows some physiological disorders like bitter-pit and cork-spot in apples and pears, cracking in cherries, and other degradation of the fruit.

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Chapter 10

MAGNESIUM (MG)

10.1. GENERAL FEATURES

Magnesium is another secondary macronutrientelement having the atomic number of 12 and atomic weight of 24. It is an alkaline earth metal and eighth most abundant-element in the earth crust. The Mg concentration in earth crust ranges from 0.5 g kg^{-1} in sandy soil to 5 g kg^{-1} in clay soils (Mengel et al., 2001). It has three stable isotopes namely ^{24}Mg, ^{25}Mg and ^{26}Mg. It is an essential plant nutrient element which is involved in the synthesis of chlorophyll as a central element.

10.2. MAGNESIUM UPTAKE BY THE PLANTS

Magnesium is taken up by the plant roots as Mg^{2+} ion, and the rate of uptake is lower than other cations like K^+, due to many reasons. There is lack of special transportation through transmembrane, and it may be restricted by K^+ and Ca^{2+} (Schimansky, 1981).

The flux of Mg^{2+} through plasmalemma is mediated by facilitated diffusion via one or more specific ion channel following electrochemical gradient for Mg^{2+}. The higher level of K^+ in the plant body suppresses the uptake of Mg^{2+}. Heliophytes i.e., plants growing in high light intensity require higher amount of Mg than sciophytes, the shade loving plants.

The uptake rate of Mg is suppressed by other cations namely K^+, NH_4^+, Ca^{2+}, and Mn^{2+} (Kurvits and Kirkby, 1980; Heenan and Campbell, 1981). Moreover, it is influenced by soil pH that is why acidic soil shows deficiency of Mg (Marschner, 1995).

Table 10.1. Magnesium containing fertilizer commonly used for crop production with their chemical formulae and approximate concentration

Name of Fertilizer	Chemical Formula	Concentration (% Appox.)
Epsom salt (magnesium sulfate)	$MgSO_4.7H_2O$	9.8
Potassium-magnesium sulfate	$K_2SO_4.2MgSO_4$	11.0
Kieserite	$MgSO_4.H_2O$	16.0
Magnesium chloride	$MgCl_2.10\ H_2O$	8.0-9.0
Magnesia	MgO	55.0
Magnesite	$MgCO_3$	27.0
Ground burnt magnesian lime	Mg oxide	0.6-20.0

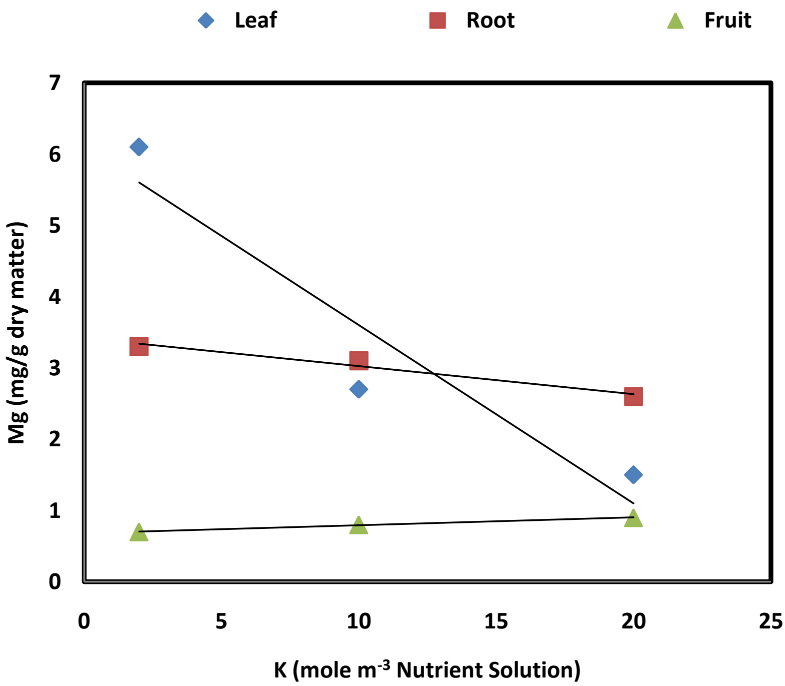

Figure 10.1. Effect of an increasing application of K on the concentration of Mg in root, leaf and fruit of tomato (*Solanum lycopersicum*) (modified from Mengel et al., 2001).

10.3. Methods of Mg Application

Generally, Mg is applied in the form of carbonate, oxide or a sulfite where sulfite fertilizers are more effective than carbonate fertilizer although more expensive. Mg-containing fertilizers are applied as broadcasting during final stage of land preparation. Normally, magnesium sulphate may be applied @ 50 kg ha^{-1} in the field during land preparation before sowing. The dose varies depending upon crop species. Foliar spray may be suggested in case of severe deficiency caused by problem soil or under drought or flooded condition, and may be applied @ 0.2 - 0.5% magnesium sulphate and 0.1 - 0.3% lime.

10.4. Factors Affecting Mg availability

Higher levels of NH_4^+ and K^+ in the soil decreases the uptake of Mg^{2+} by the plants. Mg-fertilizer application is therefore, necessary where K^+, Ca^{2+} and NH_4^+ levels are high. The best Mg-fertilizer application response is found in the light sandy soil. The following factors are considered for uptake of Mg:

- Mg concentration in soil
- Growth and nature of plant
- pH of soil

- Cation exchange capacity in soil
- Presence of NH_4^+, K^+ and ion in soil

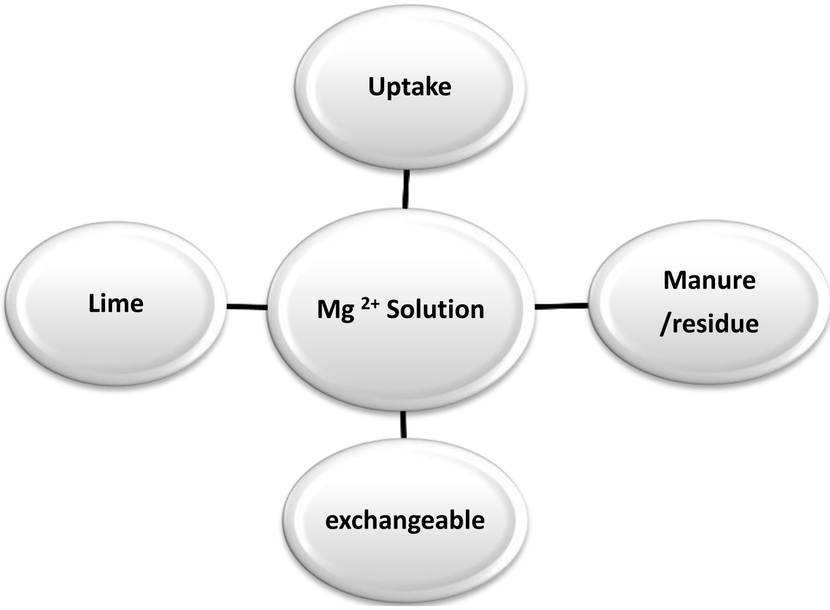

Figure 10.2. Simplified equilibrium state of Mg^{2+} in the soil showing different state of Mg.

10.5. Functions of Magnesium in Crop Plants

Magnesium is involved in a number of physiological and metabolic activities of plants, and is involved throughout the plant body.

10.5.1. Photosynthesis

Magnesium stimulates the activity of Rubisco enzyme for increasing photosynthesis. The positive effect of Mg on the assimilation of CO_2 and subsequently on the production of sugar is the result of Rubisco activation.

10.5.2. Chlorophyll Synthesis

Mg occurred in the central part of chlorophyll molecule, and essential for structure and conformation of nucleic acid viz. DNA and rRNA. It is reported that nearly 35% of the plant Mg is present in chlorophyll.

10.5.3. Protein Synthesis

It is the structural unit of ribosome, and is essential in the process of protein synthesis by the involvement of DNA and RNA.

10.5.4. Activation of Enzyme

Magnesium is a metal activator of the enzyme known as Ribozymes, found in ribosome. It is necessary for the stabilization of the ribosomal particles necessary for the synthesis of protein. It is required for the development of mitochondrion (Zielinski and Price, 1978). Essential for structure and conformation of nucleic acid in living organisms, and is required for maximal activity of almost every phosphorylating enzyme in carbohydrate metabolism. Magnesium is required in the reactions of transferring phosphate from ATP.

Table 10.2. Mg activation of enzyme concerned with carbohydrate metabolism

Enzyme	Reaction	Metal
Galactokinase	Galactose +ATP → Galactose 1-P+ADP	Mg^{2+}
Fructokinase	Fructose +ATP → Fructose-1-P +ADP	Mg^{2+}, K^+
Hexokinase	Hexose (glucose, fructose, mannose) + ATP → Hexose-6-P+ ADP	Mg^{2+}, Mn^{2+}

10.5.6. Transformation of Sugar and Regulation of Nutrient Uptake

Magnesium helps in uptake and transformation of phosphorus, and in transformation of sugar and starch in plants. It also regulates the uptake of other nutrients namely K^+, NH_4^+ etc.

10.6. Deficiency Symptoms of Mg in Plant

10.6.1. Degradation of Chlorophyll

Degradation of chlorophyll begins due to lack of sufficient amount of Mg which causes the main symptom of interveinal chlorosis i.e., vein remain green but yellowing between leaf veins, giving the leaves a marbled appearance (Thomson and Weier, 1962). Due to magnesium's mobile nature, the plant will first break down chlorophyll in older leaves and transport to younger leaves which have greater need of assimilates. Therefore, the first sign of magnesium deficiency is the chlorosis of old leaves which progresses to the young leaves as the deficiency continues. The chlorophyll of vein i.e., vascular bundle is more stable than mesophyll tissue results in the greenness of vein under deficient condition (Hopkins, 1995). Some time its deficiency may be confused with Zn and Cl deficiencies, virus infection or natural ageing since all have similar symptoms. Reduction in plant growth is also the results of less photosynthetic area. The typical Mg-deficiency symptom has been shown in the Figure 10.3.

Plate 10.3. Typical Mg-deficiency symptom showing the interveinal chlorosis.

10.6.2. Anatomical and Cytochemical Effects of Mg

Deficiency of Mg results in the deformation of tissue system i.e., small pith cells and densely packed chlorenchyma tissue with chlorophyll. Microsomal particles, containing RNA and protein appear to consist of sub-microsomal particles which are considered to be bound together by Mg ions combined with phosphate radical of RNA (Hewitt, 1963; Marinos, 1963).

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Chapter 11

IRON (FE)

11.1. GENERAL FEATURES

Iron is the most important micro nutrient element for crop plants which comprises around 5% of the earth crust. The major part of the iron in the world is present as crystal lattice comprising of numerous minerals. It is relatively less mobile in the plant system, and mobility is affected by several factors namely occurrence of Mg and K deficiency, high P and high light intensity. Under aerobic condition free Fe^{2+} is oxidized to ferric iron (Fe^{3+}) which is not readily soluble (Neilands, 1995).

Iron is found in the soil as following forms:

- Primary and secondary Fe-minerals
- Adsorbed iron in clay minerals
- Organic-Fe in soil
- Solution-Fe in soil

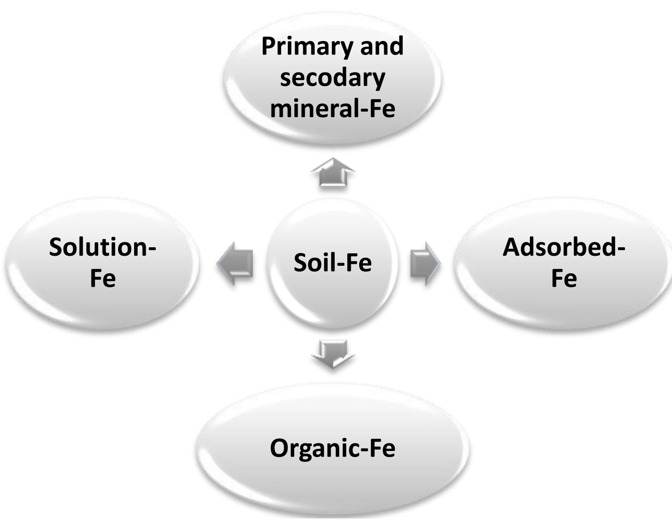

Figure 11.1. Iron equilibrium state showing different forms in soil.

Table 11.1. Fe containing fertilizer with their chemical formulae and approximate concentration

Name of Fertilizer	Chemical formula	Amount of Fe (%) appox.
Ferric sulfate	$Fe_2(SO4)_3.4H_2O$	23
Ferrous sulfate	$FeSO_4.7H_2O$	19
Ferrous oxide	FeO	77
Ferric oxide	Fe_2O_3	69
Ferrous ammonium sulfate	$(NH_4)_2SO_4.FeSO_4.6H_2O$	14
Ferrous ammonium phosphate	$Fe(NH_4)HP_2O_7$	29
Iron chelates	NaFeEDTA	22
	NaFeHEDTA	10
	NaFeEDDHA	7
	NaFeDTPA	10

11.2. SIDERPHORE

The term siderphore stands for iron carriers or bearer which are water soluble having low molecular weight, organic legends with high affinity and specific for iron binding (Kraemer, 2004). Recently, siderphore production by plant growth promoting rhizobacteria is gaining prominence in crop productivity through enhancing iron nutrition in plants (Scavino and Pedraza, 2013). A specialized mechanism for iron uptake is also observed in Poaceaeous crops via production of nonproteinogenic amino acid, called phytosiderphore (Romheld and Marschner, 1986). Microbes play important role in the Fe uptake by the plant from the soil. Root exudates released by plants alter the physico-chemical properties of soil and turn into microbial community of the rhizosphere. The altered microbial community thus enhances the Fe uptake by the production of siderphore.

11.3. PHYTOSIDERPHORE

Recently, the concept of using phytosiderphore in iron nutrition is gaining prominence. Phytosiderphores are organic chemicals e.g., mugeneic acid, released from the roots as exudates, which are capable to bind ferric ion (FeIII) and makes a complex for transporting into cytoplasm thereafter release into cytosol for utilization (Figure 11.2).

11.4. IRON-CHELATORS

These are water soluble organic compounds (Figure11.3) which make bond with metals namely Fe, Zn, Cu and Mn resulting enhanced solubility and thereby supply to the plant roots. They catch the Fe^{3+} and make a complex which enables to transport the iron into the cell. The most common iron chelate is the Fe-EDTA (iron salt of ethylene diamine tetra acetic acid) (Figure 11.3). The schematic diagram shows the absorption of iron through chelator (Figure 11.4).

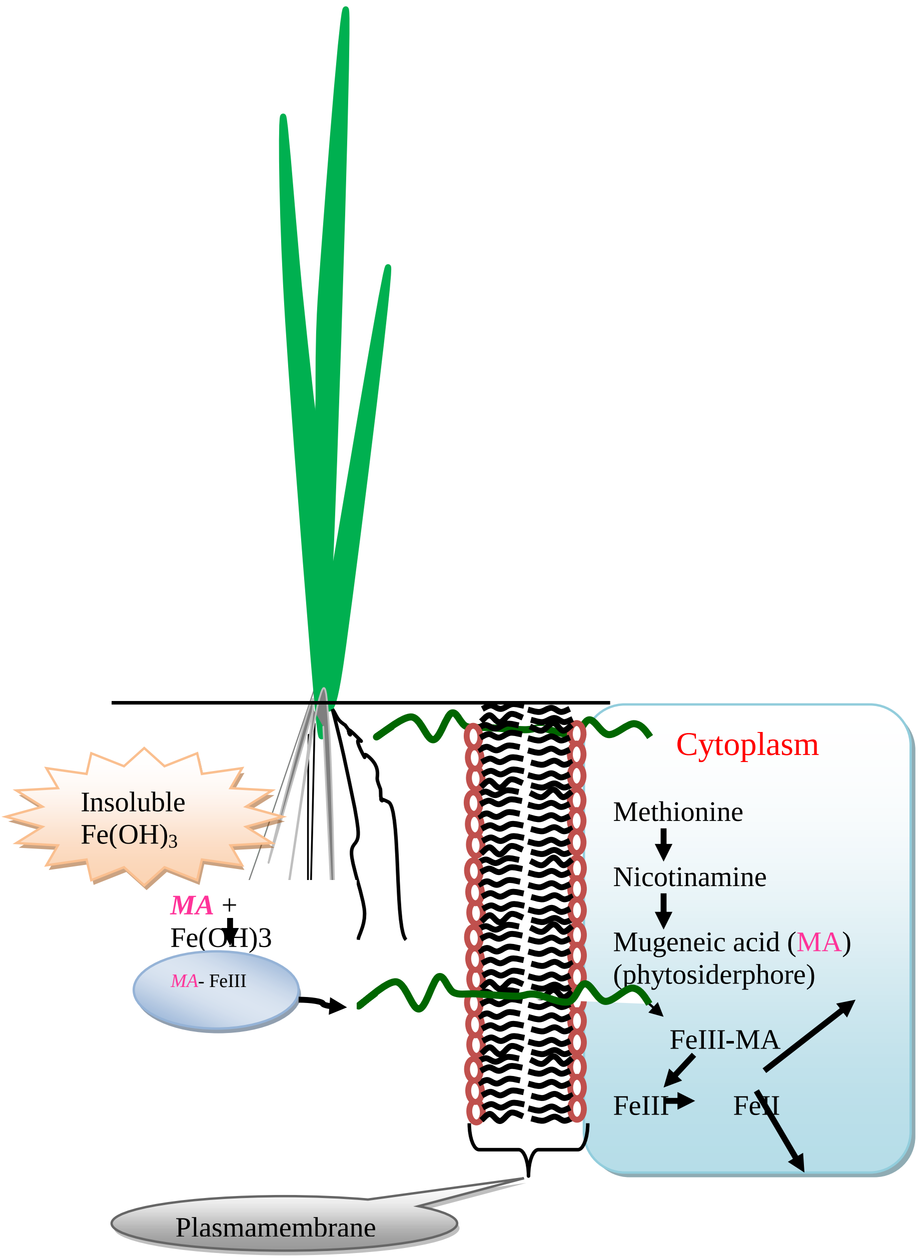

Figure 11.2. Diagrammatic scheme of phytosiderphore activity of Fe^{3+} absorption in plant roots.

