Mineral Nutrition of Plants

20

Renu Pandey

Abstract

In this chapter, a brief overview of the history of plant mineral nutrition is provided. Soil serves as the source of nutrient elements, and so the availability of nutrients is governed by soil properties. The term 'essential mineral element' has been defined, and these elements are grouped according to their biochemical behaviour and physiological functions. In addition to these elements, another group of elements called beneficial elements has been discussed. The pathway of movement of elements (as ions) through roots and different mechanisms for absorption and transport of nutrients has been outlined in this chapter. Besides the inorganic nutrient elements, the use of biofertilisers in agriculture has been discussed. These biofertilisers associate with the plants either symbiotically or non-symbiotically, to help them enhance absorption of nutrient elements from soil and improve growth.

Keywords

Plants • Mineral nutrition • Macro- and microelements • Essential mineral element • Transport and absorption of nutrients • Biofertilisers

20.1 Introduction

Inorganic minerals present in the Earth's crust are used for nutrition by plants by extracting them from soil or the aquatic environment. These mineral elements are formed by the complex interaction involving weathering of rock minerals, decay of organic matter, animals and microbes. Among nutrient elements, nitrogen is exceptional as its primary source is gaseous nitrogen of the atmosphere and little occurs in minerals. Roots absorb

R. Pandey (✉)
Mineral Nutrition Laboratory, Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: renu.pandey.iari@gmail.com

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mineral nutrients in the form of their salts dissolved in soil water. The study of absorption of inorganic mineral elements and their assimilation by plants is called mineral nutrition. Once the elements are absorbed by roots, they are translocated to various parts of the plant where they are involved in carrying out important biological functions resulting in normal growth and development.

In agriculture, the addition of mineral elements to soil to improve plant growth dates back to more than 2000 years. About 150 years ago, the function of mineral nutrients in plant growth was a topic of scientific debate. However, it was Justus von Liebig (1803–1873) who collected, compiled and summarised the scattered information pertaining to the importance of mineral elements for plant growth. This established the mineral nutrition of plant as a scientific discipline. These achievements led to a rapid increase in the use of mineral fertilisers in agriculture. By the end of the nineteenth century, particularly in Europe, large amount of potash, superphosphate (phosphorus) and, later, inorganic nitrogen was used in agriculture and horticulture to improve plant growth. It was concluded based solely on observation and speculation rather than by precise experimentation that mineral elements such as nitrogen, sulphur, phosphorus, potassium, calcium, magnesium, silicon, sodium and iron are essential for plant growth.

By the end of nineteenth century, a large number of studies were undertaken to establish the 'mineral element theory'. From the extensive investigation carried out on mineral composition of different plant species growing on different types of soils, it was concluded that neither the presence nor the concentration of mineral element in a plant is the criteria for essentiality. Plants have a limited capability to absorb selectively those mineral nutrients that are essential for their growth. They also take up mineral elements that are not essential for growth and may be even toxic. Therefore, it was evident that the mineral composition of plants growing in soils cannot be used as a criterion to judge the essentiality of a mineral element. Once this fact was established, both water and sand culture experiments were carried out in which particular elements were omitted. The technique of growing plants in soilless culture media was termed as hydroponics. These techniques were used to characterise the essentiality of individual mineral elements more precisely and led to a better understanding of their role in plant metabolism. Discovery of essentiality of micronutrients by various workers using hydroponics is presented in Table 20.1.

Table 20.1 Discovery of essentiality of micronutrients for higher plants (Marschner 1995)

Element	Year	Discovered by
Iron	1860	J. Sachs
Manganese	1922	J. S. McHargue
Boron	1923	K. Warington
Zinc	1926	A. L. Sommer and C. B. Lipman
Copper	1931	C. B. Lipman and G. Mackinney
Molybdenum	1938	D. I. Arnon and P. R. Stout
Chlorine	1954	T. C. Broyer et al.
Nickel	1987	P. H. Brown et al.

20.2 Soil as Source of Nutrient

Soil is a complex physical, chemical and biological substrate which acts as a matrix for various organic and inorganic nutrients. Soil contains all the three phases, viz., solid, liquid and gaseous, which interact with mineral elements. The solid phase provides a reservoir of both inorganic nutrients (potassium, calcium, magnesium and iron) as well as organic compounds which provide nitrogen, phosphorus and sulphur among other elements. The liquid phase or the soil solution is very important from the view point of absorption of nutrients by roots because it contains dissolved nutrient ions and facilitates the movement of ions towards root surface. The airspaces between soil particles which are occupied with gases such as oxygen, carbon dioxide and nitrogen constitute the gaseous phase of soil. From the biological perspective, soil constitutes a diverse ecosystem in which plant roots and microorganisms compete strongly for mineral nutrients. In spite of this competition, roots and

microorganisms can form associations for their mutual benefit (nitrogen-fixing bacteria, arbuscular mycorrhiza).

Based on the bioavailability of inorganic nutrient elements to the plant roots, the soil may be considered as fertile or non-fertile. The term fertility refers to the inherent capacity of soil to supply nutrients to plants in adequate amounts and appropriate proportions. In non-fertile soils, in order to increase the availability of nutrients in a balanced proportion, inorganic elements in the form of fertilisers are added externally. Most fertilisers are formulated to overcome the deficiencies of mineral elements in soil.

20.2.1 Soil Properties Affecting Nutrient Availability

The chemical (surface charge, pH) and physical (soil texture and structure) properties of soil determines the availability of nutrients on the root surface. The soil particles, both organic and inorganic, are negatively charged. This negative charge is due to the fact that many inorganic soil particles are crystal lattices which are arranged in a tetrahedral form of aluminium and silicon (Al^{3+} and Si^{4+}). These tetrahedral arrangements are covalently bound to oxygen atoms forming aluminates and silicates. These tetrahedral structures undergo isomorphic substitution wherein another cation of lesser charge replaces the Al^{3+} or Si^{4+} thus making them negatively charged particles. Microbial decomposition of dead plants, animals and microorganisms leads to the formation of organic soil particles. The dissociation of hydrogen ions from carboxylic acid and phenolic groups present in organic soil particles provides negative surface charges.

Charge on the surface of soil particles has an important role in plant nutrition. Mineral cations such as ammonium (NH_4^+) and potassium (K^+) adsorb to the negatively charged surface of the organic and inorganic soil particles. This adsorption is an important factor in governing soil fertility. The major advantage of adsorption is that the cations are not easily lost from the surface of soil particles when the soil is drained with water (leaching loss). However, these adsorbed nutrients can be replaced by other cations in a process known as cation exchange. The capacity of a soil to adsorb ions and exchange it with other ions is termed as cation exchange capacity (CEC) and is highly dependent on soil types. Soils with small particles like clays have a high ratio of surface area to volume resulting in a higher CEC. A soil with higher CEC generally has larger reserves of mineral nutrients. On the other hand, mineral anions such as nitrate (NO_3^-), chloride (Cl^-) and phosphates (PO_4^{3-}) do not get adsorbed because of repulsion due to the negative charge on the surface of soil particles and thus remain dissolved in the soil solution. These anions are highly prone to leaching loss.

Another important chemical property affecting nutrient availability is pH or the hydrogen ion concentration of soil solution. The reaction of soil solution, whether it is neutral, alkaline or acidic, has a distinct effect on the availability of mineral elements to plant roots. The range of pH for most crops lies approximately between 5.5 and 6.5 at which the greatest average levels of all essential plant nutrients become available (Fig. 20.1). This pH range also favours the root growth. Extreme fluctuations of soil pH on either side can cause nutrient imbalances in plants resulting in deficiency or toxicity symptoms.

The physical property of soil includes different sizes of soil particles. The relative proportion of various soil particle types is referred to as soil texture. The arrangement of soil particles, that is, sand, silt and clay in soil, is known as soil structure. The soil particles are named as gravel, coarse sand, fine sand, silt and clay based on two systems as shown in Table 20.2. Practically, all soils are mixtures of sand, silt and clay. Soils with 10–25 % clay and the rest about equal parts of sand and silt are called loams. The soil texture governs the fertility of soil.

Soil aeration is another important physical property affecting the availability of some of the nutrient elements. Soils rich in clay and humus can hold more water expelling air from the space between the particles. Such soil lacks oxygen and is not ideal for plant growth. In these waterlogged (reduced) soils, the availability of reduced elements

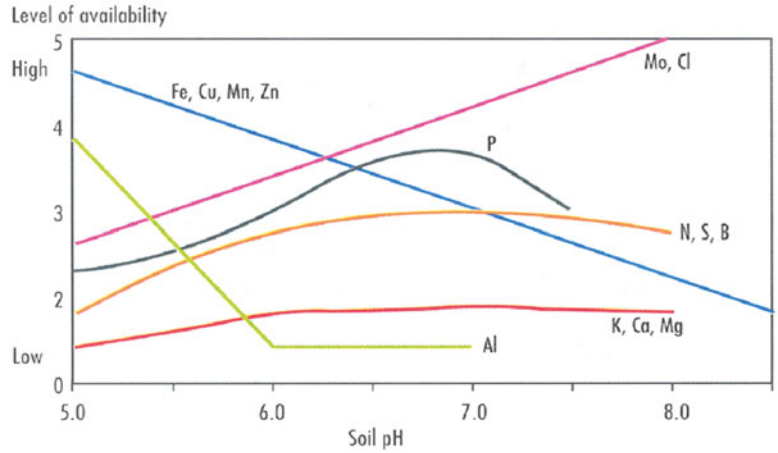

Fig. 20.1 Relationship between level of availability of different elements and soil pH (Source: Goedert et al. 1997)

Table 20.2 Classification of soil particles by diameter (Salisbury and Ross 1985)

Particle	USDA system (mm)	World system (mm)
Coarse sand		2.0–0.2
Fine sand	2.0–0.05	0.2–0.02
Silt	0.05–0.002	0.02–0.002
Clay	<0.002	<0.002

such as Fe^{3+} increases. The microorganism present in waterlogged soil utilises Fe^{2+} as an electron acceptor and thus Fe is reduced. This reduced form of Fe^{2+} is absorbed in excess by some plants adapted to wetland such as paddy and suffer from iron toxicity (bronzing). The best agricultural soil from the point of view of good plant growth and nutrient availability are sandy loams and clay loams.

20.3 Criteria for Essentiality of Elements

The nutrient elements essential for healthy growth of plants are called essential nutrients or essential mineral elements. Till date, only 17 elements (nickel being the recently enlisted) are considered as essential. Arnon and Stout in the year 1939 proposed the term 'essential mineral element'. They established that the following three criteria must be met for an element to be considered essential.

1. A plant must be unable to complete its life cycle in the absence of the mineral element.
2. The function of the element must not be replaceable by another mineral element.
3. The element must be directly involved in plant metabolism, for example, as a component of an essential plant constituent such as an enzyme or it must be required for a distinct metabolic step such as an enzymatic reaction.

The second criterion has some exceptions; it cannot be followed as such because there are some elements that can be replaced by others without causing any adverse effect on the plant. For example, the monovalent cation K^+ can be replaced by Na^+; both these ions play important roles in osmoregulation. On the other hand, this strict definition of essentiality excludes those mineral elements that compensate for toxic effects of other elements or replace mineral nutrients involved in some specific function. Such elements are not essential but perform certain important functions and are classified as beneficial elements. By definition, beneficial elements are those that stimulate growth but are not essential or might be essential for certain plant species under specific conditions. The optimum genetic potential of crop plants cannot be achieved if the beneficial elements are excluded from the agricultural production system.

Based on the essentiality criteria, mineral elements have specific and essential functions in plant metabolism. Therefore, depending on the requirement of a nutrient element to produce

optimum plant growth, the nutrient is referred to as either macronutrient or micronutrient. The macronutrients are required in larger quantities and are present in plant tissues in amounts ranging between 0.2 and 4.0 % (on a dry weight basis), while the concentration of micronutrients in plant tissue ranges from 5 to 200 ppm or less than 0.02 %.

The macronutrients are further divided based on their requirements into primary macronutrients consisting of nitrogen (N), phosphorus (P) and potassium (K) and secondary macronutrients including calcium (Ca), sulphur (S) and magnesium (Mg) (Table 20.3). Another classification based on physiochemical properties divides nutrients into metals (K, Ca, Mg, Fe, Mn, Zn, Cu, Mo, Ni) and non-metals (N, S, P, B, Cl). However, the most widely used classification is based on the quantity of requirement of mineral element rather than the physiochemical properties.

Table 20.3 Essentiality of mineral elements for higher plants

Classification	Element	Higher plants
Macronutrient	N, P, S, K, Mg, Ca	+
Micronutrient	Fe, Mn, Zn, Cu, B, Mo, Cl, Ni	+
Beneficial elements	Na, Si, Co, Al	±

20.3.1 Quantitative Requirements of Nutrient and Tissue Analysis

The concentrations of essential elements required by plants for maintaining optimum growth and preventing any deficiency symptoms are given in Table 20.4. Such values serve as useful guide to plant physiologists and farmers because concentration of elements in plant tissue is more reliable than in soil indicating whether the plant will grow faster if more of a given nutrient is applied. However, for determining the optimum growth of a plant, a farmer should take into consideration the soil analysis of his field. Soil analysis is the chemical determination of the nutrient content and their quantification in soil sample. The soil

Table 20.4 The form of nutrient taken up by plant and its average concentration in shoot dry matter required for adequate growth

Element	Abbreviation	Form taken by plants	Concentration in dry tissue (ppm)	Concentration in dry tissue (%)	Relative no. of atoms
Molybdenum	Mo	MoO_4^-	0.1	0.00001	1
Nickel	Ni	Ni^{2+}	~0.1	0.00001	1
Copper	Cu	Cu^+, **Cu^{2+}**	6	0.0006	100
Zinc	Zn	Zn^{2+}	20	0.002	300
Manganese	Mn	Mn^{2+}	50	0.005	1,000
Iron	Fe	Fe^{3+}, **Fe^{2+}**	100	0.01	2,000
Boron	B	H_3BO_3	20	0.002	2,000
Chlorine	Cl	Cl^-	100	0.01	3,000
Sulphur	S	SO_4^{2-}	1,000	0.1	30,000
Phosphorus	P	**$H_2PO_4^-$**, HPO_4^{2-}	2,000	0.2	60,000
Magnesium	Mg	Mg^{2+}	2,000	0.2	80,000
Calcium	Ca	Ca^{2+}	5,000	0.5	125,000
Potassium	K	K^+	10,000	1.0	250,000
Nitrogen	N	**NO_3^-**, NH_4^+	15,000	1.5	1,000,000
Oxygen	O	O_2, H_2O	450,000	45.0	30,000,000
Carbon	C	CO_2	450,000	45.0	35,000,000
Hydrogen	H	H_2O	60,000	6.0	60,000,000

Source: Modified after Salisbury and Ross (1985)

Between one of the two forms of element, bold-faced type is the preferred form by plants

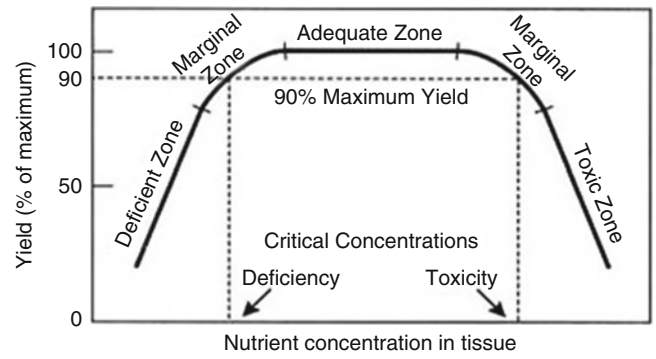

Fig. 20.2 Generalised plot of growth rate as a function of the concentration of a nutrient element in plant tissue

analysis reflects overall levels of nutrients potentially available to plant roots. However, it fails to evaluate the uptake conditions and amount of nutrients actually absorbed by the plants. This additional information can be obtained by plant tissue analysis.

Proper use of plant tissue analysis requires an understanding of the relationship between plant growth and the mineral content of plant tissue samples. Figure 20.2 shows an idealised plot of growth rate as a function of the concentration of any given nutrient element in the plant. When the nutrient content in tissue sample is low, growth is reduced called the deficient zone. In this zone, an increase in tissue mineral content is directly related to an increase in growth or yield. As the nutrient content in tissue sample increases further, a point is reached at which additional increases in tissue mineral content do not appreciably affect growth or yield. This region of the curve is called adequate zone. The transition between deficiency and adequate zone of the curve represents the critical concentration of the nutrient in question, which may be defined as the minimum tissue concentration of the nutrient that is sufficient to give maximal growth or yield. The adequate zone also represents the luxury consumption of elements during which there is no increase in growth and the nutrient taken up in excess is stored in vacuoles. This occurs for a few elements like K, otherwise increases in tissue concentration beyond the adequate zone results in toxicity limiting the growth or yield. Plant analysis and soil analysis data are useful in establishing fertiliser schedules that sustain yield and ensure the food quality of many crops.

The distribution of mineral nutrients between different types of cells within a given tissue (e.g. epidermis, guard cells, mesophyll cells of leaf) also provides important information about function of mineral nutrients. This is particularly true for the distribution of ions in different cellular compartments. In the last decade, much progress has been made in this respect by applying techniques, such as X-ray microanalysis, nuclear magnetic resonance (NMR), ion-selective microelectrodes or fluorescent dyes, for studies on ion distribution in cytoplasm and the organelles contained within it (e.g. chloroplast) and the vacuole. New insight into the functions of mineral nutrients, for example, of calcium as a secondary messenger, is based on these studies of cellular compartmentation. David E. Salt and his group (2008) have proposed the concept of 'ionome'. They defined 'ionome' as the entire mineral nutrient and trace element composition of an organism representing the inorganic component of cellular and organismal systems. Hence, ionome includes both essential and non-essential elements. Ionomics is the study of the ionome, involving quantitative and simultaneous measurement of the elemental composition of living organisms and changes in this composition in response to physiological stimuli, developmental cues and genetic modifications. This study requires application of high-throughput elemental analysis technologies such as inductively coupled plasma-optical emission spectroscopy (ICP-OES) or ICP-mass spectroscopy (ICP-MS)

and their integration with both bioinformatics and genetic tools. The information about functional state of an organism under different conditions brought about by the genetic and developmental differences and by biotic and abiotic factors can be captured by ionomics.