11.5. Physiological and Metabolic Functions of Iron

Iron is physiologically indispensible and required as a part of many proteins and enzymes, especially the enzymes involved in the redox reactions. In plant cells Fe is mainly found in the form of porphyrin.

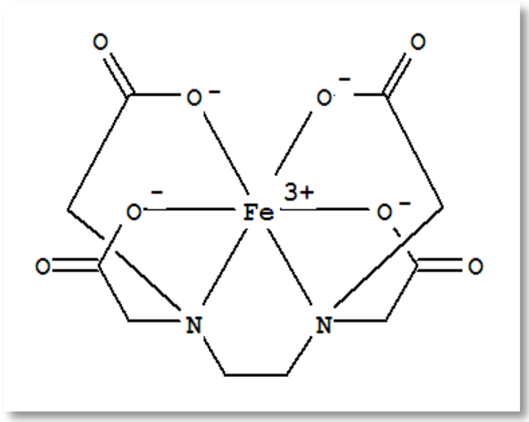

Figure 11.3. Commercial fertilizer of Iron-EDTA.

Among the important functions of Fe in the plant body the constituent of enzyme as metal activator namely, haem or haemin is the most important. Here, Fe plays similar role as Mg in chlorophyll. The most common haem-containing enzymes are catalase, peroxidase, cytochrome oxydase. In legume-*Rhizobium* symbiosis Fe-containing protein is known as leghemoglobin.

11.5.1. Iron Act As Constituent of Protein and Enzyme

There are two types of iron protein found in plants; they are:

i. Hemoprotein
ii. Iron-sulfur protein

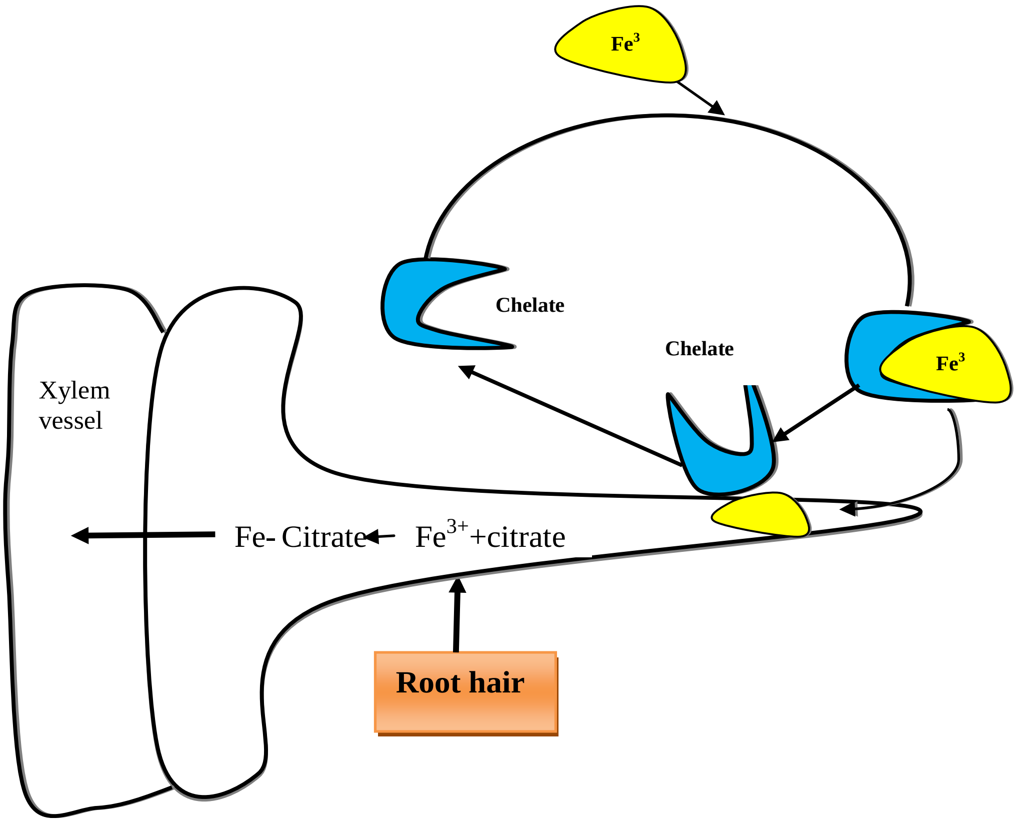

Figure 11.4. Diagrammatic scheme of flow of ferric ion absorption in the root hair through iron chelator.

Iron Containing Enzymes

Iron is a component of many enzymes in the plant system like catalase, peroxidase etc.. The role of catalase is to dismutase the H_2O_2 to H_2O and O_2. The equation has been illustrated as follows:

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

There are two types of Fe-containing proteins namely Fe-protein and Fe-Mo- protein; the latter performs the fixation of N_2 and converts to NH_4^+.

11.6. Deficiency Symptoms of Iron

11.6.1. The main deficiency symptom of iron is the failure of chlorophyll formation as Fe is essential for biosynthesis of chlorophyll molecule. The size of chloroplast is decreased due to Fe-deficiency.

11.6.2. Interveinal chlorosis is observed in tender leaves, principal veins remain green in color (Figure 11.5). The youngest leaves may be totally devoid of chlorophyll and often become necrotic.

11.6.3. Reduction of plant growth is observed despite of chlorosis.

11.6.4. Fe-deficiency causes the disruption of thylakoid membrane formation.

11.6.5. The accumulation of anions and amino acids are characterized by the deficiency of Fe in crop plants.

11.6.6. In extreme deficient condition scorching of leaf margins and tips may occur.

Figure 11.5. Deficiency of Fe in rice seedling showing chlorosis of leaf.

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Chapter 12

MANGANESE (MN)

12.1. GENERAL FEATURES

Manganese is a metal element found in the soil as mineral like Fe-Mn-rocks, pyrolusite (MnO_2), manganite (MnOH), rhodochrosite ($MnCO_3$). The concentration in the earth's crust is 1000 ppm in an average. The Mn content in soils generally ranges between 20-3000 ppm. The critical concentration of Mn in plants varies from 10-20 ppm in dry leaves. Its uptake is performed as Mn^{2+} through facilitated diffusion across plasmalemma (Fox and Guerinot, 1998). It is relatively immobile in plant system, and is sparingly translocated through phloem, and preferentially translocated to meristems in tender organs.

12.2. FACTORS INFLUENCING THE AVAILABILITY OF MN

- Water logged condition with low aeration capacity in the soil
- Presence and content of organic matter in soil
- Weather and climatic condition of crop field
- Interaction with other ions in the soil and plant body
- Physiology of plants
- Microbial activity of soil

Table 12.1. Mn-containing fertilizer with their chemical formulae and approximate concentration (%)

Name of fertilizer	Chemical formula	Percent Mn (Approx.)
Manganese oxide	MnO	41-68
Manganese sulfate	$MnSO_4.4H_2O$	26-28
Manganese chloride	$MnCl_2$	17
Synthetic chelate	Mn-EDTA	5-12

12.3. APPLICATION OF MN-FERTILIZER

Mn-fertilizer may be applied as broadcasting during final land preparation before seed sowing or transplanting. In deficient condition, foliar spray may be recommended where proper dose should be strictly maintained. Foliar spray is more effective than soil application.

12.4. METABOLIC FUNCTIONS OF MANGANESE

Manganese is absorbed as Mn^{2+} and is translocated predominantly as free divalent cation in the xylem. It can be replaced the functions of Mg^{2+}.

It is involved in the photosynthetic O_2 evolving system

i. It is required for Hill reaction in the photosystem I
ii. It is involved in the electron transport chain

12.4.1. Mn- containing Enzyme

Superoxide dismutase (SOD) is an enzyme containing Mn (around 90% is present in chloroplast), enhances resistance to oxygen radicals (oxidative stress tolerance).

i. Peroxidase
ii. Phosphatase

12.4.2. The functions of Mn are some times resemble with the function of Mg which bridge ATP with enzyme complex namely phosphokinase and phosphotransferase. It activates the enzyme functions like decarboxylase and dehydrogenase found in TCA cycle. Activation of RNA polymerase is influenced by Mn where in low concentration is much more effective (Ness and Woolhouse, 1980).

12.5. DEFICIENCY SYMPTOMS OF MANGANESE IN CROP PLANTS

12.5.1. Mn Deficiency in Soil and Plant

Alluvial silt and clay soils and marsh soils which originate from calcareous materials is prone to Mn deficiency for crop plants. Poorly drained calcareous soils having high organic matter content and high sandy acid mineral which are low in native Mn content is also favorable for Mn deficiency for plants.

12.5.2. The first symptom of Mn deficiency appears in younger a leaf which is the result of disorganization of lamellae of chloroplast. Some time the deficiency of Mn is confused with that of iron and magnesium. Additionally, deficiency of Mn results in intercostals chlorosis of the younger leaves in dicotyledons and appearance of greenish gray spots on the basal leaves of cereal crops.

Production of dry matter through photosynthesis and chlorophyll are markedly affected by Mn deficiency but the respiration remains unaffected. Manganese deficiency makes the plants more susceptible to freezing temperature. The deficiency of Ca and Mg is stimulated by the deficiency of Mn.

Figure 12.1. Manganese deficiency in wheat showing chlorosis of younger leaves.

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Chapter 13

ZINC (ZN)

13.1. GENERAL FEATURES

The average Zn concentration of earth crust ranges from 17 to 160 ppm (Reed and Martens, 1996), and is present as the lattice structure of primary and secondary minerals. Morphologically it is similar to Fe and Mn especially in case of radius of ion.

Zinc is dissolved in the solution in ionic or complex form and in following state:

- Exchange site of clay minerals
- Organic matter content
- Adsorbed on solid surfaces
- Non-extractable

Plant normally up takes Zn as divalent cation form Zn^{2+}, but in calcareous soil it is taken up as mono-valent cation $ZnOH^{+}$ under high pH condition Presence of higher concentration of cations like Ca^{2+} inhibits the uptake of Zn.

Its availability in the early stage of plant growth is critical to ensure yield and quality of crops. Recently, it has been reported that higher plants absorb Zn from the rhizosphere by transporters.

13.2. FACTORS INFLUENCING THE AVAILABILITY OF ZN IN SOIL

- pH of the soil i.e., condition of acidity and alkalinity
- Presence and content of organic matter
- Adsorption by clay minerals
- Interaction with other nutrients i.e., oxidation-reduction reaction and chelators etc.
- Physiological status of plants
- Climatic condition of crop area viz. humidity, light, soil temperature etc.
- Microbial activity of soil

Table 13.1. Zn containing fertilizers with their chemical formulae and approximate concentration in percentage

Name of fertilizer	Chemical formula	Concentration of Zn (% approx)
Zinc oxide	ZnO	78
Zinc sulfate monohydrate	$ZnSO_4.H_2O$	35
Zinc phosphate	$Zn_3(PO_4)2$	51
Zinc carbonate	$ZnCO_3$	52
Zinc chelators	NaZnEDTA	14
	NaZnNTA	13
	NaZnHEDTA	9

13.3. Metabolic and Physiological Functions of Zn

13.3.1. Metal Activator of Enzyme

Zinc performs either as a metal component in an enzyme or as functional cofactor of a number of enzyme reactions. It acts as metal activator of many enzymes where the Zn is actively involved in the biological reactions. The common Zn-containing enzymes are as follows:

i. Alcohol dehydrogenase: this enzyme catalyze the reaction of acetaldehyde to ethanol. This reaction occurrs in meristematic zones namely root and shoot apices of plant.
ii. Zn is associated with Cu-Zn-super oxide dismutase (Cu-Zn-SOD)
iii. Carbon anhydrase- is found in cytoplasm and chloroplast, and it catalyze the conversion of CO_2 to H^+ and HCO_3^-
iv. RNA-polymerase is involved in the RNA proliferation

13.3.2. Activation of Enzyme

Activity of several enzymes namely ribonuclease, dehydrogenase, aldolase, isomerase, RNA and DNA polymerase is influenced by Zn (Dwivedi et al., 1974).

13.3.3. Synthesis of Phytohormone Like Auxin

Zn is required for the synthesis of tryptophan which is the precursor of IAA (indoleacetic acid). Zn deficient plants show lower level of auxins which results stunting of internodes and leaf size.

13.3.4. Influence of Zn on Metabolism Process

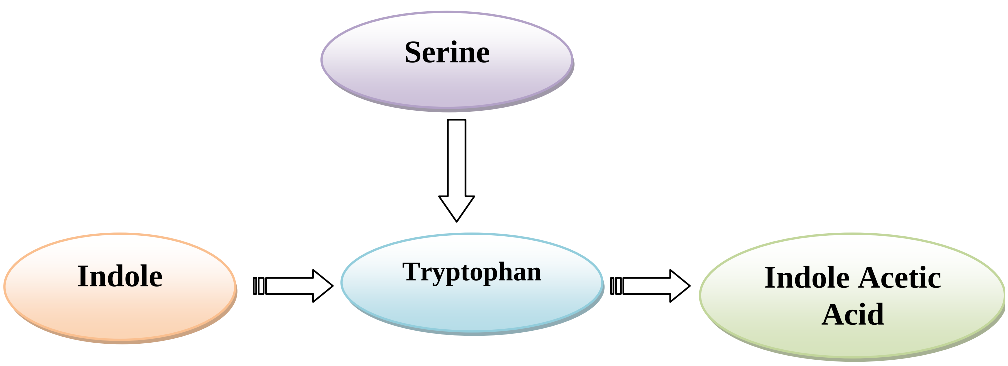
Grain or seed yield are affected if there is deficiency of Zn, it plays a specific role in fertilization of mega-gamete as pollen grains contain higher level of Zn. At flowering stage, most of the Zn is taken up during fertilization, and is incorporated into the developed seed. Zn is involved in the metabolism of protein, carbohydrate, nucleic acids and lipid. Moreover, it also controls the gene transcription and coordination of other biological processes regulated by proteins containing DNA-binding Zn-finger motifs (Rhodes and Klug, 1993).

13.3.5. Influence of Zn on Synthesis of Protein

Application of Zn increases the seed protein content. Zinc is the metal activator of the enzyme-RNA polymerase which is responsible for protein synthesis. Higher rate of RNA-ase activity is the common phenomenon of Zn deficiency which induces the degradation of RNA.

13.3.6. Role of Zn in the Synthesis of Phytohormone

Zinc influences the synthesis of tryptophan which is a precursor of indole acetic acid (IAA), a phytohormone. Marschner (1995) suggested that higher level of Zn is required for the synthesis of IAA from tryptophan through tryptamine.



13.3.7. Interaction Effect of Zn with Phosphorus

Higher level of P in the plant cell induces Zn deficiency in plants, which is the indirect effect of higher uptake of Ca. The higher uptake of Ca is the result of greater availability due to Ca-containing phosphorus fertilizer (TSP) application. This is called P-induced Zn deficiency found in different crop plants like bean, wheat, cotton, tomato etc. It is not clear how P inhibit the translocation of Zn in the plant system (Gianquinto et al., 2000).

13.3.8. Zinc functions on the structural and functional integrity of membrane and protein. It may be linked with phospholipids of membrane or sulfhydryl compounds and finally protect the oxidation damage of membrane (Domingo et al., 1992; Cakmak et al., 1989).

13.4. Zinc Deficiency and Toxicity

Zn Deficiency Symptoms in Crop Plants

- Zinc deficiency is widespread among plants grown in highly weathered acid soils and in calcareous soils.
- It decreases the productivity and quality of crop plants.
- Presence of higher concentration of P in shoot hinders Zn translocation which affects seed development.
- Maintaining the ratio of P: Zn in soil to predict the availability of zinc to the plant is very important.
- Zinc deficiency produces leaf morphology and cellular changes in different crop plants. Some of the well-known disorders including little leaf, rosette, mottle leaf white bud sickle leaf, bronzing and yellowing of different crop plants (Figure 13.1). In histological attributes Zn-deficient plants show compact cells of palisade and spongy parenchyma where the intercellular spaces are almost absent.

Figure 13.1. Little leaf and stunted growth showing Zn-deficiency in rice plants.

- In calcareous soils, Zn deficiency is often associated with iron deficiency (lime chlorosis)
- The most characteristic visible symptoms of Zn deficiency in dicotyledons are stunted growth due to shortening of internodes (rosette) and a drastic decrease in leaf size.
- Zinc deficiency is associated with iron deficiency, and often accompanied with chlorosis.
- Symptoms of chlorosis and necrosis of older leaves of Zn deficient leaves are the characteristics of P deficiency.

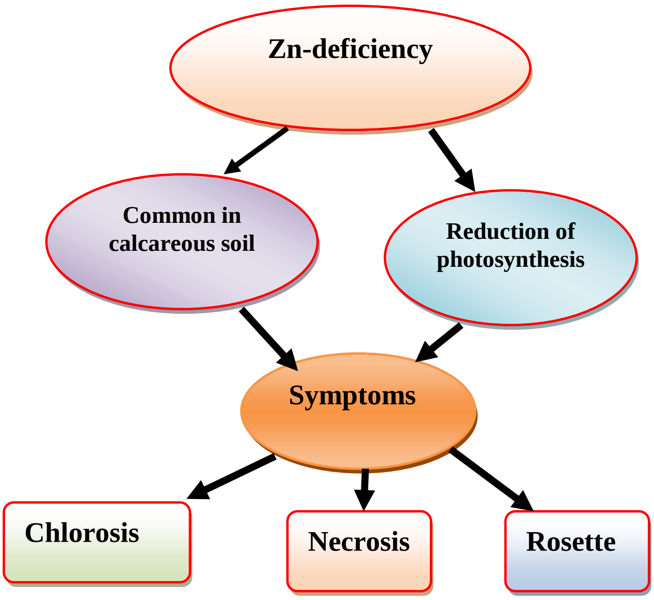


Figure 13.2. Diagrametic scheme of Zn deficiency in crop plants.