The progress towards a better understanding of the function of mineral nutrients helps in comparing genotypes or mutants within a given plant species. The advanced techniques and concept in mineral nutrition like ionome analysis provides a powerful approach to not only analyse the functions of genes and gene networks directly controlling the ionome but also the extended gene networks that control physiological and developmental processes which indirectly affects the ionome.

20.4 Importance of Macro and Microelements

For easy understanding, both the macro- and micronutrient elements have been classified into four groups as given below (Malik and Srivastava 1982):

Group 1: N and S – present in reduced form and are covalently bonded constituents of organic matter

Group 2: P, B and Si – occurs as oxyanions such as phosphate, borate or silicate

Group 3: K, Na, Mg, Ca and Cl – involved in osmoregulation and ionic balance and have specific functions of enzyme conformation and catalysis (e.g. metalloprotein complexes)

Group 4: Fe, Cu, Mo and Zn – present as structural chelates or metalloproteins; also involved in oxidation-reduction (redox) reactions (first three elements)

The following paragraphs describe the occurrence and physiological functions of macro- and micronutrients in detail with recent developments in the field of mineral nutrition. A summary of physiological functions and the deficiency symptoms of mineral elements in plants are presented in Table 20.5 (modified after Malik and Srivastava 1982).

20.4.1 Physiological Functions of Macronutrients

20.4.1.1 Nitrogen

The Earth's atmosphere consists of about 80 % N, but the extremely stable form of atomic N (dinitrogen, N_2) is not available to plants. However, the microorganisms both free-living and symbiotic can fix atmospheric N_2 to ammoniacal form (NH_4^+) which is then directly taken up by the plants or converted into nitrate (NO_3^-) by nitrifying bacteria. The preferred form in which N is taken up by plants depends on soil conditions and plant species. Plants adapted to low pH and waterlogged soil conditions tend to take up NH_4^+, for example, paddy. In aerobic soils with higher pH, NO_3^- is the predominant form preferred by most of the plants. Also, organic N compounds such as amino acids are found in soil, and there is growing evidence that these can also form important N sources.

20.4.1.1.1 N Uptake and Distribution

The uptake of nitrate or ammonium into plants from soil via the roots involves two basic categories of transport system, high-affinity transport system (HATS) and low-affinity transport system (LATS), identified by kinetic studies of N uptake. The HATS operates under conditions of low external nitrate concentration (*Km* <200 μM), and LATS operates under higher external concentrations even as high as 50 mM without saturation. Based on molecular, physiological and biochemical studies, the transport systems were further subdivided into inducible and constitutive depending on substrate induction process. The HATS is comprised of inducible and constitutive HATS (iHATS and cHATS) and are encoded by the members of *NRT2* gene family (Williams and Miller 2001). The constitutive system (cHATS) is available when plant has been previously starved for nitrate, and the inducible system (iHATS) is stimulated by supplying nitrate. Similarly, LATS encoded by members of *NRT1* gene family is also comprised of both constitutive and inducible elements, evidence of which was shown in *Arabidopsis thaliana.* First inducible LATS gene

Table 20.5 Summary of physiological functions and the deficiency symptoms of mineral elements in plants

Elements	Primary function	Specific deficiency symptoms
Oxygen	Final electron acceptor in aerobic respiration; element in carbohydrates, nucleic acids and many other organic compounds	
Carbon	Building block of all organic compounds in the plants' body	
Hydrogen	Component of water and all organic compounds	
Nitrogen	Constituent of amino acids, proteins, chlorophyll, nucleic acid and some coenzymes	'V' shape chlorosis starting from the tip in lower leaves, plant becomes pale green, poor growth
Potassium	Involved in enzyme activation, protein metabolism, cell membranes, ionic balance, cell extension growth, opening and closing of stomata, cell turgor	Chlorosis of older leaves later turns into necrotic lesions on leaf tip, rolling of leaves, shortening of internodes leading to stunted growth
Calcium	Constituent of cell wall, middle lamella, enzyme cofactor, controls cell permeability and cell wall configuration, involved in cell signalling	Twisting and deformation of growing tips and youngest leaves, later necrosis occurs at the leaf margin
Phosphorus	Involved in photosynthesis, constituent of high-energy intermediaries like ATP, nucleic acids, phospholipids in membranes	Stunted growth, foliage turns dark green, high root/shoot ratio, delayed maturity of plants
Magnesium	Central element of chlorophyll molecule and an activator of several enzymes	Interveinal chlorosis of older leaves, purple coloration with necrotic spots, leaves become stiff and intercostal veins twist
Sulphur	Component of some amino acids (cysteine and methionine) and coenzymes	Symptoms appear in younger most formed leaves, a general chlorosis of entire leaf including vascular bundles
Iron	Involved in chlorophyll biosynthesis, structural component of cytochromes and ferredoxin	Symptoms appear first in younger growing organs, interveinal chlorosis; in severe cases, leaves become white which later dries out
Chlorine	Stomatal regulation in some plants (e.g. onion), stimulation of proton pumping ATPase located at tonoplast, involved in photosynthetic oxygen evolution, osmoregulation	Symptoms appear first in younger leaf, reduction in leaf surface area, wilting of leaf margins, interveinal chlorosis of mature leaves, highly branched root system
Copper	Activator of some enzymes like Cu-Zn SOD	Young leaves turn dark-green colour, twisted with necrotic spots, depressed internode growth, bushy appearance, stunted growth, reduction in panicle formation
Manganese	Activator of some enzymes (Mn-SOD), involved in PS I as water-splitting complex	Small yellow spots and interveinal chlorosis on younger leaves
Zinc	Activator of some enzymes, involved in synthesis of auxin	Stunted growth due to shortening of internode called 'rosette', drastic decrease in leaf size
Molybdenum	Cofactor of enzymes involved in nitrogen metabolism	Chlorosis and stunted growth in younger leaves, drastic reduction in size and whiptail appearance of leaf blade
Nickel	Metal cofactor of urease enzyme	Symptoms closely related to Fe deficiency

isolated was *Chl1* from *A. thaliana* (renamed as *AtNRT1.1*) primarily expressed in roots, and its induction was caused by high external nitrate concentrations up to 25 mM. Later, kinetic studies revealed that *AtNRT1.1* possessed both low- and high-affinity components and therefore classified as dual-affinity transporter (DATS) (Wang and Crawford 1996; Wang et al. 1998; Liu et al. 1999). Another exception of DATS was reported in *Medicago truncatula*, *MtNRT1.3* (Morère-Le

Paven et al. 2011). Very recently, the molecular mechanism of switching affinity of NRT1.1 from low to high revealed that it is controlled by phosphorylation and dephosphorylation of a key amino acid residue, threonine, at position 101 (Parker and Newstead 2014). In Arabidopsis, the entire family of *NRT1* and *NRT2* genes have been characterised comprising of nine and seven members, respectively, that control the flux of nitrate from soil into root tissues and throughout the plant body. These transport systems are induced by NO_3^- concentration in soil solution as well as the N status of the plant. Besides nitrate transporters, a large number of high- and low-affinity ammonium transporters are encoded by the AMT family in plants grown under reduced soil conditions (Howitt and Udvardi 2000). Organic N forms are also transported throughout the plant by proton-dependent oligopeptide transporters of the POT/PTR family.

20.4.1.1.2 Assimilation and Biological Functions of N

Plants cannot use inorganic N as such so it has to be reduced. Two important enzymes of N assimilation, nitrate reductase and nitrite reductase, are involved in reducing the oxidised form of N, that is, NO_3^- to NH_4^+. Other enzymes of N assimilation pathway include glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (AspAT) and asparagine synthetase (AS). These enzymes are responsible for the incorporation of NH_4^+ into amino acids such as glutamine, glutamate, asparagine and aspartate. The primary function of N is to provide amino groups in amino acid constituent of bases in nucleotides of purine and pyrimidine. Besides these, N is an essential constituent of many nonprotein compounds such as coenzymes, photosynthetic pigments, secondary metabolites and polyamines and vitamins. When N is in ample supply, the NO_3^- form is stored in the vacuole where it contributes to generation of turgor pressure.

20.4.1.1.3 Symptoms of Deficiency and Excess N

Deficiency symptom is seen as a general chlorosis starting in the lower leaves due to loss of chlorophyll. Typical symptom of N deficiency is the formation of 'V' shape of chlorosis starting from the tip of the leaf (Fig. 20.3). This yellowing later can be seen in younger leaves, and in case of severe N deficiency, the older leaves fall off. The plant becomes pale green and the leaf petiole and veins become purple due to anthocyanin pigment synthesis. The overall plant growth is poor and stunted due to low protein synthesis. The symptom appears later in the young leaves because N is highly mobile in plant, and so it is translocated from older leaves to the young growing points.

Plants grown with excessive N usually have dark-green-coloured leaves, produce excess foliage but have a poorly developed root system and, thus, a low root/shoot ratio. For example, potato plants supplied with excess N produce profuse foliage with a few small tubers and poor root growth; perhaps sugar translocation to tubers and root is affected due to hormonal imbalance. Flowering and seed development in several agricultural and horticultural crops are reduced; the flowering is delayed due to excessive vegetative growth. However, short-day plants given abundant N flower faster. Excess N also causes tomato fruits to split as they ripen.

20.4.1.2 Phosphorus

Almost 90 % of P is fixed in soil in the form of aluminium/iron phosphates or calcium/magnesium phosphates depending on soil pH. Plants cannot use these fixed or non-labile forms of P. Another part of insoluble P, called the labile fraction, exchanges with the soil solution. The inorganic P released from the labile fraction into the soil solution, called solution P, is extremely slow and can take a few years. This is the only form of P accessible to plants for uptake. Therefore, P deficiency is a widespread phenomenon. Since phosphatic fertilisers are made from rock phosphates, P is considered as a non-renewable resource which is expected to be exhausted within the next 50–60 years. The form in which P is found in soil solution is pH dependent (Fig. 20.1), but at typical soil solution pH, P occurs exclusively as $H_2PO_4^-$, the preferred form of inorganic P (Pi) taken up by plants.

Fig. 20.3 Deficiency symptoms of macronutrients created in maize seedlings (15 days old) grown in hydroponics. The element in question was omitted from the nutrient solution thus producing severe deficiency symptoms. –N leaf showing characteristic 'V' shape chlorosis starting from tip of leaf, stubby root growth without laterals. –P shoot growth more depressed than root growth, dark-green leaves. –K leaves with marginal necrosis, poor root growth without lateral roots. –Ca characteristic symptom is death of growing point, bushy roots. –Mg interveinal chlorosis in older leaves, purple coloration due to anthocyanin. –S interveinal chlorosis in younger leaves (Symptoms developed by Pandey R.)

20.4.1.2.1 P Uptake and Distribution

In response to persistent Pi deficiency, plants have developed many adaptive morphological, physiological and molecular mechanisms to cope with low Pi. These includes changes in root growth (increased root surface area, fine root hairs, root length) and architecture, induction of high-affinity Pi transporters, increased secretion of acid phosphatase enzyme and low-molecular-weight organic acids, symbiotic associations with mycorrhizal fungi and changes in the activity of several key photosynthetic enzymes (reviewed by Lopez-Arredondo et al. 2014). Several plant species (e.g. Proteaceae) form dense clusters of fine lateral roots called proteoid roots under P deficiency. These clusters release large amounts of organic acids that act as chelators and help in bringing sparingly soluble calcium phosphates into soil solution.

A wide variety of Pi transporter family has been identified in plants recently based on genome sequence analysis and experimental evidences.

These transporters are involved in the uptake of Pi, movement within the cell and around the plant body. Based on their protein sequence, structure, localisation in membrane and functions, they have been grouped into different families. These include Pht1 (plasma membrane), Pht2 (plastid inner envelope), Pht3 (mitochondrial inner membrane), Pht4 (chloroplasts, heterotrophic plastids and Golgi) and pPT (plastid inner envelope). Among the Pi transporters, Pht1 family is most widely studied which belongs to high-affinity Pi transporter family involved in Pi uptake by roots. High-affinity Pi transporter operates at low external Pi concentrations (l–10 μM), whereas the low-affinity Pi transporter, which is a Pi/H^+ symporter, has an apparent K_m of 0.4 mM (Rausch and Bucher 2002). The Pht1 families from Arabidopsis and rice contain 9 and 13 members, respectively, and have been well characterised (Mudge et al. 2002). The presence of mycorrhizal associations also influences the expression of specific transporters. Vacuolar sequestration of P occurs during seed development where large amounts of P and other minerals (Ca, Mg, Zn, Fe) are stored in seeds forming a complex inositol-hexaphosphate called phytate.

20.4.1.2.2 Assimilation and Biological Functions of P

Unlike N and S, P remains in its highly oxidised form once it enters the plant. The inorganic P is either found as soluble Pi (orthophosphate) or as PPi (pyrophosphate). Organic P is mainly bound to hydroxyl groups to a carbon chain (C-O-P) as a simple phosphate ester (e.g. sugar phosphate) or attached to another phosphate by the energy-rich pyrophosphate bond (P~P), such as in ATP. Another type of phosphate bond is the diester state (C-P-C) with relatively high stability. In this association, phosphate forms a bridging group between connecting units resulting in more complex macromolecular structures. P as a structural element is a constituent of nucleic acids (DNA, RNA) (responsible for their strongly acidic nature) and phospholipids of biomembranes forming phosphatidylcholine (lecithin). In membranes, Pi acts as a link between glycerol-fatty acid (lipophilic part) and the choline (hydrophilic) part of the lipid. Choline is strongly hydrophilic because of the negative charge on phosphate group, and this helps in proper orientation in the membrane. Further, P has a major role in energy transfer reactions. This involves formation and disruption of pyrophosphate bond to maintain energy homeostasis in cellular processes. Hydrolysis of one mole of ATP releases 30 kJ of energy. ATP is the basis of many synthetic pathways, and other similar energy-rich phosphonucleotides (UTP, CTP and GTP) play a central role in nucleic acid metabolism. UTP is involved in synthesis of sucrose, starch and cellulose, while CTP provides energy during phospholipid biosynthesis.

The energy-rich phosphates like ATP, GTP or ADP modulate enzyme activities by reversible phosphorylation (Fig. 20.4). This regulatory

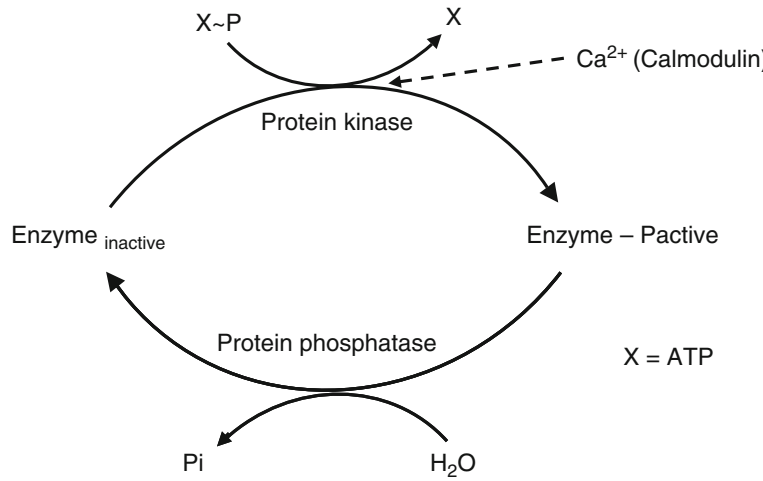

Fig. 20.4 Modulation of enzyme activity by reversible protein phosphorylation

phosphorylation is mediated by protein kinases and can result in activation, inactivation and/or changes in the allosteric properties of the target molecule. Protein kinase phosphorylates serine or threonine residues of proteins and the enzyme becomes activated, while dephosphorylation is carried out by phosphatases that release Pi, hence inactivating the enzyme protein. Protein phosphorylation also has an important role in signal transduction.

The large amounts of P stored in seeds as phytic acid facilitate development of embryo, seed germination and seedling growth. The concentration of Pi has a very important role in the process of photosynthesis. Generally photosynthesis is limited by Rubisco activity or the capacity to regenerate ribulose 1,5-*bis*phosphate (RuBP). In light, for optimal photosynthesis, a Pi concentration in the range of 2.0–2.5 mM is required in the chloroplast; however, photosynthesis is inhibited if the Pi concentration falls below 1.4–1.0 mM (Marschner and Marschner 2012). This may be due to many of the intermediary steps involving sugar phosphates during carbon fixation. Therefore, the demand for Pi is higher in chloroplast which is achieved by the Pi/triose phosphate (Pi/TP) translocator present in the chloroplast envelope. The synthesis of starch in chloroplast is regulated by Pi/TP ratio. A high Pi/TP ratio in chloroplast inhibits the key enzyme ADP-glucose pyrophosphorylase, thus decreasing starch synthesis. However, a low Pi/TP ratio activates this enzyme, and TPs are diverted towards sucrose synthesis in the cytoplasm.

20.4.1.2.3 Symptoms of Deficiency and Excess P

Deficiency of P causes stunted growth of plants and foliage is often dark green. The dark-green colour of leaves is because of the accumulation of starch and sugars in the leaves. Under P deficiency, shoot growth is much more repressed than root growth leading to a higher root/shoot ratio (Fig. 20.3). In severe cases, the roots also develop purple coloration (observed in hydroponically grown maize plants). The leaf expansion is affected due to reduced cell division and enlargement thereby producing smaller leaves. Because P is highly mobile inside the growing tissues in plants, the older leaves are first to show chlorosis. Plant maturity is also delayed.

Excess P application leads to P toxicity in plants leading to delayed formation of reproductive organs. This may be because P and N have synergistic effect, meaning presence of P will lead to more uptake of N.

20.4.1.3 Potassium

The Earth's crust contains around 2.3 % K. Mostly K is bound in primary minerals or present in secondary clay minerals making clayey soils rich in K. Examples of some of the minerals containing K as K_2O are alkali feldspar (4–15 %), muscovite or K mica (7–11 %), biotite or Mg mica (6–10 %) and illite (4–7 %). A typical concentration of K in soil solution varies between 0.1 and 1 mM K^+. Soil K occurs in three forms: K present in soil solution (readily available to plant), K adsorbed in exchangeable form to soil colloids such as clay minerals and K as a structural element of soil minerals. Generally, K deficiency is a rare occurrence, but plant growth is usually stimulated by additional K supply.