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Chapter 14

COPPER (CU)

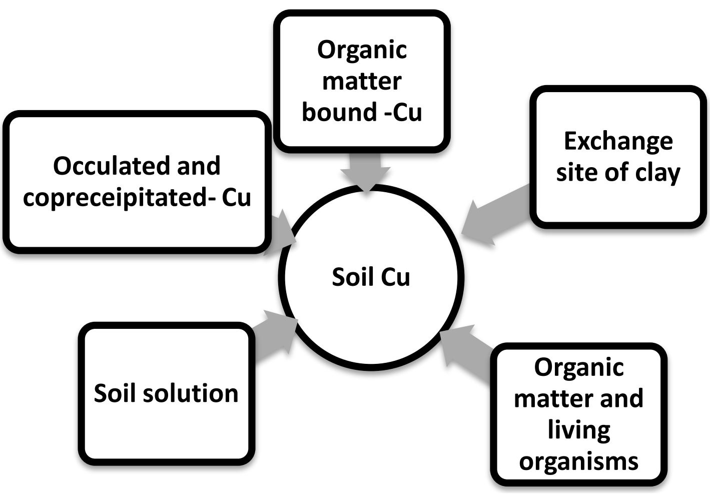
14.1. GENERAL FEATURES

Copper is a micro nutrient element which is essential for plant growth and development having atomic weight of 63.54. It has 29 isotopes where ^{67}Cu is radioactive. In soil, Cu is present in the crystal lattices of primary and secondary minerals, and bound with humic and fulvic acid very strongly forming Cu-organic matter complex. Around 98% of the Cu form complex with low molecular weight organic matter. It is mostly present as divalent and taken up by the plant as Cu^{2+}. The divalent ion of Cu is stronger than other divalent mineral ions in order to form complex in the following order.

Cu> Ni >Pb >Co >Ca> Zn> Mn >Mg

14.2. FORMS OF CU IN SOIL

Forms of Cu present in the soil are as follows:



14.3. Copper Uptake by the Crop Plants

Copper is taken up by the plants as Cu^{2+} ion in small quantities. Under physiological condition it exists as Cu^{2+} and Cu^{+} form. It enters into cytosol through copper transporter protein family (COPT) (Sancenon et al., 2003). The concentration of Cu in the plant tissue ranges from 5 to 20 ppm on dry matter basis. Its uptake is performed by active transport or metabolic processes, and strongly inhibits the uptake of Zn and *vice versa* (Bowen, 1969).

14.4. Copper Containing Fertilizer

The most common Cu-containing fertilizer is $CuSO_4.5H_2O$, although CuO and copper chelator have also been used as fertilizers. The fertilizers which are generally used as a source of Cu have been summarized as Table 14.1.

Table 14.1. Copper containing fertilizers with their chemical formulae and approximate concentration in percentage

Name of fertilizer	Chemical formula	Concentration (appox. % Cu)
Copper oxide	CuO	80
Copper sulfate	$CuSO_4.5H_2O$	25
Copper sulfate monohydrate	$CuSO_4. H_2O$	35
Copper ammonium phosphate	$Cu (NH_4)PO_4.H_2O$	32
Copper chelator	Na_2Cu EDTA	13
Copper chelator	Na_2Cu HEDTA	9

14.5. Factors Affecting Availability of Cu in Soil

- Interaction with other ions viz. divalent cations like Zn^{2+}
- Soil reaction i.e., pH of soil
- Organic matter content of soil
- Texture of soil
- Nature of crop plants grown in the soil

14.6. Metabolic Functions of Copper in Crop Plants

Copper performs various metabolic functions in plant such as metal activator of enzyme or as a part of protein. It has a role in photosynthesis, respiration, cell wall metabolism and anti oxidation activity (Pilon et al., 2006).

14.6.1. Copper Containing Proteins

There are three types of protein containing Cu:

- Blue protein which does not have any oxidase activity e.g., plastocyanin
- Non-blue protein-having oxidase activity e.g., peroxidase
- Multi-copper proteins- at least four Cu molecules are present per mole of protein e.g., ascorbate oxidase

14.6.2. Superoxide Dismutase (SOD)

There are various types of SOD enzymes which contain Cu and Zn as metal activator found in the chloroplast especially in the stroma. They detoxify the super oxide radicals namely singlet oxygen ($O_2^{\cdot -}$), and Cu atom is involved in the detoxification process.

14.6.3. Plastocyanin

Copper is present in plastocyanin, a monomeric protein having molecular weight of around 10500 Dalton. All plastocyanins have a hydrophobic surface surrounding the exposed histidine of the Cu binding site. It is present in the chloroplast and is involved in the electron transfer process in the electron transport chain. It is an electron transfer agent between cytochrome complex that follows photo-system II and the entry point to photo-system I of the non-cyclic electron transfer process.

14.6.4. Cytochrome Oxidase

This enzyme contains copper as metal activator which contributes significant role in electron transport in mitochondria.

14.6.5. Ascorbate Oxidase

It is a Cu-containing enzyme, and a member of oxidoreductase family which acts on diphenols and related substances. It catalyzes the ascorbate and forms dehydroascorbate as follows:

L-ascorbate +O_2 $\rightleftharpoons$ Dehydroascorbate +2H_2O

14.6.6. Phenolase and Laccase

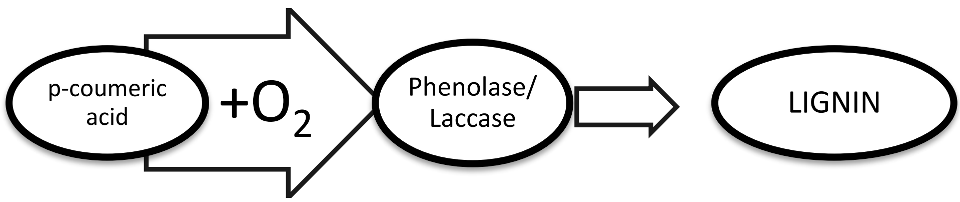
Copper is attached as metal activator with these enzymes, and is involved in the oxygenation reaction of plant phenols. It is also found in thylakoid membrane of chloroplast.

14.6.7. Amine Oxidase

Cu-containing enzyme amine oxidase influences on the breakdown of amine and amino acid to liberate NH_4. Polyamines like putrescine and spermidineare found in legumes during seedling stage, and are used as substrates for the reaction mediated by the above enzyme.

14.6.8. Formation of Lignin in Secondary Cell Wall

Copper has great impact on the biosynthesis of lignin in the secondary cell wall, and its effect is more pronounced in the sclerenchymatous cell of the plant. In vascular bundle, vessel is deformed due to deficiency of Cu. The Cu-containing enzymes namely, phenolase and laccase mediate the biosynthesis of lignin from *p*-coumeric acid, a precursor of lignin.



14.6.9. Copper Influences the Formation of Pollen and Helps Fertilization

Copper has significant influence on microsporogenesis which consequently helps the formation of microgamete (male gamete). High concentration of Cu is found in viable pollen and embryo sac.

14.7. Deficiencies of Copper in Crop Plants

14.7.1. Deficiency of Cu is generally less common than that of other micronutrients. However, deficiency of Cu resulted in the decrease of photosynthesis.

14.7.2. Copper deficiency indirectly affects thylakoid membrane by controlling SOD activity, and enhances Zn uptake consequently expedites biosynthesis of chlorophyll.

14.7.3. Stunted growth, twisted of young leaves, necrosis of the apical meristem are the general characteristic features of Cu-deficiency.

14.7.4. Deficiency of Cu results in bleaching of young leaves and summer die back in trees.

14.7.5. Necrosis of the apical meristem caused by Cu deficiency produces more tillers in cereals and of auxillary shoots in dicotyledons.

14.7.6. Crop plants which are susceptible to Cu-deficiency, are wheat, barley, oat, lettuce, onions, carrot, spinach, and beet.

14.8. Deficiency Symptoms

Copper is found to be evenly distributed in the plant; however, it is relatively immobile. Deficiency symptoms of Cu in plants are primarily observed in young tissues which show chlorosis, distortion, and necrosis (death). Excessive tillering in monocotyledons and branching in the point of dead organ occurs due to the deficiency of Cu. The deficiency of Cu often causes a complete failure of flower setting, and reduced seed and fruit yield (mainly caused due to male sterility).

Figure 14.1. Deficiency symptoms of Cu in tomato leaf.

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Chapter 15

BORON (B)

15.1. GENERAL FEATURES

Boron is a nonmetal essential micronutrient required for growth and development of crop plants. Balanced application of boron can be of great economic importance for crop plants. An exciting development in boron research in the last decade contributed tremendously in understanding of the role of boron in plants. In 1923, Warington first discovered the B deficiency in higher plants. It is now established that continuous supply of B is required for the completion of life cycle and adequate growth and developments (Glass, 1989). Both water-soluble and insoluble form of B is present in the plants. It is assumed that the insoluble B is the functional form of B, on the contrary water soluble is the surplus, and the appearance of B deficiency coincides with the decrease of water soluble B. As a micronutrient, plant requires very small amount of B which varies from species to species. Generally, under optimum condition, B content ranges from 20-100 ppm in leaves,.

In light textured soil, it is more severe as compared to dark soils. Adequate B is required not only for increasing the yield but also for quality of the crop plants.

15.2. BORON CONTAINING FERTILIZERS

Table 15.1. Boron containing fertilizer with their chemical formula and approximate concentration (%)

Name of boron fertilizer	Chemical formula	Concentration (% B)
Boric acid	H_3BO_3	17
Borax	$NaB_4O_7.10H_2O$	11
Sodium tetraborate	$NaB_4O_7.5H_2O$	14
Borate-65	NaB_4O_7	20
Solubor	$NaB_4O_7.5H_2O$ $NaB_{10}O_{10}.10H_2O$	20-21

The most commonly used boron fertilizer is borax, although it can be leached easily in the sandy soil.

15.3. Forms of B in Soil

Boron is found in soil as following forms (Samuel et al., 1993):

i. Soil solution B
ii. Adsorbed B
iii. Organically complexed B
iv. Mineral-B

Figure 15.1. Schematic presentation of B cycle in soil system (modified from Tisdale et al., 1993).

15.4. Application of B-fertilizer for Crop Cultivation

Boron should be applied as basal dose with a narrow range between deficiency and toxicity. The most common methods are the broadcast and banded, in perennial plants it can be used as foliar spray. Under adverse conditions like water stress and severe deficiency it is suggested to apply as foliar spray. The rate of B application depends on the nature of crop plants, texture and organic matter content of soil.

15.5. Uptake of B through Roots

Boron is taken up by the roots through passive absorption from un-dissociated solution of boric acid. It is absorbed as the form of borate ions and it has some sort of antagonistic relation with calcium and potassium. The un-dissociated form of boric acid is membrane permeable (Hening and Brown, 1997). At physiological pH (8) mainly the un-dissociated boric acid is present in soil or nutrient solution. The rate of uptake depends on the concentration of boric acid in soil solution and the transpiration rate of crop plants.

15.6. Physiological and Metabolic Functions of Boron

15.6.1. Mobility of B in Xylem and Phloem

Boron moves in transpiration stream in crop plants through xylem and accumulates in growing points of leaves and stem. Boron is generally considered as phloem immobile; however, phloem mobility has been reported in various *Brassica* crops namely, mustard, cauliflower, radish etc. (Blevins and Lukaszewski, 1998)

15.6.2. Boron in Sugar Transport

Photosynthesis converts light energy into chemical energy as the product of sugar which needs to be transported from source to sink. It increases the rate of transport of sugars as sugar-borate complex to actively growing regions and also in developing fruits. It is also needed for normal root growth and nodule formation in legume crops.

15.6.3. Boron in Flowering and Fruiting

Boron is needed in high amount particularly in reproductive organ than in vegetative growth in most crop plants, as it increases flower initiation and retention, pollen tube germination and elongation and consequently in seed and fruit development. The pollen growth and development requires adequate amount of sugar for energy.

15.6.4. Boron in Phytohormone Regulation

Plant growth and development are regulated by phytohormone namely, auxin, cytokinin, gibberelins. Flower initiation, fruit development, cell wall and tissue formation, and root elongation are all influenced by hormones. Boron plays an important role in regulating these hormone levels in plants.

15.6.5. Boron in Plant Anatomical Features

Boron influences on the cell and tissue structure especially the meristems of shoot and root apices of crop plants. More cambial tissues are produced in B-deficient plants, parenchyma tissues are produced by the expenses of vascular tissue. It is essential for generation and differentiation of new cell. However, high content of boric acid showed the antimitotic activity of cell.

15.6.6. Boron in Cell Wall Formation and Stabilization

A close relationship between B nutrition and formation of primary cell wall is found where B accumulates in the cell wall as boric acid and makes the cell wall development steadily. Up to 90% of the cellular B is localized in the cell wall fraction, and it is helpful in the uptake and movement of Ca which acts in cell wall stabilization. A mechanism for cross linkage of cell wall polymers by the formation of borate esters with hydroxyl groups of cell wall carbohydrates and/or glycoprotein has been proposed by Loomis and Durst (1992).

15.6.7. Boron in Nucleic Acid Metabolism

Boron has a role in nitrogen base synthesis or utilization leading to RNA metabolism (Johnson and Albert, 1967). Deficiency or decrease of B in the plant results in decreases the RNA content, however, the DNA content remains unchanged. This pattern of deficiency symptoms coupled with a decrease in RNA content has been observed by many researchers and it appears that B has an early effect on RNA metabolism.

15.6.8. Boron in Differentiation of Tissue

In crop plants, cells are organized in proper manner for performing particular functions; new cells are differentiated for specific function and organ development. Boron has some significant roles on cell division and differentiation.

15.7. Deficiency Symptoms of Boron in Crop Plants

Boron deficiency in plants is more extensive than any other micronutrients. Determination of the deficiencies of B in crop plants is very complex as the majority of functions are apparently have secondary effects in plant nutrition. Nevertheless, some of the most common deficiency symptoms are presented here.

i. Plants become stunted, meristems are broken down
ii. Terminal leaves become necrotic and shed prematurely
iii. Sterile grains are observed in cereals due to impaired germination of pollen tube
iv. Fruits become deformed namely papaya (Figure 15.1)
v. Boron deficiency is sever in reproductive organ than vegetative parts
vi. Heart rot and crown symptoms are the common deficiency in sugar beet

Figure 15.1. Boron deficiency symptom in papaya showing deformed fruits.

15.8. References

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Chapter 16

MOLYBDENUM (MO)

16.1. GENERAL FEATURES

Molybdenum is a transitional element is an essential micronutrient for plants, animal and most microorganisms (Sigel and Sigel, 2002). Its atomic weight is 95.5 having atomic number of 42. Molybdenum is present in oxide state, and does not occur as free metal in nature. Majority of the Mo-compounds have low solubility in water but molybdate (MoO_4^{2-}) ion is soluble. Molybdenum largely occurs in the soil as an oxycomplex (MoO_4^{2-}). There are 35 known isotope of Mo ranging from ^{83}Mo to ^{117}Mo, the most common one is ^{99}Mo which is radioactive and emits γ ray. The content of Mo in soil ranges from 0.2 to 3.5 ppm and average concentration is 2 ppm.

The requirement of Mo for plants was discovered by Arnon and Stout (1939) using hydroponically grown tomato. Recent progress in Mo research indicates that the role of Mo in plants are essesntial. Molybdenum is taken up by the plants as molybdate anion (MoO_4^{2-}) which requires specific uptake systems to search molybdate in the presence of competing anions. It is present in the solution as MoO_4^{2-}, $HMoO_4^-$ and H_2MoO_4 forms, and plant absorb this element as MoO_4^{2-} ion.

16.2. FACTORS AFFECTING MO AVAILABILITY

The availability of molybdenum for plant growth is highly dependent on the following factors:

- Soil pH, in alkaline soils, Mo becomes more soluble and is accessible to plants mainly in its anion form as $MoO4^{2-}$. In contrast, in acidic soils (pH <5.5) Mo availability decreases as anion adsorption to soil oxides increase (Reddy et al., 1997).
- Concentration of adsorbing oxides (e.g., Fe oxides)
- Extent of water drainage in the field
- Soil organic matter content in the field

16.3. MOLYBDENUM CONTAINING FERTILIZER

Table 16.1. Commonly used Mo-containing fertilizer with their chemical formulae and approximate concentration

Name of Fertilizer	Chemical formula	Concentration of Mo (% Approx.)
Sodium molybdate	$Na_2MoO_4.2H_2O$	39
Ammonium molybdate	$(NH_4)_6MoO_{24}.2H_2O$	54
Molybdenum trioxide	MoO_3	66
Molybdenum fruit	Fritted glass	1-30

16.4. METABOLIC AND PHYSIOLOGICAL FUNCTIONS

Molybdenum is needed by the plants for nutrition of N like NO_3^- and N_2 as Mo is the part of nitrate reductase and nitrogenase enzymes.

16.4.1. Molybdenum Containing Enzymes

Molybdenum itself is not biologically active but is rather predominantly found to be an integral part of an organic protein complex called the molybdenum co-factor (Moco). Moco binds to molybdenum-requiring enzymes (molybdoenzymes) found in most biological systems including plants, animal and prokaryotes (Williams and Frausto da Silva, 2002).

16.4.2. Nitrogenase

This enzyme is present in certain bacteria having nif gene viz. *Rhizobium, Azospirillum, Azotobacter* etc. There are three components to its action like Mo at the active site and Fe-S cluster are involved in transporting electrons needed to reduce nitrogen.

16.4.3. Nitrate Reductase

The enzyme nitrate reductase contains Mo as prosthetic group, and is responsible for the formation of nitrite from nitrate.

Xanthine dehydrogenase, a key enzyme for degradation of purine that oxidizes hypoxanthine to xanthine and xanthine to uric acid by simultaneous release of electrons from the substrate.

16.5. Deficiency Symptoms of Molybdenum in Crop Plants

16.5.1. The major deficiency symptom of Mo is stunted growth and de-greening of leaves of non-legumes. The affected leaves show pale green or yellowish green in color. The legume crops need more Mo to fix N_2 than to utilize NO_3^-. Plants of Brassicaceae and Fabaceae require higher amount of Mo than others.

16.5.2. The deficiency of Mo first appears on the leaves of intermediate. The leaves become chlorotic and the margins roll, and its deficiency occurs in acidic soil condition.

16.5.3. Interveinal and marginal leaf chlorosis occurs followed by death of tissue on the leaf margin.

16.5.4. Patchy distribution of affected plants is the characteristic symptoms of Mo deficiency. The patch may be distributed throughout the whole plant.

16.5.5. Occurrence of whiptail in cauliflower is the characteristic symptom of Mo deficiency.

Figure 16.1. Molybdenum deficiency in tomato leaf showing necrosis in leaf blade.

16.6. References

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Chapter 17

CHLORINE (CL)

17.1. GENERAL FEATURE

Chlorine, a strange mineral micro nutrient element mainly present in plant and soil as Cl^- ion, and is essential for higher plants having numerous physiological functions. Its requirement for crop production is around one gram Cl per kilogram dry mass (Marschner, 1995). It moves readily in the soil and does not form any complex with clay or other materials, and the movement is determined by the water flux (White and Broadley, 2001).

Its atomic number is 17 and the mean atomic weight is 35.453. Its radioactive isotope ^{36}Cl has also been identified as an environmental contaminant which has detrimental effect on human health (Sheppard and Ostedgaard, 1996).

17.2. UPTAKE OF CHLORINE BY THE CROP PLANTS

Chlorine is taken up by the plants as chloride ion (Cl^-) by roots, and is highly mobile in the plant system both in short and long distance transport. It is quickly taken up by plants. The concentration of chloride is about 0.2 to 2%, all these values are much higher than the normal physiological requirement of plants. The transmembrane movement of Cl^- is mediated by up-hill process as well as by transport protein (White and Broadley, 2001). Chlorine is highly mobile in the plant system, and the distribution pattern reflects its higher concentration in leaf than in other parts of the plant (Chen et al., 2010).

Table 17.1. List of chlorine containing fertilizer with their chemical formulae and approximate concentration (%)

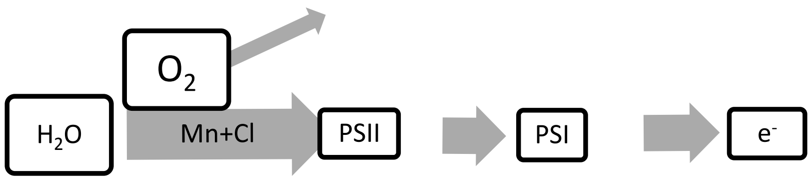
Name of Fertilizer	Chemical Formula	Concentration (% Appox.)
Potassium chloride	KCl	47
Ammonium chloride	NH_4Cl	66
Magnesium chloride	$MgCl_2$	74
Sodium chloride	$NaCl$	60

17.3. Application of Fertilizer for Supplying Chlorine

Around 4-5 kg chlorine is required per hectare of land of average crop production. Chloride containing fertilizers are applied during final land preparation.

17.4. Metabolic and Physiological Functions of Chlorine

Chlorine is involved in the photosynthesis process in Photosytem II for the splitting of H_2O in the Hill reaction. It is recognized as a cofactor of the Manganese-containing O_2-evoving complex.



i. Photosynthesis and stomatal movement are mediated by chlorine, it is an active osmoticum present in the vacuole, and involve in turgor and osmoregulation.
ii. Chlorine improves the yield and quality of plants such as onion, cotton etc. especially in deficient soils.
    a. Cl is required in Hill reaction of photosynthesis process for splitting the H_2O and evolving of O_2.
    b. Chloride acts as cofactor of the Mn-containing O_2-evolving system in the photosynthesis.
    c. Chloride can affect photosynthesis and plant growth indirectly via stomatal regulation i.e., closing and opening of stomata.
    d. Stomatal closer is correlated with corresponding efflux of accompanying anion (Cl^-) in the guard cell.
iii. It helps in activation of membrane bound proton pumping ATP-ase enzyme.
    a. Chloride stimulated H^+-ATP-ase is probably of particular importance.
iv. Other effects
    b. A specific role of chloride in N metabolism is indicated by its stimulating effect on asparagines synthetase especially for the plant species where asparagines are the major compound in the long-distance transport of soluble N.
    c. Chloride is one of the major osmotically active solutes in the vacuoles and thus affects the turgor potentiality of cell and maintains the ionic balance.
    d. When excessive in the plant body it acts as salinity stress and creates toxicity to the plant.

17.5. Deficiency Symptom of Chlorine in Crop Plants

Chlorine deficiency is generally not observed under field condition. However, crop growth is reduced substantially by the deficiency of chlorine (Broyer et al., 1954; Xu et al., 2000). Under deficient condition, reduced leaf growth and wilting, followed by chlorosis, bronzing and, finally, necrosis are observed in different crops. Root growth is inhibited and becomes stunted. Wilting of leaf margin is a typical symptom caused due to transpiration loss of water in crop plants.

Figure 17.1. Deficiency of Cl in tomato leaf showing little leaf and burning of leaf margin.

17.6. References

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Chapter 18

BENEFICIAL ELEMENTS

18.1. GENERAL FEATURES

Plants absorb around 60 elements, among them only 16 are essential for growth and development process. Some mineral elements are not essential but beneficial to plants. Mineral elements which either stimulate growth but are not essential or essential only for certain plant species, or under given conditions, are usually defined as beneficial elements. These beneficial elements perform the following functions:

- Enhance plant growth and development
- Essential for certain plant species but not for all plant species
- Essential at certain condition only

Table 18.1. List of important beneficial elements with their molecular weight and available form of uptake

Name of Element	Molecular weight	Available form
Sodium (Na)	23	Na^{+}
Silicon (Si)	28	SiO_2^{2-}
Aluminium (Al)	27	Al^{3+}
Cobalt (Co)	59	Co^{2+}
Nicle (Ni)	59	Ni^{2+}

18.2. SODIUM (NA)

It is a mono-valent mineral element which is abundant in the earth crust, and around 2.8% is present in the earth. Its atomic number and weight are of 11 and 23, respectively. The hydrated Na ion has a radius of 0.358 ηm on the other hand K has a radius of 0.331 ηm.

Some of the plants have affinity for Na than other element, plant species are characterized as natrophilic and natrophobic depending upon their growth response in sodium. The role of Na on plant nutrition is to be considered for its essentiality in certain metabolic functions as well as the replacement of the function of K. Still now Na is not considered as the essential nutrient element (except of some C_4 plant species). Ions of sodium and potassium are chemically and structurally similar ions and they can compete each other. Certain plants absorb Na luxuriantly which is beneficial for phytoremediation of saline soils.

18.2.1. Essentiality

Sodium has been considered to be essential for certain C_4 plant species namely *Atriplex vesicaria, A. tricolor, Kochia childsii, Panicum miliaceum* and *Distichlis spicata* (Subbarao et al., 2003; Marschner, 1995; Pessarakli et al., 2001). Those plants show deficiency symptoms such as chlorosis and necrosis, or failed to form flowers in absence of Na, and overcome the said deficiencies by the application of Na.

18.2.2. Growth Stimulation

The application of Na in the growth medium has been shown to stimulate the growth of many species, including asparagus, barley, broccoli, brussels sprout, caraway, carrot, chicory, cotton, flax, millet, oat, pea, rutabaga, tomato, vetch, wheat, cabbage, celeriac, horseradish, kale, ohlrabi, mustard, radish, rape, celery, mangel, sugar beet, red beet, swiss chard, and turnip (Subbarao et al., 2003)**.** Visual leaf symptoms have been shown in halophytes, where Na is clearly beneficial if not essential, it is also required only at the micro amount level (Flowers et al., 1977). Based on the requirement and substitution the function of K, plants can be classified into following types :

a. Type A- a higher amount of K can be replaced by the application of Na, and stimulate the growth, which is not achievable by the application of K.
b. Type B-specific growth responses to Na are observed but they are not much significant and visible.
c. Type C-only minor replacement of K is possible and Na has no effect on the growth and development on plants.
d. Type D- No replacement of K is found and no beneficial effects are observed especially the function of K.
    i. Sodium has significant effects on the stimulation of growth through increasing leaf area, stomatal regulation and, maintaining the water balance.
    ii. It functions in metabolic process in C_4 plants especially in the C_4 cycle through stimulation of PEP-carboxylase activity.
    iii. Sodium can replaces the functions of K in the following ways
        a. It acts as internal osmoticum under normal and stressfull condition
        b. It influences positively on the function of stomatal regulation
        c. It increases the photosynthetic activity
        d. It counteractions the long distance transport of nutrients
        e. It influences on the enzyme activation
    iv. Na improves the crop quality e.g., improve the taste of carrots by increasing sucrose in the fruit crops.

Thus, Na has potential benefit of acting as a growth retardant for floricultural crops in which height control is an important quality issue.

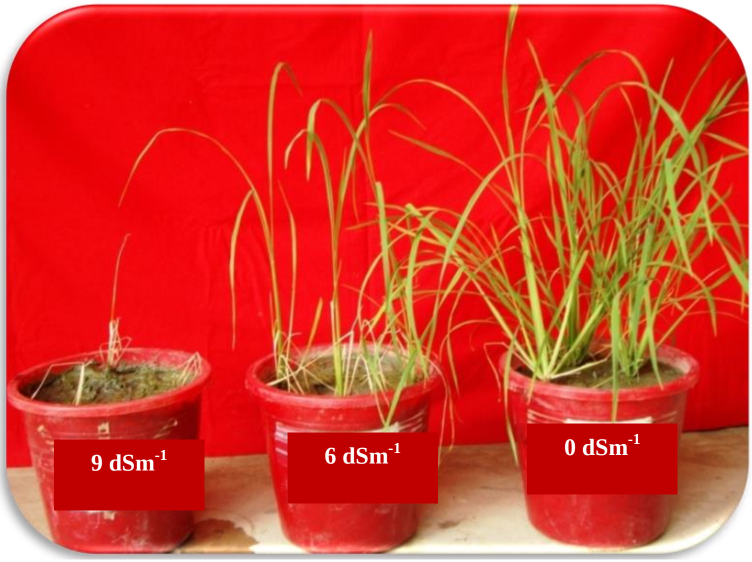


Figure 18.1. Sodium toxicity in rice plants showing total damage of the plants under 9 dSm^{-1} saline conditions and reduced plant growth under $6dSm^{-1}$.

18.3. SILICON (SI)

Silicon is the second most abundant element in the earth's crust. There is a wide variation in capacity to take up silicon in higher plants. Plants of Poaceae family absorb greater amount of Si than plants of other families. Depending on their content of SiO_2they can be divided into three major types:

i. Type A- wetland Poaceae like wetland rice, horse tail uptake greatest amount of Si (10-15%)
ii. Type B- dry land Poaceae namely sugar cane, most of the cereal species and few dicotyledons species absorb less amount (1-3%) of Si than type A
iii. Type C- most of dicotyledons especially, legumes absorb minimum amount of Si (<0.5%).

Transport of Si through Vascular Bundle

Silicon is confined in the xylem incase of long distance transport. Its distribution within the shoot organ is therefore determined by transpiration rate in the organs.

Beneficial Effects

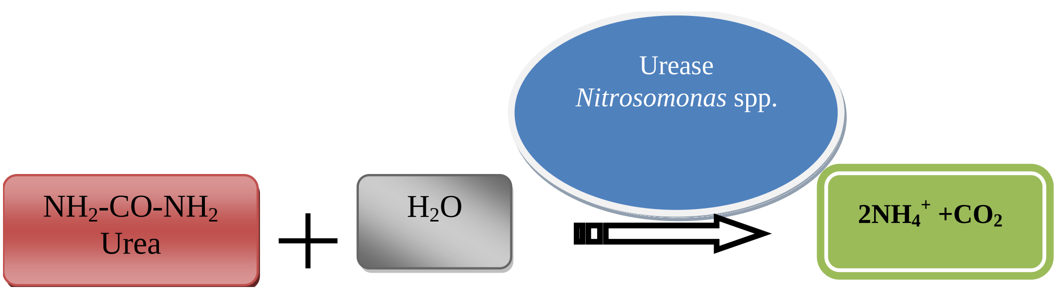
Silicon can stimulate growth and yield by several indirect actions including decreasing mutual shading by improving leaf erectness, decreasing susceptibility to lodging, preventing Mn and Fe toxicity. The epidermal cell walls impregnated with a film layer of silicon act as effective barrier against water loss and, cuticular transpiration rate in the organs. Silicon influences on the lignifications process of the cell wall which increases the capacity of plants to resist lodging.

18.4. Cobalt (Co)

Cobalt is essential for N_2-fixing plants having bacteria in symbiosis or associative process. It is required for nitrogenase enzyme in bacteria but not for the host plants. Similarly it is required in free-living and associative N_2-fixing bacteria including Cyanobacteria. Cobalt is present in the coenzyme cobalamine (vitamin B-12) as a metal component in the center as like as hemin. In *Rhizobium* three cobalamin dependent enzymes are present. Protein synthesis of *Rhizobium* is impaired due to Co deficiency.

18.5. Nickel (Ni)

Nickel is a mineral element having atomic weight of 59. Relatively little is known about the role of Ni in plant nutrition. However, recently it has been found as beneficial element (Dalton et al., 1988), and the requirement of Ni for plants is extremely low (about 1-100 ŋg g^{-1} dry weight) and it ranges from 0.05 -5 mg kg^{-1} (Bai et al., 2006). It is taken up by the plant as Ni^{2+}, and acts as metal component of certain enzymes viz. urease, hydrogenase. The enzyme hydrolyze the urea into $NH4^+$, and the enzyme is found in the microbes namely, *Nitosomonas* spp., Gram negative rod-shaped prokaryote. The enzyme urease contains two Ni ions in the active site (Ciurli, 2001).



Nickel also influences on the mobilization of N from seed to seedling during germination. It is suggested that both carbon respiration and nitrogen metabolism are sensitive to Ni nutrition. In ureide transporting plants especially in the legumes, urea is produced which does not breakdown and may create toxic effect on the plant. Ureides are produced in root nodule and are transported to leaves via xylem. They are also produced in senescing leaves and transported out to the developing seeds for storage (Hopkins, 1995). Nickel is present in hydrogenase enzyme which is involved in nitrogen fixation process. A deficiency of Ni decreases the activity of this enzyme and subsequently reduces the BNF process.

18.6. Aluminium (Al)

Aluminium is another beneficial metal element in plant nutrition with the atomic weight 27, and has been found as beneficial for plant nutrition. It is the third most abundant element and metal in the earth. Aluminium has following beneficial effects on crop plants:

i. Tea is very tolerant to Al toxicity and the growth is stimulated by Al application possibly due to prevention of Cu, Mn and P toxicity effects (Marschner, 1995).
ii. There have been reports that Al may serve as fungicide against certain types of root rot.
iii. It has been shown that application of Al exerts an enhancement of dry matter production and nutrient accumulation in rice under hydroponic condition using lower concentration of Al (Hai et al., 1998).

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Chapter 19

BIOFERTILIZER

19.1. GENERAL FEATURES

Recently, the use of microbial inocula for increasing growth and yield of diversified crop plants is getting popular. In addition to increased yield its application improves the environment as well, and ensures the sustainable agriculture system. Various types of biofertilizer and their formulations are available in agricultural field. Biofertilizers, the microbial inoculants can increase growth and yield of inoculated plants through the following processes:

- By fixing atmospheric N_2
- Increasing phytohormone production and thereby root growth
- Enhancing uptake of certain unavailable nutrient elements namely P, K, Zn, Fe etc.
- Improving uptake capacity of water
- Acting as biocontrol agent against diseases and pests

These microbial inocula are mainly prokaryotic bacteria (Sahoo et al., 2013), mycorhizal fungi, cyanobacteria (previously known as blue green algae) and *Azolla-Anabaena* are the most common biofertilizers.

The most important mechanism is the biological nitrogen fixation (BNF), the process of conversion of atmospheric nitrogen (N_2) to available-N (NH_4^+). It is estimated that total world BNF is much higher than industrial fixation, and is assumed that total annual BNF ranges from 200 to 250 mMt per year (Marschner, 2011). Eight families of Cyanobacteria and 11 families of bacteria are capable for the BNF process.