20.4.1.3.1 K Uptake and Distribution

Plants take up K as monovalent cation, K^+. Uptake of K in plant tissues occurs at high rates due to relatively high permeability of plant membranes to K. This high permeability of membranes to K results from ionophores located in membrane that enables facilitated diffusion. Besides the passive uptake, K also enters plant roots via high- and low-affinity transporters (Giertha and Maser 2007; Wang and Wu 2013). Studies employing electrophysiology techniques indicated that passive transport of K occurs through ion channels with millimolar K_m denoting low-affinity and active transport through H^+-cotransporters with micromolar K_m denoting high-affinity transporters. Once inside the plant, K is highly mobile at all levels, i.e. within individual cells, tissues and in long-distance transport via the xylem and phloem. The bulk of K is taken up during vegetative phase. A large flux of K from shoot to root is maintained through phloem, which is crucial to maintain K homeo-

stasis and to provide a constant supply of cations to accompany anions like NO_3^- for their movement towards the shoot.

20.4.1.3.2 Biological Functions of K

Unlike other elements, K is not metabolised in the plant and it forms only weak complexes in which it is readily exchangeable. K has an exceptional role in plant-water relations. Besides maintaining turgor, it is required for activating an array of enzymes in metabolic reactions. It is maintained in the range of 100–200 mM in the cytosol, and almost similar concentration is found in chloroplast. This storage pool is called 'metabolic pool', which is not replaceable by other inorganic cations such as Na^+. However, K concentration in vacuole may vary between 10 and 200 mM or sometimes may even reach 500 mM, and this storage pool is called 'non-metabolic pool', frequently replaced by other cations.

In cytosol, K is involved in enzyme activation; specific enzymes include vacuolar pyrophosphatases (PPases) that accumulate protons into the vacuolar lumen and are strictly dependent on K. Besides this, many enzymes involved in carbon metabolism such as pyruvate kinase, phosphofructokinase and ADP-glucose starch synthase are also activated by K. It is essential for chlorophyll development and catalyses normal carbohydrate breakdown during respiration. Protein synthesis mediated by ribosomes is another key process that requires high concentrations of K. The metabolic enzymes involved in transcriptional and post-transcriptional regulation are also affected by K status of the plants, thereby influencing the metabolism. K is involved in the up-regulation of malic enzyme and assimilation of nitrate via the GS/GOGAT pathway, while the uptake of nitrate and its reduction are downregulated (Armengaud et al. 2009). It plays an important role in photosynthesis since it is the dominant counterion to the light-induced proton flux across the thylakoid membrane and for establishing the transmembrane pH gradient required for ATP synthesis (photophosphorylation). The dominant role of K is in turgor maintenance and water homeostasis termed as osmoregulation. The turgor pressure-driven solute transport causes cell extension, stomatal movements and other photonastic and seismonastic movements. Moreover, the loading and unloading of sucrose in phloem are also dependent on K concentration in the sieve tubes.

20.4.1.3.3 Symptoms of Deficiency and Excess K

As with N and P, K is also easily redistributed in plant tissue, so the deficiency symptoms first appear on the older leaves. The typical symptom of K deficiency is the development of chlorosis which later turns into necrotic lesions on the leaf tip spreading downwards on the margins. Under severe K deficiency, the younger leaves also become chlorotic (Fig. 20.3). In maize, root development is poor without lateral roots and the stalks are weak. Lignification of vascular bundle is generally impaired making the plant prone to lodging. Other characters of K deficiency are rolling of leaves and shortening of internodes leading to stunted growth. Severe K deficiency causes accumulation of reducing sugars and depletion of organic acids and synthesis of toxic amines such as putrescine and agmatine by the decarboxylation of arginine.

High K content in the growing medium does not usually produce any toxic symptoms in plant. This may be because uptake of K in excess represents 'luxury consumption' and it is stored in the vacuole without any increment in growth.

20.4.1.4 Sulphur

Soils contain inorganic and organic forms of S, but the major soil S reservoir is organically bound S. The organic S is mostly present in the form of phenolic and choline sulphates as well as lipids and amino acids, and the C/N/S ratio in soil organic matter is approximately 125:10:1.2. In saline and sodic soils, inorganic salts are predominant. Under aerobic soil condition, inorganic S is present primarily as sulphate (SO_4^{2-}): the preferred form for uptake by plants. Under waterlogged conditions, inorganic S occurs in reduced forms such as FeS, FeS_2 and H_2S. Sulphate reduction under waterlogged (anaerobic) condition is carried out by bacteria belonging to genus *Desulfovibrio* leading to formation of H_2S. The

H_2S undergoes oxidation to elemental S by chemotrophic S bacteria such as *Beggiatoa* and *Thiothrix*. The total S content in soil varies in the range from 0.005 to 0.04 %.

20.4.1.4.1 S Uptake, Distribution and Assimilation

In addition to S uptake by roots as SO_4^{2-}, plants can also absorb S from the atmosphere in the form of SO_2 through stomatal openings. Uptake of SO_4^{2-} is an active process as it is absorbed via plasma membrane sulphate transporters energised by H^+ gradient. The proton-coupled cotransport occurs with $3H^+/SO_4^{2-}$ stoichiometry. Transcription of this type of mechanism is induced when S becomes deficient. Analysis of genome sequences of Arabidopsis and rice has led to the identification of 14 putative sulphate transporter genes in each genome. These genes are subdivided into 4 closely related groups and all having 12 membrane-spanning domains and a STAS domain at their carboxy-terminus (Aravind and Koonin 2000). The fifth group is more diverse but related with two smaller proteins lacking the STAS domain (Hawkesford 2003). However, only groups 1 and 2 transporters have sulphate transport activity. In roots, the genes belonging to *Sultr* family are involved in sulphate uptake that are located in epidermal and cortical plasma membranes. The high-affinity sulphate transporters which belong to group 1 operate at low sulphur concentration (K_m: 1.5–10 μM). However, group 2 sulphate transporters belong to low-affinity transport activity and are constitutively expressed under high sulphate concentration (K_m: 99.2 μM and 1.2 mM). Group 2 transporters, *Sultr2;1* (Km 0.41 mM) and *Sultr2;2* (Km 1.2 mM), have specific functions in the vascular movement of sulphate. *Sultr2;1* is expressed in the xylem parenchyma and phloem cells in leaves, but *Sultr2;2* is localised specifically in phloem of roots and vascular bundle sheath cells of leaves (Buchner et al. 2004).

Inside plants, SO_4^{2-} is highly mobile resulting in its rapid transport from root to shoot tissues through xylem. Its translocation is mainly in an upward (acropetal) direction. Once sulphate is taken up, bulk of it is reduced in shoot chloroplasts in the presence of light, while some may be reduced in root plastids. Surplus S is deposited in vacuoles as SO_4^{2-}. When SO_2 reacts with water in cells forms bisulphite (HSO_3^-), and in this form, it inhibits photosynthesis and causes chlorophyll degradation.

The S assimilation involves activation of SO_4^{2-} ions by ATP forming adenosine phosphosulphate (APS). The APS then serves as a substrate for the synthesis of sulphate esters (sulpholipids), polysaccharides or secondary metabolite (glucosinolates). Through another pathway, APS is reduced and incorporated into amino acid cysteine, the first stable product of a series of reactions involving glutathione, reduced ferredoxin and acetyl serine. The enzymes of assimilatory SO_4^{2-} reduction are localised in the chloroplasts and to some extent also found in roots.

20.4.1.4.2 Biological Functions of S

Most S is found in reduced form in the amino acids cysteine and methionine and hence a constituent of protein. Both of these amino acids are precursors of other S-containing compounds such as coenzymes (CoA), vitamins (biotin, thiamine) and secondary metabolite (glucosinolates, alliins). S is a structural constituent of these compounds (e.g. R^1-C-S-C-R^2) or acts as a functional group, sulfhydryl (R-SH or thiol), that can undergo reversible oxidation. The formation and disruption of these S bridges affect the tertiary and quaternary structure of protein, thereby influencing protein activity. The production of glutathione, a tripeptide having –SH group, serves many functions in plant. Glutathione is readily water soluble and acts as a powerful antioxidant, present mostly in chloroplasts. Glutathione may function as a transient storage pool of reduced S and also a precursor of phytochelatins or 'class III metallothioneins'. These phytochelatins have a general structure of (Glu-Cys)*n*-Gly, where *n* is between two and more than ten and functions in detoxifying heavy metals. Research in the direction of increasing phytochelatin production is being carried out in order to make (crop) plants more tolerant to pollutants such as arsenic and Cd^{2+} and to improve the phytoremediation potential. Another important family of thiols in plants is thioredoxins, which functions as regulatory

protein in carbon metabolism. In reduced form, thioredoxin activates several enzymes of Calvin cycle.

The biomembranes contain S as sulpholipids; in chloroplast thylakoids, they are essential for the stabilisation of photosystem components. Sulpholipids are also involved in the regulation of ion transport across biomembranes. High levels of sulpholipids in roots provide tolerance to plants against salt. The S as in glucosinolates is stored in vacuoles, and their hydrolysis catalysed by enzyme myrosinase releases sulphate that can be recycled under S-deficient condition (Grubb and Abel 2006).

20.4.1.4.3 Symptoms of Deficiency and Excess S

Unlike nitrogen, S deficiency symptom first appears in the younger most leaf (Fig. 20.3). Chlorosis occurs throughout the entire leaf including vascular bundles (veins). Under severe S deficiency, inhibition of protein synthesis takes place which also drastically decreases chlorophyll content of leaves. In S-deficient plants, there is an accumulation of soluble organic nitrogen and nitrate.