Important Characteristics of Biofertilizers

- It is eco-friendly and cost effective fertilizer which can increase crop yield by 20-30%
- Increase soil fertility by adding N by fixing atmospheric N_2
- Enhance uptake of other nutrients like P, K, Ca, and Fe despite of N

- Secrete or produce plant growth hormones such as auxin, gibberelin
- Act as crop enhancer which stimulate the plant growth
- Enhance water uptake and decrease drought susceptibility
- Act against diseases as biocontrol agent

19.2. Types of Biofertilizer

A. *Rhizobium/Bradyrhizobium*
B. Plant growth promoting rhizobacterial biofertilizer
C. Phosphorus solubilizing biofertilizer
D. Potassium releasing biofertilizer
E. Siderphore or iron uptake enhancing biofertilizer
F. Cyanobacterial biofertilizer
G. *Azolla-Anabaena*

19.2.1. *Rhizobia*

The symbiosis between the genus *Rhizobium* and the host of nodulated legumes results in the fixation of atmospheric nitrogen (N_2) and convert into ammonium. *Rhizobium* belongs to the family Rhizobiaceae, and it falls into two groups based on their growth characteristics:

Group	*Rhizobium*	Host plant
Group I	*Rhizobium leguminosarum*	Nodulates peas, lentil, vetch
	R. phaseolii	Nodulates beans
	R trifolii	Nodulates clovers
	R. meliloti	Nodulates alfalfa and other *Medicago* spp.
Group II	*R. lupini*	Nodulates lupin
	R. japonicum	Nodulates soybean
	Rhizobium spp.	Nodulates member of cowpea miscellany group of legumes.

Symbiosis process is the association between two organisms for mutual benefit. In the association the plant is designated as host and the rhizobial microbial partner is known as microsymbiont. The most common symbioisis occurs in nodule of legume plant roots. Nodules are also found in the stem of sesbania (*Sesbania* spp.)

Formation of Root Nodule

Roots of Fabaceous plants contain swelling gall like structure where *Rhizobium* bacteria reside, and the swelling organ is called root nodule. Roots secrete flavonoid which activates the production of nod factors by bacteria. A series of biochemical and morphological changes happen when the nod factor is sensed by the bacteria. The infection of roots by *Rhizobium* activates cell division in the cortex of the root and form nodule.

Fixation of N_2

The major N_2 fixation through BNF process is the *Rhizobium*-legume symbiosis which plays a significant role in increasing soil fertility. The *Rhizobium*-legume symbiosis is the most efficient association and has drawn attention in agricultural productivity. It is generally assumed that symbiotic N_2 fixation constitute more N to the soil pool than other systems viz. associative free nitrogen fixation system. The *Rhizobium* contains the enzyme dinitrogenase and catalize the N_2 to ammonium. The bacteroid, the N_2-fixing cell is surrounded by peribacteroid. Nitrogen fixation occurs inside the bacteroid cell by the enzyme dinitrogenase which is very much sensitive to oxygen supply. Leghemoglobin a protein found in the legumes act as O_2-carrier for limited supply of oxygen. The BNF is an energy-costly process where 16 ATP is required to reduce one molecule of N_2 into ammonia.

19.3. Plant Growth Promoting Rhizobacteria (PGPR)

Bacteria which perform beneficial effects and increase growth and yield of crop plants are called plant growth promoting rhizobacteria (PGPR) (Adesemoye et al., 2009; Kloepper et al., 1980; Singh and Singh, 2013; Shamsuddin et al., 1998). They are the root bacteria i.e., rhizobacteria which stimulate the growth of diversified crop plants upon inoculation. Nowadays, application of PGPR for increasing growth, yield and quality of crop are becoming popular. The following microbes are considered as best candidate of PGPR:

- Symbiotic- Rhizobium, Bradyrhizobium etc.
- Associative- Alcaligenes, Azospirillum, Bacillus, Enterobacter, Herbarspirillum, Klebsiella, Pseudomonas and Rhizobium
- Endophytic- Azospirillum, Bacillus, Herbaspirillum etc.
- Free living e.g., Azotobacter, Beijerinckia

19.4. Plant Growth Promoting Endophytic Bacteria

Nowadays application of endophytic plant growth promoting microbes for increasing crop yield is gaining prominence. These types of bacteria are found in the internal parts of the plant i.e., in the apoplastic region of cortex or stele without doing any harm to the host plat. They perform beneficial effects in various ways namely, growth promotion, enhancement of disease resistances capacity and are known as endophytic beneficial plant bacteria (Tyler and Triplett, 2008). Based on their mode of action endophytic bacteria may be categorized into three classes:

i. Plant growth stimulating bacteria which produce growth hormone.
ii. Neutral in plant interaction i.e., not producing any growth enhancer or retardant.
iii. Plant pathogenic i.e., harmful to the host plants where the bacteria produce toxic substances.

Previously it was assumed that these bacteria may be harmful to the host plant by causing diseases through pathogenic activity (Tyler and Triplett, 2008). But nowadays it is proved that these bacteria are beneficial to the host plant in various ways. They act as biofertilizer by adding N through BNF process, perform as biocontrol agent by suppressing pathogenic microbes, and lack of these endophytes in plant body may cause susceptibility to diseases.

Table 19.1. List of some associative and endophytic growth promoting bacteria stimulating growth and increase yield of various crop plants (modified from Mia et al., 2013)

Bacterial species	Type	Test crop	References
Azospirillum amazonense	Associative	Banana	(Magalhaes et al. (1983)
A. brasilense	Associative/ endophytic	Rice, wheat, maize, oil pulm, banana etc.	Tarrand et al. (1978), Mia et al. (2007)
A. lipoferum	Associative	Banana	(Reinhold et al., 1987)
Bacillus spp.	Associative and endophytic	Rice and banana	Rao et al. (1998)
Bacillus sphaericus	Associative and endophytic	Rice, wheat, oil pulm, banana etc.	Mia et al. (2007), Mia et al.(2013)
Herbaspirillum seropedicae	Associative	Sugarcane and banana	Weber et al. (1999)
Burkholderia spp.	Associative	Banana and rice	Weber et al. (1999) Naher et al. (2009) Doty (2011)

Azospirillum

Azospirillum, a Gram-negative diazotropohic bacteria, fixes atmospheric N_2 in association with roots of grasses and cereals (Tarrand et al., 1978). It is the most extensively studied plant growth promoting bacteria are found both in externally and internally in plant roots. The following species of *Azospirillum* so far have been reported as beneficial: *Azospirillum brasilense*, *A. lipoferum* (Tarrand et al., 1978), *A. amazonense* (Magalhaes et al., 1983), *A. halopraeferens* (Reinhold et al., 1987), *A. irakense* (Khamas et al., 1989) and *A. doebereinerae* (Eckert et al., 2001). They are versatile bacteria, which fix atmospheric N_2 under microaerobic condition. Application of *Azospirillum* inoculation has shown significant beneficial effects on rice, wheat, maize, grasses and fruit crops like banana, coconut, oil palm etc.(Amir et al., 2001; Bashan and Levanony, 1990; Mia et al., 2010a,b).

Bacillus

Bacillus is rod shaped, motile, endospore forming facultative Gram-positive bacterium. Bacteria of this group also have been found to fix atmospheric N_2 in association with roots of different crop plants. They have also been found in colonizing in roots along with VAM fungi and do beneficial effects (Bellone et al., 1997). Different species of *Bacillus* namely *B. licheniformis, B. macerans, B. polymyxa B. azotofixans* exert beneficial effects through BNF, stimulating root hair formation, and enhance nutrient uptake. *Bacillus* spp. inoculation can fix N_2 in association with different non-legumes which results 15- 40 % yield improvement compared to un-inoculated control (Wei 1997, Mia et al., 2010b).

Azotobacter

They are free living N_2-fixing bacteria belong to the family Azotobacteriaceae, exert beneficial effects on the inoculated plants. The following species of *Azotobacter* namely *A. chroococcum, A. agilis, A. vinelandii, A. beijernickia, A. insignis, A. macrocytogenes, A. paspili* have been found as N_2-fixer and beneficial to plants. Among the species, *A. chroococcum* is predominantly found in arable soils and capable of fixing N_2. There are some limitations of using this biofertilizer as bacteria are not symbiotic or associative and their beneficial interactions with host plants are not obligatory.

19.5. CYANOBACTERIA

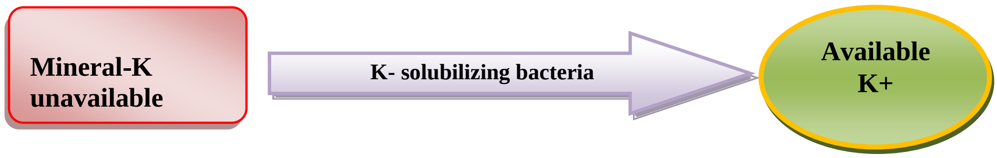
Earlier Cyanobacteria was known as blue green algae (BGA), used as a source of N-fertilizer for different crop plants especially in wetland rice cultivation. They can also be used as composite fertilizer with *Nostoc, Anabaena, Aulosira.* Previously, this type of biofertilizer was very much popular earlier for increasing the yield of rice crop, and in ideal condition it could add 20-30 kg N ha^{-1} in rice field. However, production and application of such type of fertilizer is very much laborious and difficult to keep free from contamination.

19.6. PHOSPHATE SOLUBILIZING BACTERIA (PSB)

The PSB are root associated bacteria and contribute significant role in P nutrition of plants. These types of bacteria are able to hydrolyze the unavailable organic and inorganic P and convert them into available form for the absorption of plants (Alagawadi and Gaur, 1992; Islam and Hossain, 2012a). The bacteria release low molecular weight organic acid which dissolve the rock phosphate or fixed phosphate consequently make P in the available form for absorption by the roots (Khan et al., 2006; Ahmed and Khan, 2010). Additionally, some of the PSBs produce phosphatase enzyme such as phytase hydrolyses the organic phosphate to release available P (Afzal and Bano, 2008; Ahmed and Khan, 2010; Khan et al., 2006).

19.7. POTASSIUM SOLUBILIZING BACTERIA (KSB)

They are Gram positive heterotrophic bacteria; obtain their carbon for energy from organic matter. The common KSBs are *Bacillus mucilaginous* and *B. edaphicus*. The use of K-solubilizing biofertilizer has significant impact on the enhancement of soil fertility by making K available in deficient soil. The bacteria make K available by enzymatic reaction process. They secrete organic acids which solubilize potassium rock and the K^+ for plant uptake (Han and Lee, 2005). Since KSBs are aerobic bacteria they play an important role in maintaining soil structure through various biochemical processes namely formation of water-stable soil aggregates and can act as biocontrol agent by producing bioenhancer that stimulate plant growth and inhibit root pathogen through colonizing roots earlier than pathogenic microorganisms (Han et al., 2006).



19.8. MYCORRHIZAL FUNGI

Mycorrhiza means root fungi, is effective biofertilizer and crop enhancer for both legumes and non-legumes. There are two types of mycorrhizae namely ectomycorrhiza and endomycorrhiza commonly found in nature which have beneficial effects on crop plants.

Ectomycorrhiza are found on the external area of roots, and they do not penetrate into the internal part of the roots. The fungal hyphae increase the root surface area which have influences on the absorption of nutrients especially phosphorus. Its infection process resulted in physical, chemical and biological alteration in the colonized roots. These phenomena improve the general condition for alleviating biotic and abiotic stresses (Barea et al., 2004). Ectomycorrhyzal fungi can be differentiated from other fungi through the production of hyphal sheath known as mantle.

19.8.1. Endomycorrhyzae

Endomycorrhyzae penetrate inside the epidermis and are classified as arbuscular, ericoid, arbutoid, monotropoid and orchid mycorrhyza. The arbuscular mycorrhyza was formerly known as vesicular-arbuscular mycorrhiza (VAM). The arbuscules are balloon like hyphae which penetrate into the plant cells and are dichotomously branched. Arbuscular structure greatly increases the contact surface area between the hypha and the cell of host plant thereby facilitates the transfer of nutrients.

19.8.2. Benefits of Mycorrhizal Fungi

- Increases the longevity of roots by spreading mycelia for absorption of nutrients.
- Plays a significant role for absorbing some selective ions namely P.
- Arboscular fungi inoculation increases the uptake of water.
- Transplanting shock of seedlings can be minimized by mycorrhizal inoculation process.
- Increases the disease resistance of host plant through biocontrol activity.

19.9. *AZOLLA*

Azola is a floating water fern which can fix atmospheric N_2 in association with Cyanobacteria *Anabaena-Azollae*. This biofertilizer is widely used in rice growing areas in South East Asia. It is gaining eminence for supplementing N-fertilizer for different crop plants. Around 40-60 kg N ha^{-1} could be incorporated in rice field under wetland condition by the application of *Azolla*.

19.10. MODE OF BENEFICIAL EFFECTS OF BIOFERTILIZERS

Biofertilizers exert following beneficial effects on crop plants:

19.10.1. Plant Growth and Development

Biofertilizers, increase root growth especially the root hair and lateral roots and subsequently increase the nutrient and water uptake capacity (Ahmed and Khan, 2011; Mia et al., 2009; Shamsuddin et al., 1999). The possible mechanisms of enhanced root growth could be due to the production of phytohormone as PGPR increase the synthesis of IAA via tryptophan (Wani et al., 2008**;** Ahmad and Khan ., 2011; Maziah et al., 2010**)**. Inoculation process has also been shown to increases cell division and cell size. *Azospirillum* (Sp7) has the potential to synthesize plant hormone which can replace indole acetic acid (IAA) to stimulate root growth (Molla et al., 2001).

19.10.2. Nitrogen Fixation and Utilization

Nitrogen fixation is the main mechanism of beneficial activity of biofertilizer to support improved plant growth following inoculation with PGPR (Podile and Kishore, 2007; Mia et al., 2013). This was mainly because of an increase in N compound and nitrogenase enzyme activity in inoculated plants (Bashan and Holguin, 1997). Diazotrophic plant-associated bacteria can fix substantial amounts of N_2 in non-legumes like banana, rice, sugarcane, wheat, maize etc. (Mia et al., 2007; James, 2000; Chalk, 1991). Inoculation with *Azospirillum brasilense* strain Sp7 and *Bacillus sphaericus* strain UPMB10 can fix around 38% nitrogen of their requirements in association with banana seedlings (Mia et al., 2007; 2010a). Research using N-balance, ^{15}N isotope dilution and ^{15}N natural abundance studies has provided strong evidences of those findings. The BNF occurs when atmospheric dinitrogen (N_2) is converted to ammonia mediated by an enzyme dinitrogenase, and the overall reaction is given below:

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,Pi$$

The reaction is energy consuming process where 16 ATP is required and is accompanied by the formation of H_2.

19.10.3. Dinitrogenase Enzyme

The di-nitrogenase enzyme complex consists of two proteins viz. Fe-protein and Fe-Mo-Protein. The first protein (Fe-protein) is reduced by electrons received by ferredoxin and then the reduced Fe-protein binds and reduces the Fe-Mo-protein which finally donates electrons to N_2 for the production of HN=NH. The process of cycles further converted into $2NH_3$. The dinitrogenase enzyme is very much sensitive to oxygen which is controlled by leghemoglobin.

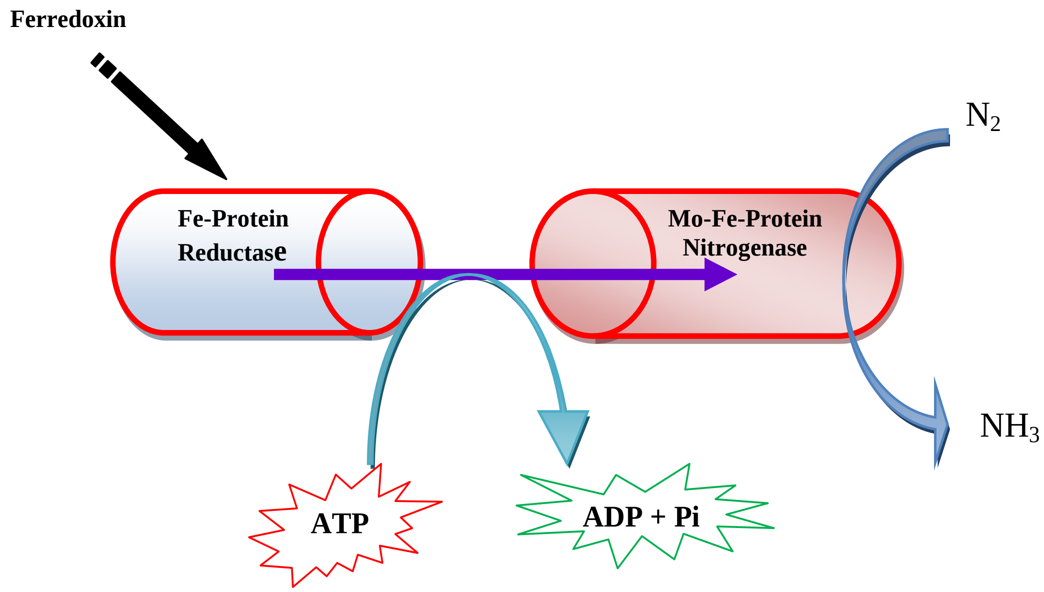


Figure 19.1. Schematic diagram of reduction process of N_2 to NH_3.

19.11. Nutrient Uptake

Application of biofertilizer results in the enhancement of mineral uptake in inoculated plant, and the major nutrient elements are N (nitrate and ammonium) P and K in rice, wheat, sorghum corn and bananas (Bashan et al., 1989; 1990, 2000; Mia et al., 1998,2010a, 2013; Bashan and Holguin 1997; Kucey, 1988; Murty and Ladha, 1988) (Table 19.2).

Table 19.2. The N, P, K, Ca and Mg concentrations in root of banana plantlets inoculated with PGPR strains Sp7 and UPMB10 grown for 45 days (modified from Mia et al., 2009)

Treatments	N	P	K
	Concentration in (%) dry matter		
N_0-PGPR	1.67 b	1.09 ab	4.08 b
N_0+Sp7	1.63 b	1.14 a	4.72 b
N_0+UPMB10	2.28 a	0.98 b	5.27 a

Following are the mechanisms of enhanced nutrient uptake:

i) Increased root growth especially the root hairs by phytohormone production, the presence of root hairs to increase the surface area of a root system which aid in ion uptake by roots,
ii) Inoculation improves the efficiency of applied mineral nutrients by helping the plant scavenge limiting nutrients, a role similar to that of mycorrhiza in the phosphate recovery.

19.12. PHYSIOLOGICAL PROPERTIES

Plant growth promoting rhizobacterial inoculation increases the physiological properties of host plants namely, photosynthetic activity, stomatal conductance and decrease proline accumulation (Bashan et al., 1992; Mia et al., 2000). *Azospirillum brasilense* inoculated sorghum plants improve leaf water potential under field condition (Fallik et al., 1994). The water regime of sorghum plants may be improved by inoculation via their higher leaf water potential, lower canopy temperature, and greater stomatal conductance and transpiration (Sarig et al., 1988,1990).

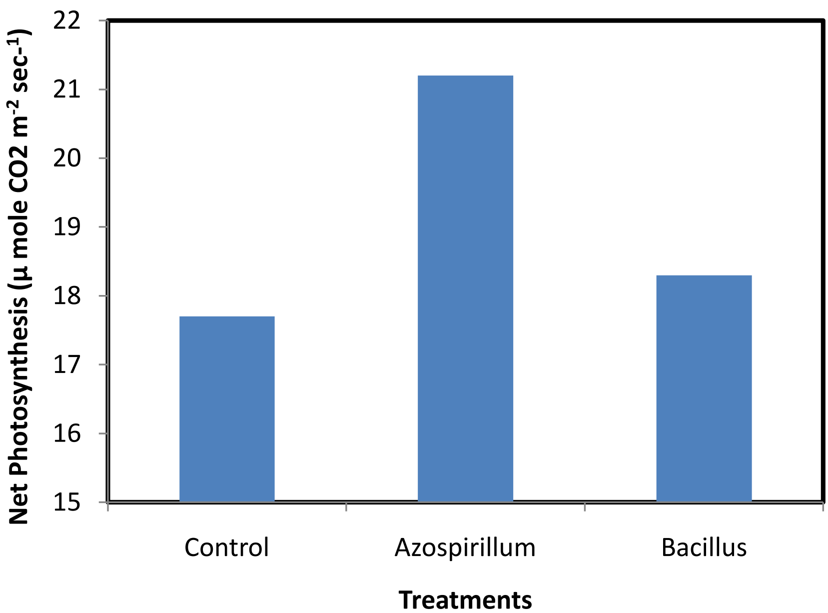


Figure 19.2. Effect of *Azospirillum brasilense* strain Sp7 and *Bacillus sphaericus* strain UPMB10 inoculation on net photosynthesis of bananas (modified from Mia et al., 2010a).

Photosynthetic capacity of plants inoculated by diazotrophic bacteria is higher compared to that of fertilizer-N applied plants. The possible physiological reasons are that the inoculated plants require more photosynthate to meet the higher demand by diazotrophs during the N_2-fixing process (Quilici and Medina, 1998; De Veau,1990).

19.13. BIOCONTROL AGENT

Control of plant diseases by means of beneficial microorganisms is said to be biological control. It involves the use of organisms, their products, and genes to control pathogenic organisms and favor desirable organisms, such as crops, trees, beneficial organisms and insects. The microorganism that suppresses the disease or pathogen is referred to as the biological control agent (BCA). Plant growth promoting bacteria can do beneficial effects in bananas and other cereal crops in controlling soil and seed borne diseases in bananas (Yuan et al., 2012).

The possible mechanisms are early root colonization by beneficial bacteria which make barrier for pathogenic microorganism's invasion (Bais et al., 2006), secondly antibiosis with biosurfactant activity and finally development of induced systemic resistance in the host plants (Islam and Hossain, 2012b). In bananas, *Fusarium* wilt can be controlled by PGPR inoculation has been documented by various researchers. *Bacillus sphaericus* inoculation increased the fungal diseases resistance in bananas. The onset of *Fusarium* wilt diseases symptoms was delayed due to *Bacillus sphaericus* UPMB10 and Strain *Bacillus amyloliquefaciens* NJN-6 inoculation (Yuan et al., 2012).

19.14. REFERENCES

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Chapter 20

ROLE OF PLANT NUTRIENTS ON PEST AND DISEASES

20.1. GENERAL FEATURES

Plant nutrients are very vital for growth and development of plants as well as pathogens which are important for causing and controlling diseases (Agrios, 2005). They have profound effects on control and management of insects and diseases. Plant nutrients have diversified effect on the morphology, anatomy, cytological and biochemical attributes of crop plants. Altered biochemical and anatomical characters have influence on the prevalence of pests infestation. Alteration of chemical compounds in the plant tissue may also increase or decrease the pest infestation.

20.2. ROLE OF NITROGEN ON DISEASES AND PEST INFESTATION

Nitrogen has great impact on plant growth, development and diseases infestations. Increased application of N-fertilizer enhances the pest infestation. Nitrogen influences on cell division, cell enlargement and decreases lignification. Cell wall becomes thin due to N application, which consequently increases the chance of pathogen invasion into apoplastic and symplastic region. Concentration of amino acids, amides and other organic acids are higher due to increased application of N. These phenomena positively influences on the germination and multiplication of microbial spore of pathogens. However, the influence of N on diseases infestation depends on the form and level of N-fertilizer application.

The effect of N-fertilizer application on diseases is quite inconsistent depending on the type of pathogens. For example, under high N condition, the infestation of *Puccinia graminis* and *Erysiphe graminis* are increased; on the other hand, infestation of *Alternaria*, *Fusarium* and *Xanthomonas* spp. is decreased. Table 20.1 shows the infestation of different diseases under high and low N-fertility levels.

The phenolic content of nitrogen deficient plant is high and both the levels of these substances and their fungistatic effects decrease when the supply of N increases.

Table 20.1. Effect of high and low levels N-fertilizer on the disease infestation of crop plants

Disease	Pathogen	High N	Low N	References
Powdery mildew	*Erysiphe graminis*	Increase	Decrease	Büschbell and Hoffmann (1992)
Powdery mildew of tomato	*Oidium lycopersicum*	Increase	Decrease	Hoffland et al. (2000)
Canker of wood	*Pseudomonas syringae*	Increase	Decrease	Hoffland et al. (2000)
Bacterial leaf spot	*Xanthomonas vesicatoria*	Decrease	Increase	Chase (1989)
Early blight of potato	*Alternaria solani*	Decrease	Increase	Blachinski et al. (1996)

20.3. Effect of K on Infestation and Control of Diseases

Potassium has significant effect on the disease infestation and especially the pest attack when higher level of K-fertilizer is applied. Resistant power of host plant is increased by the application of K. The high susceptibility of K-deficient plant is related to the metabolic activity of K where low molecular weight organic compounds are produced despite of higher molecular weight compound (starch, protein etc.). Balance nutrition of K enhances the lignifications of secondary cell wall resulting a defense mechanism against invasion of microbes. Balance between N and K influences the disease infestation. It has been reported that application of K fertilizer can reduce the intensity of several infectious diseases as shown in Table 20.2.

Table 20.2. Effect of high and low level of K-fertilizer on disease infestation of crop plants

Disease	Pathogen	High K	Low K	References
Rust of cereal	*Puccinia graminae*	Decrease	Increase	Lam and Lewis (1982)
Brown spot of rice	*Xanthomonas oryzae*	Decrease	Increase	Chase (1989)
TMV	Virus	Decrease	Increase	Ohashi and Matsuoka (1987)
Root-rot of field peas	*Fusarium oxysporum*	Decrease	Increase	Srihuttanum and Sivasithamparam (1991)
Early blight of potato	*Alternaria solani*	Decrease	Increase	Blachinski et al. (1996)

20.4. ROLE OF CALCIUM (CA) ON DISEASE MANAGEMENT

Calcium is another mineral nutrient which influences on the infestation of diseases. Calcium-deficient plants reduce the resistant capacity of host plants. It plays significant role on the maintenance of stability of cell membrane and cell wall. Low molecular weight compounds like amino acid, organic acids, amides are drained out from the cytosol to apoplastic region when the cell membrane is disintegrated due to lack of Ca. This phenomenon influences positively in the invasion and colonization of pathogenic microorganism in apoplastic region. Calcium plays significant role on the binding of cell wall acting as cementing material forming polygalacturonates compound which help in preventing the invasion of microorganism. When Ca concentration in plant tissue decrease, there is an increment of susceptibility to fungal diseases which preferentially invades the xylem and dissolve the cell walls of the conducting vessels leading to wilting symptoms. In addition, plant tissues low in Ca is also much more susceptible than tissues with normal Ca levels to parasitic diseases during storage. Therefore, application of Ca before storage is very much effective for storage vegetables as well as fruits.

20.5. ROLE OF SILICON (SI) ON DISEASES MANAGEMENT

Application of Si significantly influences on the control of a number of diseases namely blast (*Magnaporthe grisea*) in Augustine grass, brown spot (*Cochliobolus miyabeanus*) and sheath blight (*Thanatephorus cucumeris*) in rice, and increases the tolerance of various turf grasses to different diseases. The possible mechanism is that Si creates a physical barrier to hypal penetration as it influences on thickening of cell wall through lignifications. Silicon also increases the formation of organosilico compounds which increase the stability of cell walls to enzymatic degradation. It also acts as a defense mechanism in hypal access, and the function of silicon accumulation in this defense reaction might be similar to synthesis of polyphenol and lignin (Marschner, 1995).

20.6. ROLE OF MICRONUTRIENTS ON DISEASES MANAGEMENT

Application of micronutrients can reduce the disease infestations of crop plants significantly. They suppress the diseases indirectly by creating the defense mechanisms and producing certain compounds which act as repellent for microorganisms. Acquired systemic resistance can be achieved by application of micronutrients. Management of diseases namely powdery mildew by foliar application of H_3BO_3, $CuSO_4$, $MnCl_2$ or $KMnO_4$ in different crops has been reported by several researchers (Reuveni et al., 1997a, b).

20.7. ROLE OF OTHER MICRONUTRIENTS ON CONTROL OF PLANT DISEASES

Chlorine is an essential micronutrient, influences on the control of several diseases of crop plants such as stalk rot in corn, stripe rust in wheat, downy mildew of millet and septoria in wheat (Graham and Webb, 1991; Mann et al., 2004). The precise mechanism for controlling plant diseases by chlorine is still obscure.

Boron is another essential micronutrients for stabilization of cell wall and membrane which directly influences on the reduction of infestation of diseases. It acts for preventing disease attack through following ways: i) by improving cell wall structure, ii) increasing the cell membrane stability and iii) its role in metabolism of lignin (Brown et al., 2002).

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Chapter 21

Diagnosis of Nutrient Deficiency in Crop Plants

21.1. General Features

Diagnosis of nutrient deficiency is very important for fertilizer management in achieving sustainable crop production system. The precise diagnosis of nutrient deficiencies is vital in maintaining optimum crop growth and development. Diagnosis of deficiency is a systematic approach to identify the causes of deficiency in crop plants, and it is very critical to find out the actual reason of deficiency. Generally, deficiency appears due to lower level of nutrients in soil. However, presence of plenty amount of nutrients in soil cannot give assurance to avoid the deficiency. The deficiency is influenced by various factors, and may also be occured due to biotic and abiotic stresses. For overcoming the deficiency, availability of nutrients must be maintained to a certain level in achieving higher yield of crop and the availability of nutrients depend on various edaphic and other environmental factors.

21.2. Deficiency of Nutrients

It is a condition of physiological disorder that when the concentration of nutrients becomes low enough to limit the plant growth severely, the yield is lost drastically, and characteristics deficiency symptoms are distinct. The yield of crop plants drastically reduced and plant may even die under extreme deficient condition. Deficient in nutrients appears due to metabolic disorder in the plant system which may hidden or become prominent.

21.3. Hidden Hunger of Plant for Nutrients

The subclinical deficiency often refers to as hidden hunger, a situation below the optimum level of nutrient. Sometimes it happens that a crop needs a particular nutrient but it does not be shown any deficiency symptom (Tisdale et al., 1993). It may so happen that nutrient content is above the deficiency symptoms zone but still considered below that is needed for optimum growth. Hidden hunger of plants cannot always be correctly identified by visual observation, and the best method of identifying deficiency of symptoms is to test the plant thoroughly for its nutrient status. Loss of crop yield can be minimized if the hidden deficiency of certain element can be detected by plant analysis and required nutrient can be applied. Significant crop response can be found by the application of specific nutrient at this stage.

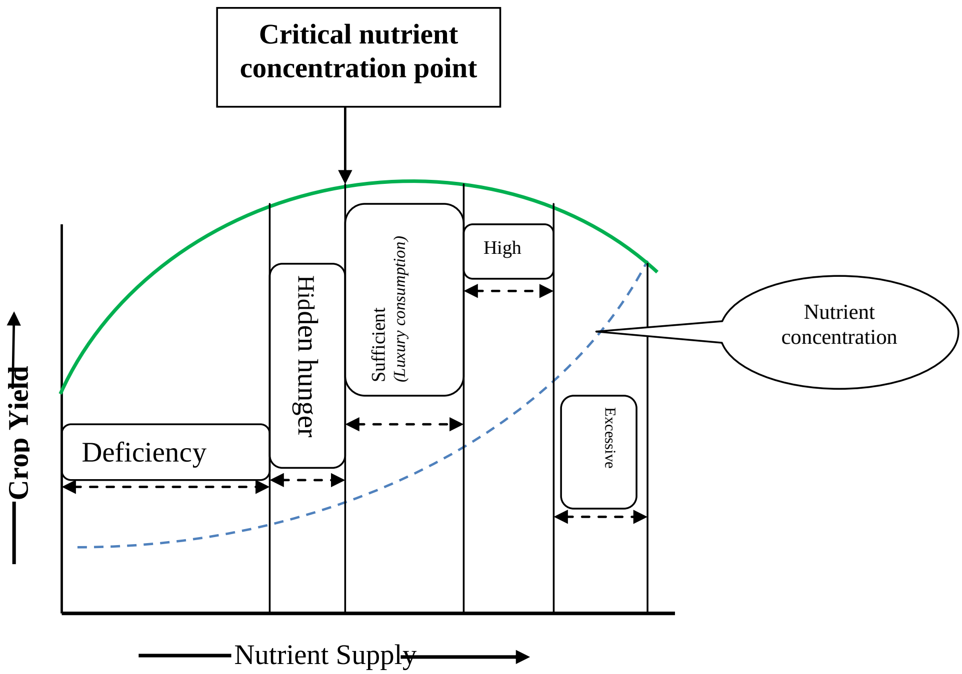


Figure 21.1. Response of crop yield due to nutrient supply.

21.4. Critical Nutrient Concentration Point (CNCP)

The CNCP is very crucial for identifying the hidden hunger and excessive uptake of nutrients. The Figure 21.1 shows the CNCP in between hidden hunger and excessive uptake of nutrients. It implies a single point on a curve that relates nutrient concentration with yield. The concept is well established as a basis for diagnosing nutritional problems in crop plants. However, it is difficult to establish a single point experimentally because the critical point may vary under different environmental conditions and crop varieties.

21.5. Critical Nutrient Range (CNR)

The CNR is defined as that range of nutrient concentration above which is reasonably confident the crop is sufficiently supplied with nutrients and below is deficient (Figure 21.1). This is very useful for determining the deficiency of nutrients than a single point i.e., CNCP (critical nutrient concentration point). For practical implication, CNR values must previously be established at various growth stages during the season.

21.6. Sufficiency Level

This is the nutrient concentration status of the plant where luxury uptake is performed. At this level, higher concentration of nutrients is found in the plant tissue without giving any yield improvement. The added nutrient element will not increase the growth but enhance the nutrient concentration. The extra nutrient ions are stored in the vacuole or cytosol without participating in metabolic activities. The stored nutrients do not show any toxic activity in the plant system. The luxury consumption implies in this situation.

21.7. Toxic or Excessive Level of Nutrients in Plants

In this situation excess amount of nutrients are taken up by the plants resulting in imbalance ionic ratio of nutrient elements and consequently metabolic disorder. The impaired metabolic disorder results in the toxicity of plants. The excessive level of nutrients can reduce the plant growth directly through toxic effect or indirectly declining the concentration of other nutrients. The excessive nutrient ions create severe interaction of other ions which ultimately resulted in heavy or zero absorption of nutrients.

21.8. Possible Causes of Nutrient Deficiencies in Crop Plants

- Presence of insufficient nutrients i.e., lower levels of nutrients *vis-a-vis* low fertility status of the soil.
- Adverse soil pH i.e., low or high pH value which affects the uptake of nutrients. The soil physical condition may influence on developing the deficiency symptoms.
- Water stress i.e., drought or flooding makes nutrients unavailable to plants.
- Excessively wet or dry soils, cold weather, or soil compaction can also significantly restrict the access of roots to soil nutrients.
- Poor root growth to acquire sufficient amount of nutrients which can be due to poor growing conditions, excessively wet or dry soils, cold weather, or soil compaction.
- Root injury due to mechanical, insect, disease, or herbicide injury.
- Genetical character of the plant i.e., certain plants are incapable of absorbing sufficient nutrients.