Plants are comparatively insensitive to high SO_4^{2-} concentrations in the nutrient media. However, if the SO_4^{2-} concentration increases beyond 50 mM as in some saline soils, plant growth is adversely affected. The symptom of excessive S is a reduction in growth rate and dark-green leaves. High SO_2 in the environment is toxic to plants as it causes bleaching of chlorophyll characterised by necrotic symptoms in leaves.

20.4.1.5 Calcium

Calcium is abundant in the lithosphere, constituting about 3.64 % of the Earth's crust. The Ca-containing minerals are Al-silicates (feldspars and amphiboles), Ca phosphates and Ca carbonates. Severely weathered soils followed by leaching lead to Ca-deficient soil, which is accelerated by low soil pH. The Ca^{2+} adsorbed on soil colloids may be exchanged with the soil solution resulting in 'free' Ca^{2+} that forms insoluble compounds with elements such as P, thus making it less available. Most soils contain enough levels of Ca^{2+} in soil solution, and their exchange sites are well enough saturated with Ca^{2+} to adequately meet the crop demand.

20.4.1.5.1 Ca^{2+} Uptake and Distribution

Plants absorb calcium as a divalent cation, Ca^{2+}. The uptake potential of Ca is lower than other cations such as K^+ even though the Ca concentration of soil solution is ten times higher than K. This is because Ca absorption takes place only through young root tips in which the endodermis cell walls are still unsuberised. Uptake of Ca from soil solution by roots is mainly a passive process, and even within the plant tissues, Ca translocation occurs passively. The upward translocation of Ca in xylem sap occurs with the transpiration stream. Since Ca precipitates as Ca-phosphate in the phloem sap, the downward translocation of Ca is very slow. Ca enters the root through Ca^{2+}-permeable channels. Some of these channels are Ca^{2+} selective, but others are 'nonselective' ion channels. The identity of the specific protein(s) that mediates Ca^{2+} uptake is still unknown (Demidchik and Maathuis 2007). Within the plant, the major Ca^{2+} transporter at the plasma membrane and also at endoplasmic reticulum is a Ca-pumping ATPase (Ca^{2+}/H^+ antiporter). However, inside the plant, Ca is rather immobile; the ions have a tendency to be sequestered in the large vacuole of mature cells. No transporters have been identified that are responsible for loading Ca^{2+} into xylem vessels. A proportion of xylem Ca^{2+} is contributed by the apoplast. However, Ca^{2+} mobility is low in the vascular system.

20.4.1.5.2 Biological Functions of Ca

Cellular functions of Ca^{2+} are mainly concerned with structure and as a secondary messenger. It is a constituent of middle lamella in the form of calcium pectate and helps to cement the wall of cells together. The proportion of calcium pectate in the cell walls is of particular importance as it is responsible for the susceptibility of tissue to fungal and bacterial infections and also for fruit ripening. Ca is also present as insoluble crystals of Ca-oxalate called raphides and sphaeraphides in the vacuoles of some plant species (e.g. in *Colocasia*, raphides are responsible for itching

sensation). Ca is essential for the formation of cell membranes and lipid structures. Most of the Ca present in plant tissues is localised in the apoplast and in vacuoles. The Ca^{2+} concentration of cytoplasm is low ranging from 10^{-6} to 10^{-8} M. Such a low cytosolic concentration is of vital importance because higher Ca^{2+} concentration inhibits various enzymes located in cytoplasm as well as in chloroplasts. However, Ca concentration is much higher in the mitochondria. In cytosol, Ca is reversibly bound to a small protein, a polypeptide of 148 amino acids, called calmodulin (*cal*cium-*modul*ated prote*in*). Calmodulin then activates downstream events in the signalling cascade by phosphorylation of soluble and membrane-bound proteins. Ca in small amounts is essential for normal mitosis and is associated with chromatin or mitotic spindle organisation.

The formation of secretory vesicles and their fusion with plasma membrane leading to exocytosis require Ca. This helps in the formation of cell wall from the precursor cellulose as well as the formation of mucilage and callose. In root-caps, secretion of mucilage depends on extracellular (apoplasmic) Ca concentration. One of the vital functions performed by Ca is cell membrane stabilisation which is brought about by bridging phosphate and carboxylate groups of phospholipids and protein at membrane surfaces. Ca present in vacuole contributes to cation-anion balance by acting as a counterion for inorganic and organic anions. Free Ca at a very low cytosolic concentration (0.1–0.2 μM) acts as secondary messenger. A wide range of stimuli evokes rapid changes in free cytosolic Ca^{2+} in plants including responses to abiotic and biotic stresses, stomatal regulation and physical damage.

20.4.1.5.3 Symptoms of Ca Deficiency

The characteristic symptom of severe Ca deficiency is the twisting and deformation of growing tips and youngest leaves (Fig. 20.3). At a more advanced stage, necrosis occurs at the leaf margin. Ca deficiency also causes disintegration of cell wall and collapse of the affected tissues such as the petioles and upper part of the stems. In fast-growing tissues, Ca^{2+} levels may fall below a critical level leading to development of diseases such as 'blossom-end rot' in tomatoes, 'black heart' in celery and 'bitter pit' in apples. Ca influences the permeability of plasma membrane, thus its deficiency makes the membrane leaky. Ca also mediates starch hydrolysis to sugar, hence its deficiency results in the accumulation of starch in leaves. The number of mitochondria in wheat roots reduces under Ca deficiency.

20.4.1.6 Magnesium

Magnesium is derived from the Greek word '*Magnesia*'. The Mg content of soil varies between 0.05 and 0.5 %. Easily weatherable ferromagnesian minerals such as biotite, serpentine, hornblende and olivine and other secondary clay minerals add Mg to the soil. In soil solution just like Ca^{2+}, Mg^{2+} is also present in fairly high concentration between 2 and 5 mM. The size of hydrated ion is small (0.428 nm radius), due to which Mg adsorption to soil particles is relatively weak resulting in high leaching loss to the tune of 2–30 kg per hectare. Such losses lead to recurring Mg deficiency in crops.

20.4.1.6.1 Mg Uptake and Distribution

In plants, the free cytoplasmic Mg^{2+} remains at a concentration of about 0.5 mM (Yazaki et al. 1998), but total Mg^{2+} levels may vary from 0.3 to 1.0 %. Transport of Mg^{2+} is passive, mediated by ionophores in which Mg^{2+} moves down against the electrochemical gradient. In this transport system, Mg^{2+} faces cation competition; therefore, if an excess of other cations such as K^+ or NH_4^+ is present in the medium, the uptake of Mg^{2+} is adversely affected. Mg^{2+} is very mobile in the phloem and so can be translocated from older to younger leaves or the meristem. Since Mg^{2+} has a primary role in photosynthesis, the highest tissue Mg^{2+} concentration is usually measured in shoots. Most of the Mg^{2+} taken up by plants is stored in the vacuole where it contributes to turgor generation and charge balancing of anions.

20.4.1.6.2 Biological Functions of Mg

Mg occupies the central position in the porphyrin structure of chlorophyll molecule where it coordinates covalently with four N atoms. Insertion of

Mg^{2+} atom in the porphyrin structure during chlorophyll biosynthesis is catalysed by the enzyme Mg^{2+}-chelatase (Sirijovski et al. 2008). Mg plays a crucial role in photosynthesis, particularly in promoting the light reactions in the stroma of chloroplast. Carbohydrate partitioning is also regulated by Mg^{2+} concentration in the tissues. The loading of sucrose into the phloem in source leaf requires H^+-pumping ATPase, and for the optimal activity of this enzyme, about 2 mM Mg^{2+} is essential. The majority of cellular Mg^{2+} functions as enzyme cofactors in the stabilisation of nucleotides and nucleic acids. Some important enzymes activated by Mg^{2+} are phosphokinases, dehydrogenases and enolases. Activation of ribulose 1,5 *bis*phosphate carboxylase during light reaction occurs due to Mg^{2+} influx into the thylakoid. The most prominent enzyme reactions where Mg^{2+} is indispensable are those associated with energy transfer and phosphorylation/dephosphorylation. In these reactions, Mg^{2+} forms a bridge between the pyrophosphate structure of ATP or ADP and the enzyme molecule (Fig. 20.5). Mg plays an important role in gene transcription and translation as it readily binds to nucleic acids. As a result, DNA-melting temperatures remain considerably higher in the presence of Mg^{2+}. In RNA also, Mg^{2+} has similar roles and helps in maintaining secondary structure. Mg stabilises the ribosomal subunits necessary for protein synthesis and also has a similar stabilising effect in the matrix of the nucleus. Further, the transfer of aminoacyls from aminoacyl tRNA to the polypeptide chain is also activated by Mg^{2+} (Fig. 20.5).