Table 21.1. Nutrient concentration of different plant parts of different crop plants

	Nutrient Concentration Range										
Nutrient elements	N	P	K	Ca	Mg	S	Fe	Zn	B	Cu	Mn
Crop	Nutrient concentration range (%)						Nutrient concentration range (ppm)				
Rice (at panicle initiation stage, third leaf from the top)	2.20-3.90	0.22-0.99	2.10-3.10	0.16-0.39	0.12-0.21	0.12-0.39	76-261	15-36	5-25	6-25	40-740
Wheat (before heading stage, third leaf from the top)	2.0-3.0	0.25-0.5	2.0-3.0	0.2-1.0	0.10-0.80	0.25-0.5	10-250	25-70	3-25	3-25	20-100
Corn before taselling (3rd leaf from the top)	2.7-4.0	0.25-0.5	1.70-3.0	0.30-1.0	0.15-0.5	0.2-0.4	30-300	20-30	10-15	4-10	15-25
Sugarcane	2.0-2.6	0.22-0.30	1.0-1.6	0.22-0.45	0.15-0.32	0.3-0.6	55-105	17-32	4-6	4.0-8.0	20-100
Barley (3 to 4 uppermost leaves prior to heading)	2.5-4.5	0.25-0.5	1.6-3.0	0.15-1.0	0.10-.80	0.15-0.5	10-250	15-70	2-25	2-25	25-100
Millet (3 to 4 uppermost leaves prior to heading)	2.5-4.5	0.25-0.50	1.6-3.0	0.15-1.0	0.10-.80	0.15-0.5	10-250	15-70	2-25	2-25	25-100
Oat (3 to 4 uppermost leaves prior to heading)	2.5-4.5	0.25-0.50	1.6-3.0	0.15-1.0	0.10-0.80	0.15-0.5	10-250	15-70	2.0-25	2.0-25	25-100
Jute (most recently matured leaf)	2.6-2.9	0.41-0.43	1.24-2.0	0.3-0.8	0.2-0.5	0.15-0.2	-	-	-	-	-
Cotton	3.0-4.5	0.15-0.6	0.75-2.5	2.0-4.0	0.3-0.9	0.3-0.9	50-300	20-100	20-60	4-20	10-100
Cucumber (6th leaf from growing tip at flowering)	4.0-6.0	0.4-1.25	3.5-5.0	1.75-3.5	0.3-2.0	0.3-1.5	50-300	20-200	25-75	5-25	50-300
Tomato (4th leaf from growing tip at early bloom)	2.5-4.0	0.3-0.8	2.5-4.0	0.5-4.0	0.4-1.0	0.30-1.2	50-300	20-30	30-100	5-10	50-100
Soybean youngest fully expanded leaf (YFL)	4.0-5.5	0.26-0.5	1.70-2.5	0.5-3.00	0.1-1.0	0.25-.35	25-350	12-80	20-55	4-30	14-100
Beets (table) (9weeks after seeding)	2.6-4.0	0.2-0.3	1.7-4.0	1.5-3.0	0.30-1.0	0.60-0.80	40-300	15-30	60-80	5-10	70-200
Broccoli	3.0-4.5	0.30-0.50	1.5-4.0	1.20-2.50	0.23-0.40	0.20-0.4	40-300	45-95	30-50	5-10	25-150
Cabbage (most recently matured leaf)	3.0-6.0	0.3-0.6	2.0-4.0	1.5-2.0	0.25-0.60	0.3-0.5	30-60	30-50	20-40	3-7	20-40
Carrot (most recently matured leaf)	1.5-2.5	0.2-0.4	1.4-4.0	1.0-1.5	0.4-0.5	0.2-0.5	20-30	20-60	20-40	4-10	30-60
Cauliflower (most recently matured leaf)	2.2-4.0	0.3-0.7	1.5-3.0	1.0-2.0	0.25-0.60	0.3-0.6	30-60	30-50	30-50	5-10	50-80
Cucumber (most recently matured leaf)	3.5-6.0	0.3-0.6	1.6-3.0	2.0-4.0	0.58-0.70	0.3-0.8	40-100	20-50	20-60	5-20	30-100
Egg plant (most recently matured leaf at early fruit set)	4.2-5.0	0.3-0.6	3.50-5.0	0.8-1.5	0.25-0.6	0.4-0.6	50-100	20-40	20-40	5-10	50-100
Lettuce (wrapper leaf)	2.0-3.0	0.3-0.5	2.5-5.0	1.4-2.0	0.3-0.7	0.3-0.5	50-150	25-50	15-30	5-10	20-40
Muskmelon (most recently matured leaf at early fruit set)	3.5-4.5	0.3-0.4	1.8-4.0	1.8-5.0	0.3-0.4	0.2-0.4	40-100	20-60	20-80	5-10	20-100

	Nutrient Concentration Range										
Nutrient elements	N	P	K	Ca	Mg	S	Fe	Zn	B	Cu	Mn
Okra (most recently matured leaf at prior to harvest)	2.5-3.0	0.3-0.6	2.0-3.0	1.0-1.5	0.25-0.5	0.4-0.6	50-100	30-50	25-50	5-10	30-100
Pepper (most recently matured leaf at early harvest)	2.5-3.0	0.2-0.4	2.0-3.0	1.0-1.5	0.3-0.4	0.3-0.4	30-150	25-80	20-50	5-10	30-100
Potato (most recently matured leaf at tops-down)	2.0-3.0	0.2-0.4	1.5-3.0	0.6-2.0	0.2-0.5	0.2-0.5	40-150	30-60	20-30	5-10	20-100
Radish (most recently matured leaf at harvest)	3.0-4.5	0.3-0.4	1.5-3.0	1.0-2.0	0.3-0.5	0.2-0.5	30-150	25-80	20-50	5-10	30-100
Squash (most recently matured leaf at early fruit)	3.0-5.0	0.3-0.5	2.0-3.0	1.0-2.0	0.3-0.5	0.2-0.5	40-100	20-50	25-40	5-20	40-100
Pumpkin (most recently matured leaf at 8 weeks from seeding)	3.0-4.0	0.3-0.4	2.0-3.0	0.9-1.5	0.3-0.5	0.2-0.4	40-100	20-50	20-40	5-10	40-100
Spinach (most recently matured leaf 30 days after seeding)	3.0-4.5	0.3-0.5	3.0-4.0	0.6-1.0	1.0-1.6	0.20-0.4	20-100	50-70	20-40	5-7	50-100
Strawberry (most recently matured leaf at midseason)	2.8-3.0	0.2-0.4	1.1-2.5	0.4-1.5	0.2-0.4	0.8-1.0	50-100	20-40	20-40	5-10	25-100
Sweet potato (most recently matured leaf at root enlargement)	3.0-4.0	0.2-0.3	2.0-4.0	0.8-1.6	0.25-0.50	0.2-0.6	40-100	25-50	20-50	5-10	40-100
Tomato (most recently matured leaf at 5 leaf stage)	3.0-5.0	0.3-0.6	3.0-5.0	1.0-2.0	0.3-0.5	0.3-0.8	40-100	25-40	20-40	5-15	30-100
Turnip Greens (most recently matured leaf)	3.0-5.0	0.3-0.8	2.5-4.0	0.8-1.5	0.25-0.60	0.2-0.6	30-100	20-40	20-40	5-10	30-100
Water melon (most recently matured leaf)	3.0-4.0	0.3-0.5	3.0-4.0	1.0-2.0	0.25-.50	0.2-0.4	30-100	20-40	20-40	5-10	20-100
Lentil (youngest mature leaf at flowering)	1.8-3.4	0.30-0.5	1.8-2.0	0.2-0.8	0.15-0.8	0.15-0.25	25-300	20-25	20-25	3-4	3.51
Mung bean (youngest mature leaf at flowering)	0.60-1.6	0.20-.29	2.2-3.0	1.1-1.9	0.40-0.66	0.07-.35	30-350	19-22	3-5	3-6	19-25
Pea (Leaf from the third node of the plant)	4.25-5.5	.25-5.0	1.35-2.0	0.35-4.0	0.25-1.5	0.35-1.25	25-500	20-70	20-50	4.0-20	25-150
Chick pea (shoot at flowering)	2.3	.24	1.5	-	-	.15-.20	25-300	12	40	3.0	-
Cow pea (youngest mature leaf at flowering)	4.25-5.5	0.25-.50	1.35-2.0	0.35-4.0	0.25-1.5	0.35-1.25	25-500	20-70	20-50	4.0-20	25-150
Onion (tallest leaf at mid season)	3.0-4.5	0.27-.45	3.5-5.0	1.0-3.5	0.25-.50	0.50-1.0	50-300	25-100	20-75	10-50	50-250
Banana (lamina of youngest fully expanded leaf)	2.6	3.0	0.20	0.50	0.30	0.23	80	18	11	9.0	25
Orange (most recently matured leaf)	2.4-3.5	0.15-0.30	1.2-2.0	3.0-7.0	0.25-0.70	.20-.35	60-120-	25-60	30-70	6.0-15	25-125

21.9. Nutrient Deficiency Symptoms May Be Categorized As Follows

- Complete death at seedling stage or early growth stage
- Severe stunting of plants in the seedling or maturity stage
- Appearence of specific leaf symptoms during crop growth
- Delayed or abnormal maturity of crop either mature earlier or delay in maturity
- Significant reduction of yield even showing no deficiency symptom in leaf
- The quality of crop may be decreased despite of yield loss

21.10. Visual Observation

Visual observation and identification of nutrient deficiency in crop plants by naked eye is very easy and simple technique. Diagnosis can be made by specific deficiency symptoms caused by specific nutrient. Symptoms are easily visible when nutrient deficiency is much severe and yield is lost drastically. However, this technique requires systematic and careful approaches for a valid identification and diagnosis. Nowadays visual observation for diagnosis of nutrient deficiency is becoming popular due to numerous advantages compared to other techniques (Marschner, 1995).

21.10.1. Advantages of Visual Observation of Deficiency

- Visual observation of deficiencies is a very powerful tool for determining nutrient deficiency quickly and it is cost effective (i.e., no cost is required for the determination of deficiency).
- Very easy and minimum cost is required for operating the process in the field. Farmers may be given training for practicing this technique. No technical knowledge is required in operating this system.
- It traces to identify plant nutrient deficiency symptoms, and can alert farmers to take necessary precaution. Required corrective measures can be taken during crop growth if it is identified earlier. Large area of farm can be examined within a very short time.
- In order to find the actual deficiency it is necessary to gather additional information namely soil pH, moisture content, nutrient status and organic matter content of soil.

21.10.2. Disadvantages of Visual Observation of Nutrients Deficiency

- It is not independent method but a supplement to other analysis system for diagnosis the deficiency of nutrients. Only naked eye observation cannot give any precise solution.
- Visual observation is often confused with biotic stress viz. pathogenic infestation and abiotic stress like water and salinity stress.

- This system may be confused with deficiency of one element to other elements as an element shows different types of deficiencies. Interaction of different ions can create deficiency which cannot be addressed by this system.
- The deficiency symptoms of nutrients are very complex because every nutrient has many physiological and metabolic functions and each function may have interaction with environmental parameters. Moreover, the expression of these symptoms varies for chronic deficiency condition.
- Sometimes the deficiency of nutrients is induced by environmental limitation namely space, drought, flood and salinity stress. A typical example of iron deficiency, which is induced by the presence of excessive amount of Mn in the soil. Moreover, transition metals namely Cu, Zn Cr and Ni may compete with Fe for the absorption by plant roots.
- Generally, the concentration of nutrients in different plant parts viz. root, stem, leaves, fruits is not uniform which may cause deficiency of nutrients in different plant parts leads to the confusion for diagnosis system.
- It is very difficult to interpret and diagnose if complete symptoms are not visible clearly in plant parts.

In addition to above precautions, visual observation is also limited by time. Between the times if a plant is nutrient deficient (hidden hunger) and visual symptoms appear, crop health and productivity may be substantially reduced and corrective actions may or may not be effective. Therefore, regular soil or plant analysis is recommended for the prevention and early diagnosis of nutrient stress. Detailed information of the soil and environmental condition should be recorded when a deficiency symptom appears, then corrective measures will be possible to take easily. The description of symptoms and time of season when they first appear is very important. Affected field locations can be marked and monitored over time using either flag or GPS (geographical positioning system) readings. This information is useful in preventing nutrient stress for subsequent years.

21.10.3. Following Precautions to Be Taken During Identifying the Deficiency of Nutrients

- Many similar symptoms may appear at a time i.e., N and S deficiency symptoms are almost alike, but they depend upon location, growth stage, and severity of deficiencies.
- Multiple deficiencies and/or toxicities can occur at the same time. Deficiency of more than one nutrient or toxicity can produce similar symptoms, or possibly a deficiency of one nutrient can induce that of another in excess amount (for example excessive P causes Zn deficiency and luxury uptake of Mn induces the deficiency of Fe or vice versa).
- Deficiency symptoms may vary in different crop species, even some cultivars of the same species may differ in their ability to adapt to nutrient deficiencies and toxicities. For example, corn is typically more sensitive to Zn deficiency than barley.

- Pseudo (false) deficiency symptoms (disease symptoms appearing similar to nutrient deficiency symptoms). Potential factors causing pseudo deficiency include, but are not limited to, disease, drought, excess water, genetic abnormalities, herbicide and pesticide residues, insects, and soil compaction. Some time deficient plants do not show any deficiency symptom under hidden hunger condition.

Field symptoms appear different than 'ideal' symptoms, as many of the plants shown in this module since photographs were taken from plants grown under controlled nutrient conditions, and deficiency/toxicity symptoms observed in the field may or may not appear as they do here. Experience and knowledge of field history are excellent aids in determining causes for nutrient stress. Finally, vast experience is needed for the person who will act as nutritionist.

The sequential schematic presentation of identifying the deficiency of nutrients through visual observation has been given on next page.

Necrosis: It is a nutrient deficiency symptom when death of cells or tissues occurs through injury or disease, especially in a localized area of the plant body.

Chlorosis: It is the symptom of nutritional disorder when the affected portion loss chlorophyll and becomes yellow in color i.e., de-greening condition.

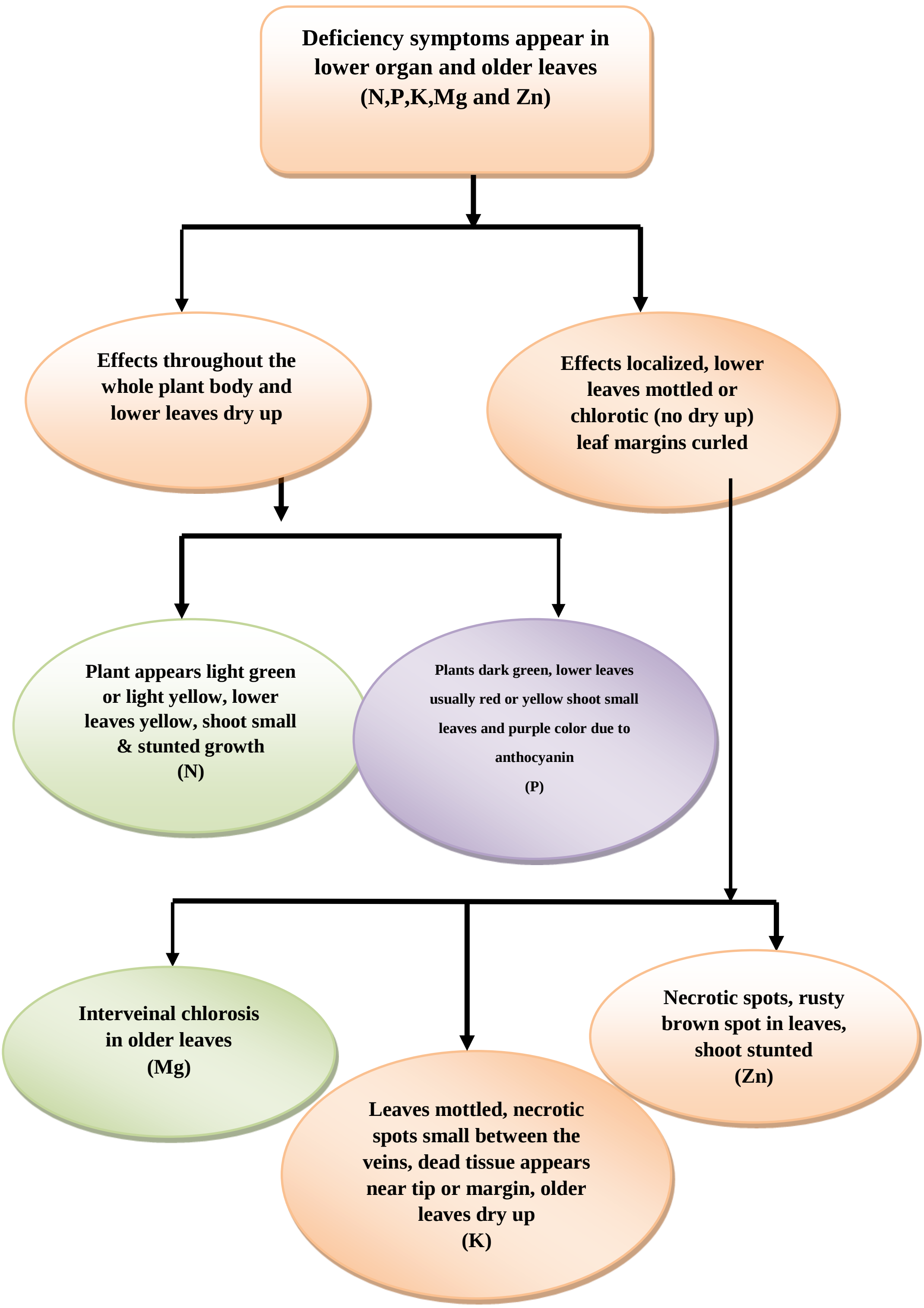
21.11. Diagnosis of Nutrient Deficiency through Analysis of Plant and Soil

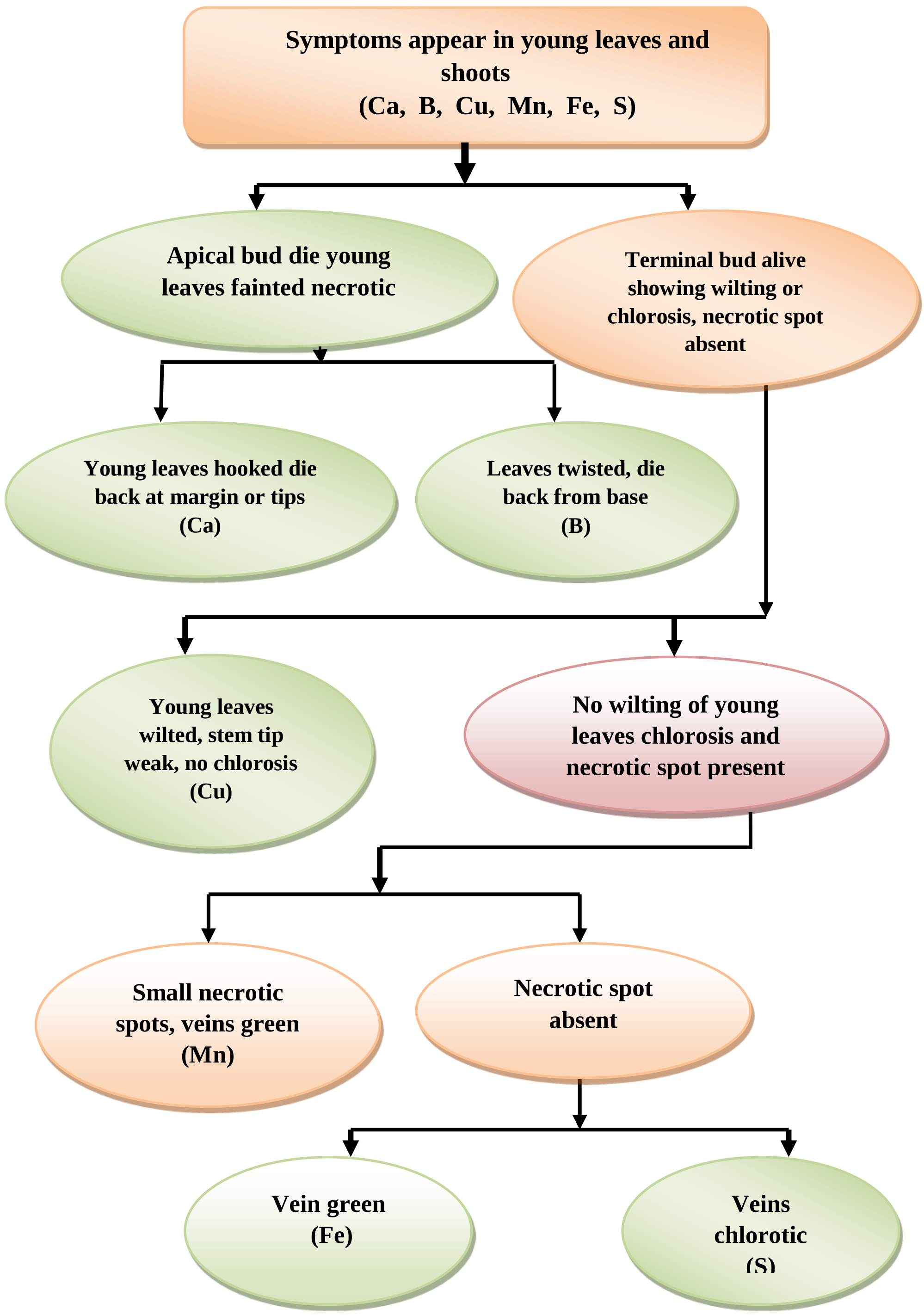
21.11.1. Soil Analysis

Analysis of soil is very important in providing information on available nutrient levels of soil, as well as, soil conditions such as pH, CEC. Problem soil like salinity may be crucial in determining the causes of a deficiency. Certain conditions, such as high pH accompanied with high Ca level in the soil can be predicted to limit Zn, Fe, Mn, and Cu availability. Combination of soil analysis and visual symptom assessment are more valuable and useful than either performed alone.

21.11.2. Plant Analyses

Chemical analysis of plant parts i.e., tissue has been practiced over a century for identifying the nutrient status of the plants for recommending fertilizer application. This is very common practices for identifying nutrient status both of hidden hunger and deficient level in nutrient management. It is very effective tool for identifying the nutrient status of crop plants, allows farmers to more precisely trail their nutrient management system. It can identify nutrient deficiency and imbalance in addition to monitoring the nutrient status of the plant. The plant analysis system helps to reveal some complicated soil-plant mechanism in relation to plant nutrition. Two approaches of plant analyses are performed namely fresh plant parts in the field and laboratory and processed plant parts in the laboratory. Plant tissues are analyzed with the following purposes:


Deficiency symptoms appear in lower organ and older leaves (N,P,K,Mg and Zn)
Effects throughout the whole plant body and lower leaves dry up
Effects localized, lower leaves mottled or chlorotic (no dry up) leaf margins curled
Plant appears light green or light yellow, lower leaves yellow, shoot small & stunted growth (N)
Plants dark green, lower leaves usually red or yellow shoot small leaves and purple color due to anthocyanin (P)
Interveinal chlorosis in older leaves (Mg)
Necrotic spots, rusty brown spot in leaves, shoot stunted (Zn)
Leaves mottled, necrotic spots small between the veins, dead tissue appears near tip or margin, older leaves dry up (K)



- To determine the nutrient supply power of the soil which are employed in conjunction with soil tests and the history of the soil management.
- To help in identifying deficiency symptoms and to determine nutrient shortage before they appear as symptoms.
- To help in fertility treatment of the soil for increasing crop productivity.

- To study the relationship between the nutrients status of the plant and crop yield.

21.11.2. Analysis of Fresh Plant in the Field and Laboratory

21.11.2.1. Plant Analysis in the Field

This is very rapid test for determining the nutrient status in the leaf or young shoot. Portable chlorophyll meter (SPAD meter soil plant analysis device); leaf color chart (LCC) are widely used for determining N-status in the plant and subsequently the fertilizer recommendation could be made properly. There is a close relationship between leaf chlorophyll content and SPAD value. It is very easy to estimate the chlorophyll content from the reading of SPAD value. Similarly content of N in the leaf can be obtained from the SPAD value as there is a close relationship between leaf N content and SPAD value.

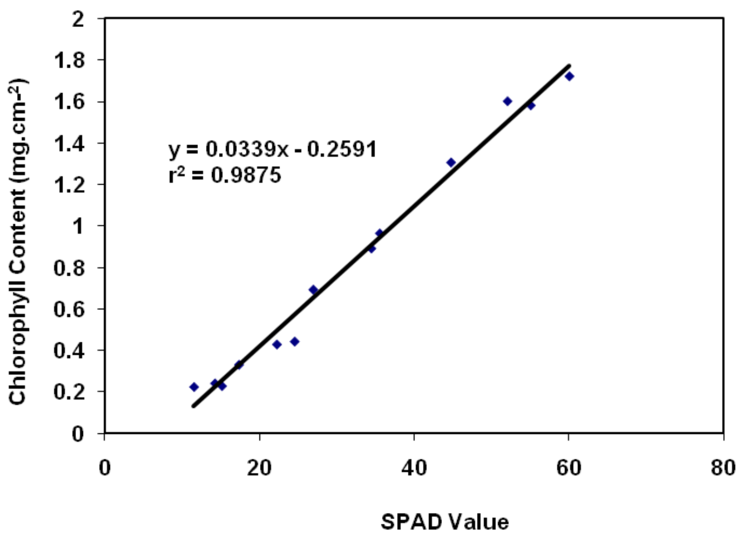


Figure 21.2. Standard curve for total leaf chlorophyll content (Chl. mg cm^{-2}) compared to SPAD value (Chlorophyll meter measurement of leaf greenness) for bananas (after Mia, 2002).

Similarly, nutrient content of cell sap is determined by portable nutrient analyzer. The nutrient status of the crop plants depends on the plant parts and position of the analyzing parts of the plant. Extraction of xylem exudates for analyzing amino acids, soluble protein and other secondary metabolites helps in determining fertilizer recommendation. In general, the vascular tissue of the fully expanded leaf is the best pinpointing for testing.

21.11.2.2. Total Plant Analysis

Analysis of total plant parts is performed after drying the sample followed by grinding and finally the nutrient content namely N is determined through Kjeldahl digestion technique. Routine assessment of plant parts namely leaf samples should be collected timely and sent to monitor nutrient levels in plants and fertility program. In case of deficient crop field the sampling should be done from both deficient and normal leaves for better comparison. Different parts i.e., root; stem, leaves and seeds of plants are analyzed for the diagnosis of the nutrient deficiency. It has several advantages over visual observation as follows:

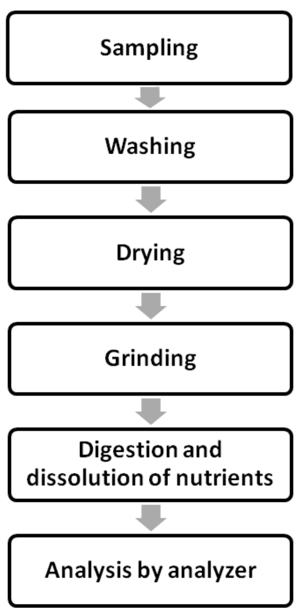
- It is very much accurate compared to visual observation, and aids in determining the nutrient-supplying capacity of soil.
- Identification of deficiency symptoms and to determine nutrient shortage before symptoms appears.
- Determination of the effect of fertility treatment on the nutrient supply in the plant.
- Possible to establish a relationship between the nutrient status and deficiency.

21.11.3. Points to Be Considered in Testing the Plant Parts

- Test different plant parts at several times during growing period of the plant to achieve a meaningful results.
- Plants generally enjoy two peaks i.e., active vegetative and reproductive phase. These two phases must be considered.
- Larger number of plants as much as possible, should be taken in analyzing purposes.
- General growth performance of the plant should be considered for finding a better interpretation of the results.

21.11.4. Plant-Analysis Methods

Chemical analysis of plant samples has been used over a century for estimating nutrient status of the deficient and normal plants for recommending the nutrient management. It is very effective technique for diagnosis, deficient, hidden hunger and toxicity levels of nutrients. The methods usually require destructive extraction method of digestion, either by dry ashing the sample or by dissolving the nutrients content of sample in acids. Plant analysis follows the steps shown in the diagrammatic scheme:



Following points should be kept in mind while sampling plant materials for chemical analysis:

- Samples should not be taken from dead and pest damaged portion of plant materials
- Check whether the samples are mechanically or chemically injured and discard the injured portion from the sample.
- Plant samples should not be taken from drought or flooding affected areas.
- Try to avoid taking samples from abnormally high or low temperature affected areas.

Table 21.2. Outline of the plant parts, sampling time and number of sample to be taken for chemical analysis for obtaining best results of some common crops

Crop	Time of sample collection	Plant parts to be collected	Number of plants to be sampled
Rice	Panicle initiation stage	Flag leaf or youngest fully expanded leaf (3rd leaf from the top)	30-40
Wheat	Before heading	Youngest fully expanded leaf (3rd leaf from the top)	50
Maize	Prior to tasseling	Youngest fully expanded leaf (3rd leaf from the top)	15-20
Barley	Before heading	Youngest fully expanded leaf (3rd leaf from the top)	50
Sorghum	Before heading	Second leaf from the top	20-25
Banana	Before shooting	3rd leaf from the top	5-10
Jute	Before flowering	Youngest fully expanded leaf	40-50
Cotton	Before flowering	Youngest fully expanded leaf	30-35
Soybean	Prior to flowering stage	Youngest fully expanded leaf	20-30
Lentil	Before flowering stage	Whole shoot	20-30
Sugar cane	Around four months old	Fourth fully developed leaf from the top	25-30
Millet	Before flowering	Youngest fully expanded leaf i.e., 3rd leaf from the top	25
Potato	Before blooming	Youngest fully expanded leaf (3rd-6th) from the top	20-30
Groundnut	Before flowering stage	Youngest fully expanded leaf	45-50
Pulses	Before flowering	Entire above ground portion	25-30
Sunflower	Florets about to emerge	Youngest fully developed leaf	20
Canola	Flowering stage	Upper leaves	25
Mustard	Flowering stage	Upper leaves	25
Broccoli	Heading	Youngest mature leaves	20
Cabbage	Mid-season	Wrapper leaves	20
Cauliflower	Mid-season	Youngest mature leaves	20
Cucumber	Prior to or at early fruit development	Youngest mature leaves	20
Pumpkin	Prior to or at early fruit development	Youngest fully developed leaf	25
Tomato	Mid- season	Youngest fully developed leaf	40
Watermelon	Prior to or at early fruit development	Youngest fully developed leaf	25
Tobacco	Early flowering stage	Youngest fully developed leaf	15
Lettuce	Middle of the growth stage	Wrapper leaves	20
Onion	Middle of the growth stage	Tops, no white portion	20
Strawberry	At first flowering stage	before fully developed leaflets mowing and petioles	40

21.11.5. Biochemical Analysis of Plant Tissue

Biochemical attributes viz. enzyme, protein, amino acid and other secondary products are closely related to nutrient deficiency and toxicity of the crop plants. Abnormal status of those chemicals present in the plant parts indicates the deficiency or toxicity of certain nutrient elements. Generally, leaf samples are analyzed for the determination of biochemical compounds like chlorophyll content, nitrate reductase activity, amino acid content etc.

21.11.6. Histochemical Method

Deficiency or toxicity of the nutrients resulted in the abnormal structure of the tissue which can be observed through microscope. Light or electron microscopy technique helps in determining the deficiency or toxicity of micronutrients in crop plants is gaining prominence in high value fruit and vegetables.

21.11.7. X-ray Spectrometry

Nutrients namely Na, K, Mg, P, S, Cl, Ca etc can be analyzed by X-ray fluorescent spectrometry using discs pressed directly from dried and ground plant material. In-situ ionic status can be monitored through this technique.

21.12. Diagnosis and Recommendation Integrated System (DRIS)

Beaufils (1973) introduced the diagnosis and recommendation integrated system (DRIS) for finding out all the nutritional factors limiting crop production and thus enhances the scope of obtaining high crop yields by upgrading the recommendation of fertilizer. It is based on collection of as many data as possible on soil properties and plant composition and factors through computer model for determining the fertilizer requirement. It is a unique method of interpreting the results of soil and plant analyses. It includes a number of assumptions which distinguishes it from “critical concentration”; approaches, the most important being the ratios of nutrient element concentrations these ratios are often better indicators of nutrient deficiency than the simple nutrient element concentrations. The nutrient balance is very important for diagnosis of deficiency rather the concentration as a whole. The relationship of different nutrients in leaves along with concentration of each nutrient is interpreted in this system. It also provides a means of simultaneous identifying imbalances, deficiencies and excesses in crop nutrients and ranking them in order of magnitude. It has been used successfully to interpret the leaf concentration of nutrients of a diverse group of crop plants namely sugar cane, potato apple, mango, sweet potato, cauliflower, rice, corn tomatoes etc. (Bangroo et al., 2010). Though several workers have shown that DRIS often produces more accurate diagnoses of nutrient element deficiency than conventional approaches, the complexity of the DRIS methodology has discouraged its use.

21.12.1. Condition for Preparing DRIS

i. All possible factors suspected of having an effect on crop yield must be addressed
ii. The relationship between these factors and yield must be described
iii. Recommendations suited to particular sets of conditions and based on correct and judicious use of these norms must be continually refined.

21.12.2. Advantages of DRIS

i. The ratio among the nutrients for identification of deficiency is better than individual nutrient concentration. The importance of nutritional balance is taken into account in driving the norms and making diagnosis.
ii. The norms for the nutrient in leaves can be universally applied to the particular crop, regardless of where it is grown. However, locally developed DRIS norm should be calibrated with the help of established wider ranged norm.
iii. Some ratios are more important than others, and maximum yield can only be achieved when the calculated ratio will be nearer to ideal ratio. Diagnosis can be made over a wide range of stages of crop development, and it is less sensitive to aged tissues than other methods.
iv. The low yield due to deficient or excessive nutrients can easily be identified through this method.

Table 21.3. The interaction among the nutrients in relation to deficiency and toxicity of various nutrient elements

N	P	K	Ca	Mg	Fe	B	Mn	Cu	Zn	May stimulate excess symptom
-	A	A	S	S	-	A	A	A	A	N
A	-	A	S	S	A	-	-	A	A	P
A	A	-	A	A	S	A	A	-	A	K
A	A	A	-	A	A	A	A	A	A	Ca
-	S	A	A	-	-	-	-	-	-	Mg
-	A	A	-	-	-	A	A+	A	S	Fe
-	-	-	A	A	A+	-	-	-	-	Mn
-	-	-	-	-	A	-	A	-	A	Cu
-	-	-	-	-	A	-	-	-	-	Zn
										May stimulate deficiency symptom
-	S	S		A	-	-	-	-	-	N
-	-	-	S	S	-	-	-	-	-	P
-	S	-	S	S	-	-	S	-	-	K
-	-	S		S	-	-	S	-	-	Ca
-	A	-	S	-	A	-	-	-	-	Mg
-	S	-	-	-	-	-	S	-	-	Fe
S	-	-	-	-	S+	-	-	-	-	Mn

A-Antagonostic, S-Synergistic

21.13. Ionic Balance of Nutrients

The ionic ratio of different nutrients in the cytosol is critical for metabolic activity of the cell. Ratios of nutrients in plant tissue are frequently used to study nutrient balance in crops. For example, N/S, K/Mg, K/Ca, (Ca+Mg)/K, N/P and other ratios are commonly used.

21.14. Interactions among the Ions

An interaction between two or more ions influences the uptake and consequently shows the deficiency or toxicity of certain nutrient elements. For example iron induces the deficiency of Mn or vice versa on the other hand K stimulates the uptake of NO_3^-. The interaction effect amongst the ions are summarized in the Table 21.3

21.15. References

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