20.4.1.6.3 Symptoms of Mg Deficiency

The first symptom of Mg deficiency is interveinal chlorosis of older leaves. In maize seedlings, leaves develop purple coloration along with necrotic spots when Mg was completely omitted from the growth medium (Fig. 20.3). In dicotyledonous plants including grapes, beans, potatoes and sugar beet, Mg^{2+}-deficient leaves become stiff and brittle and the intercostal veins twist in addition to chlorosis. Protein synthesis is also adversely affected resulting in poor growth of plants. Other ultrastructural changes in Mg^{2+}-deficient leaves include irregular shape of grana with reduction in their numbers, accumulation of starch grains in chloroplast, deformation of the lamellar structure, underdeveloped cristae of mitochondria and decrease in chlorophyll and carotenoid contents. These symptoms of ultrastructural disorganisation lead to visual Mg deficiency symptoms. In maize roots, increased suberisation of endodermis and hypodermis occurs due to Mg deficiency.

20.4.2 Physiological Functions of Micronutrients

20.4.2.1 Iron

Iron makes up about 5 % by weight of Earth's crust and is present in almost all soils. The primary minerals of Fe are ferromagnesian silicates such as olivine, augite, hornblende and biotite. The concentration of plant available Fe in soils is extremely low. The Fe solubility in soil is largely controlled by pH of soil solution. At higher pH (7.4–8.5), the solubility is at minimum, while at low pH or acid, soils Fe availability is very high. Further, in aerated soils, maintained at physiological pH range, the concentrations of Fe^{2+} and Fe^{3+} are below 10^{-15} M. Under waterlogged or reduced soil condition, Fe^{3+} is reduced to F^{2+} and this reduction is brought about by anaerobic bacteria which uses Fe oxides as electron acceptors in respiration.

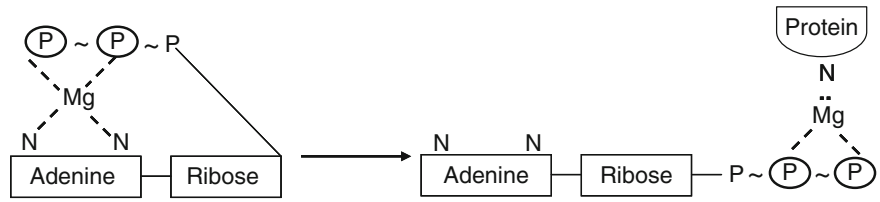

Fig. 20.5 Magnesium acts as bridging molecule between nitrogen and phosphoryl groups in ATP (Source: Marschner 1995)

20.4.2.1.1 Uptake and Distribution

The preferred uptake form of Fe by plants is the reduced cation Fe^{2+}. Fe cannot be taken up in ionic form as such; it has to chelate with other compounds that facilitate Fe uptake. Plant species differ in their ability to utilise sparingly soluble inorganic Fe and Fe chelates. Plants grown under Fe stress show physiological and morphological changes that help in Fe uptake. The mechanism of Fe uptake in plants via roots is based on two strategies: *Strategy I* involves reduction of Fe^{3+} to Fe^{2+} carried out by membrane-bound enzyme ferric-chelate reductase (FCR). The reduced form of iron is transported into root cells through the metal transporter protein called *i*ron-*r*egulated *t*ransporter (IRT). This mechanism operates in dicotyledonous plants. *Strategy II*, a chelation-based mechanism occurs in Poaceae (grass) family where roots release phytosiderophores (PS) which form stable complexes with Fe^{3+} and is taken up by plant as Fe^{3+}-PS chelate (Romheld and Marschner 1986). The gene encoding FCR enzyme belongs to the FRO family and IRT belongs to ZIP family (Guerinot 2000; Wu et al. 2005; Mukherjee et al. 2006; Jeong et al. 2008). In Arabidopsis, *FRO2* gene identified in root has been found to encode for Fe^{3+}-chelate reductase (Mukherjee et al. 2006). The mechanism of Fe uptake by roots is strongly regulated by a complex system, which involves transcription factor, bHLH, of which PYE and FIT/FER play a central role (Ivanov et al. 2012). Besides IRT, Fe uptake takes place through other transporters present in the leaf cells. These include several members of the YSL (yellow stripe-like) family expressed in leaves (such as AtYSL1, AtYSL3 and AtYSL2 in *A thaliana* and OsYSL2 and OsYSL15 in rice), but their expression is usually confined to the vascular tissue (Curie et al. 2009).

In rapidly growing plants, about 80 % of Fe is stored in chloroplasts. It is localised in the plastid stroma as phytoferritin. Some amount of phytoferritin is also detected in xylem, phloem and seeds. It also acts as storage for Fe in nodules of legumes. The major form of Fe transported through the xylem is ferric citrate.

20.4.2.1.2 Biological Functions of Fe

Being a redox-active metal, Fe plays a role in photosynthesis, mitochondrial respiration, assimilation of nitrogen, synthesis of chlorophyll and hormone (ethylene, gibberellic acid, jasmonic acid), osmoprotection, pathogen defence and production and scavenging of reactive oxygen species. Based on the type of iron ligand, there are three groups of Fe-containing proteins:

1. *Proteins with Fe-sulphur clusters (Fe-S)*: The Fe-S clusters are synthesised from inorganic Fe and sulphide (Fig. 20.6). These nonheme-proteins with Fe-S clusters have a key role in electron transfer. They constitute part of substrate-binding sites in enzymes, form iron storage moieties, are involved in transcriptional or translational regulation, control protein structure in the vicinity of the cluster and are also involved in disulphide reduction and sulphur donation (e.g. in thioredoxins). Therefore, Fe-S proteins serve as enzymes, as electron carriers (e.g. ferredoxin) and as regulatory proteins (e.g. aconitase).
2. *Fe-containing hemeproteins*: The well-characterised hemeproteins are the cytochromes involved in electron transfer reactions of photosynthetic and respiratory systems,

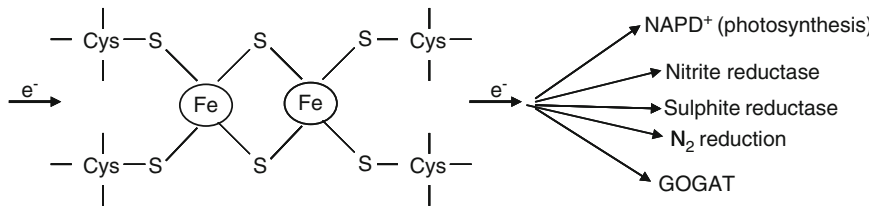

Fig. 20.6 Role of ferredoxin as an electron carrier in a number of basic metabolic processes (Source: Marschner 1995)

which contain a heme Fe-porphyrin complex. The oxidative enzymes like catalase, peroxidase, and NADPH oxidase involved in the production and scavenging of free radicals contain hemeprotein. Another very large group of enzymes called cytochrome P450 also contains hemeprotein. Globins such as leghemoglobin in nodules of legumes are involved in oxygen binding and transport. Nitrite reductase and sulphite reductase enzymes involved in N and S assimilation contain a siroheme and a Fe-S cluster in the enzyme. Hemeproteins are distributed all over the locations in subcellular organelles, for example, cytochrome P450 is localised in endoplasmic reticulum, catalase in peroxisomes and other enzymes in the cytoplasm.

3. *Other Fe proteins*: These proteins are grouped as nonhemeproteins, which bind Fe ions directly. Ferritins or phytoferritins are most common among this group of proteins. Ferritins control the interaction between Fe homeostasis and oxidative stress in plants. These are high-molecular-weight 24-mer proteins that store up to 4,500 Fe atoms in soluble and bioavailable form. Mostly nongreen plastids such as etioplasts and amyloplasts contain ferritins, but they are not found in mature chloroplasts (Briat and Lobreaux 1998).

20.4.2.1.3 Symptoms of Deficiency and Excess Fe

Since Fe is relatively immobile inside the plant tissue, the visual deficiency symptoms first appear on young growing organs. The young leaf shows interveinal chlorosis, and in severe cases, the leaf becomes white (due to loss of chlorophyll) which later dries out. In contrast, the mature leaves may not show chlorosis at all. Lack of Fe inhibits protein synthesis.

Excess Fe uptake leads to toxicity, which is a major problem in wetland rice-cropping systems. In rice, symptoms of Fe toxicity involve development of tiny brown spots that later spreads into uniform brown colour, called bronzing. The Fe concentration in rice leaves remains excessively high in the range of 300–1,000 μg Fe per g dry weight.

20.4.2.2 Copper

The soil contains Cu in divalent cation form, Cu^{2+}, in the range of 5–50 ppm. However, the concentration of Cu in soil solution is very low 10^{-8} to 60×10^{-8} M. More than 98 % of Cu is bound to organic matter in soil, which is an important factor regulating Cu mobility in soil. Cu is absorbed both as divalent cupric (Cu^{2+}) ion in aerated soils or monovalent cuprous (Cu^{1+}) ions in waterlogged soils.

20.4.2.2.1 Uptake and Distribution

Plants take up Cu in very little quantities, so the Cu content in plant tissues varies from 2 to 20 ppm. Cu uptake is an active process requiring metabolic energy. Under physiological pH, Cu exists in two oxidation states Cu^{1+} and Cu^{2+} and can interchange between these forms (monovalent copper is unstable). This property of Cu is responsible for its redox function in biochemical reactions. Cu has high affinity for N atom of amino groups, sulfhydryl groups, carboxylic groups and phenolic groups. More than 98–99 % of Cu is present in these complexed forms in soil solution and in xylem and phloem sap. Cu ions can catalyse the production of free radicals, which is potentially toxic leading to the damage of proteins, DNA and other biomolecules. Therefore, immediately following Cu uptake, the metal-scavenging proteins like metallothionein bind majority of Cu ions, thus preventing it from accumulating at a toxic level.

20.4.2.2.2 Biological Functions of Cu

Cu is of utmost importance for life. It is essential for photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection and cell wall synthesis. Three different types of Cu-protein exist in plants in which Cu is the metal component. These are:

Type 1: *Blue Cu-proteins*, without oxidase activity that functions in one-electron transfer. Common example of this is plastocyanin; more than 50 % of Cu localised in chloroplast remains in this form. Plastocyanin is a component of electron transport chain of photosystem I in photosynthesis.

Type 2: *Non-blue Cu-proteins,* which are peroxide-producing oxidases and oxidise monophenols to diphenols.

Type 3: *Multi Cu-proteins*, contains at least four Cu atoms per molecule which act as oxidases. Multi-Cu enzymes contain all three types of Cu, e.g. ascorbate peroxidase and diphenol oxidase. Cytochrome oxidase is a mixed Cu-Fe protein catalysing the terminal oxidation in mitochondria.

Recent studies have revealed that more than 100 Arabidopsis proteins are predicted to be complexed with Cu (Kramer and Clemens 2005). The high affinity of Cu to dioxygen molecules explains the role of Cu as a catalytic metal in many oxidases. Cu metabolism is also intimately linked to Fe metabolism. Depending on the bioavailability of Cu and Fe, plants possess enzymes for the alternative use of Cu and Fe, thus catalysing the same biochemical reaction with completely different apoproteins. Such reactions include Cu-nitrite versus heme-nitrite reductase, Cu/Zn-superoxide dismutase versus Fe-superoxide dismutase and cytochrome oxidase versus di-iron oxidase. Cu has also been reported to be a part of the ethylene receptor and is involved in molybdenum cofactor biosynthesis (Rodriguez et al. 1999; Kuper et al. 2004).

20.4.2.2.3 Symptoms of Deficiency and Excess Cu

Visible symptoms of Cu deficiency appear in young leaves that often become dark green in colour and are twisted exhibiting necrotic spots. However, in cereals, the leaf tip becomes white and the leaves narrow and twisted. The growth of internodes is depressed leading to bushy appearance and stunted growth. Pollen grain viability is affected resulting in reduction in panicle formation. Lignin synthesis is impaired due to lack of two important Cu-containing enzymes, phenolase and laccase. Of the total Cu concentration in plants, almost half is found in the chloroplasts where it plays an important role in photosynthetic reactions. Cu deficiency symptom in citrus is called 'die back' because young leaves die out.

The critical toxicity level of Cu in the leaves is above 20–30 µg Cu per g dry weight. The ability of Cu to displace other metal ions like Fe from physiologically active sites is associated with Cu toxicity symptoms. Thus, the Cu toxicity symptom in plants will manifest as Fe deficiency symptom. Other symptoms of toxicity include inhibition of root growth more than shoot growth. There are some Cu-tolerant species called metallophytes, which can tolerate Cu content as high as 1,000 µg Cu per g dry weight.

20.4.2.3 Zinc

The Zn content of the lithosphere is about 80 ppm and it is usually present in soil in the range of 10–300 ppm. The ionic radius of Zn^{2+} is similar to that of Fe^{2+} and Mg^{2+} due to which Zn^{2+} may replace these elements by isomorphous substitution in the mineral structure. Zn interacts with organic matter in soil and forms both soluble and insoluble Zn-organic complexes. The soluble Zn-organic complexes are mainly associated with amino, organic and fulvic acids, while the inorganic complexes are derived from humic acids.

20.4.2.3.1 Uptake and Distribution

Plants absorb Zn in the form of divalent cation. Uptake of Zn is metabolically controlled, that is, Zn uptake is an active process requiring energy. The form in which Zn is translocated from roots to shoots is not clear. Mobility of Zn inside the plant organs is relatively less, so there is accumulation in root tissues particularly when Zn concentration in the media is high. In older leaves, Zn can become very immobile. Much of the Zn absorbed is localised in the seeds and grains in the protein bodies in the form of globoid crystals. These globoids consist mainly of phytate or salts of phytic acid.

Zn transport via roots takes place through transporters belonging to ZIP family (*Z*RT, *IRT-like p*rotein). ZRT1 and ZRT2 (zinc-regulated transporter) are designated as the high- and low-affinity Zn transporters, respectively. Till now, over 25 ZIP family members have been identified which is subdivided into two subfamilies (reviewed by Guerinot 2000).

20.4.2.3.2 Biological Functions of Zn

Zn has a strong tendency to form tetrahedral complexes with N-, O- and S- ligands, which

help in regulating metabolic functions. Therefore, Zn plays a catalytic (functional) and structural role in enzyme reactions. Since it exists only as Zn(II), so it does not take part in oxidation-reduction reactions. Zn plays a crucial role in many biological processes. It is an integral component of many enzymes, alcohol dehydrogenase, carbonic anhydrase, Cu-Zn-superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase and RNA polymerase, to name a few. Large number of enzymes requires Zn for activation such as dehydrogenases, aldolases, isomerases and transphosphorylases. Zinc is important in DNA and RNA metabolism and protein synthesis and maintains the structural integrity of biomembranes. More than 1,200 protein molecules (Zn metalloprotein) have been identified including a large number of 'zinc-finger'-containing proteins and transcription factors, oxidoreductases and hydrolytic enzymes such as metalloproteases. Zn is a structural component of ribosomes thus essential for their structural integrity. It plays a major role in carbohydrate metabolism by regulating key enzymes, fructose 1,6-*bis*phosphatase and aldolase. Synthesis of auxin, indole acetic acid, is particularly impaired under Zn deficiency. Further, Zn also has a role in signal transduction through mitogen-activated protein kinases (MAPK).

20.4.2.3.3 Symptoms of Deficiency and Excess Zn

The most striking symptoms of Zn deficiency in dicotyledonous plants are stunted growth due to shortening of internodes called 'rosette' and a drastic decrease in leaf size termed as 'little leaf'. When the Zn deficiency is severe, the growing shoot apex shows 'die back' symptom. Further, these symptoms combined with chlorosis produce 'mottled leaf'.

The critical toxicity levels of Zn in the leaves ranges between 100 to 300 µg or more per gram dry weight. Symptoms of Zn toxicity are inhibition of root elongation, chlorosis in young leaves and inhibition of photosynthesis.

20.4.2.4 Boron

The soil contains B in the range of 20–200 ppm. The primary mineral tourmaline contains about 3–4 % B. Only the monomeric species $B(OH)_3$ and $B(OH)_4^-$ are present in soil solution depending on soil pH.

20.4.2.4.1 Uptake and Distribution

Uptake of B is a non-metabolic process and its distribution in the plants is also governed by transpiration stream. Boric acid channels, which are major intrinsic proteins, facilitate B transport across membranes. Nodulin26-like intrinsic protein 5;1 (NIP5;1), identified from Arabidopsis, is involved in efficient uptake of B into root cells under B-stress condition. Another channel protein, AtNIP6;1, helps in preferential distribution of B in young shoot tissues (Takano et al. 2006). Besides boric acid channels, transporters have also been identified from Arabidopsis, viz., BOR1, BOR2 and BOR4 (Takano et al. 2002). BOR1 is the first B transporter identified by analysis of Arabidopsis *bor1-1* mutant which requires high levels of B for normal leaf expansion (30 mM) and fertility (100 mM). BOR1 encodes an efflux-type B transporter which is located in the plasma membrane and is expressed in root cells including endodermis (Noguchi et al. 1997). Thus, BOR1 is required for effective xylem loading under B-limited conditions, and it is also involved in the preferential distribution of B to young leaves. BOR2 encodes a B-efflux transporter located in plasma membrane and is strongly expressed in epidermis of elongation zones of roots and lateral rootcaps. At toxic B concentrations, BOR4 is stably accumulated in plasma membrane and confers high B tolerance to plants suggesting its involvement in B tolerance by exporting the mineral out of symplast (refer to Miwa and Fujiwara 2010). Certain plant organs such as anthers, stigma and ovary contain high concentrations of B, which is twice as high as in stem.

20.4.2.4.2 Biological Functions of B

B is involved in a wide range of biological functions but the exact metabolic functions are not exactly understood. These important physiological processes include protein synthesis, transport of sugars, respiration and the metabolism of plant hormones (indole acetic acid), RNA and carbo-

hydrate. Other functions of B are related to cell wall synthesis and lignification, cell wall structure maintenance by cross-linking of polysaccharides and regulation of the structural integrity of biomembranes. B activates enzymes like plasmalemma ATPase thereby increasing the transport of chlorine and phosphorus. Stimulation of H^+ pumping by B causes hyperpolarisation of membrane potential. Since the wall-associated kinase in the plasma membrane has an extracellular matrix in connection with the pectin molecule, the membrane cell wall connection is B dependent. B promotes structural integrity of biomembranes and formation of lipid rafts. It also influences pollen germination, pollen tube growth and subsequently fertilisation.

20.4.2.4.3 Symptoms of Deficiency and Excess B

Death of the root and shoot tips occurs due to B deficiency resulting in stunted (rosette) plant growth. Leaves develop a thick coppery texture and become curled and brittle. B also affects flower retention, pollen formation, pollen tube growth or germination, N fixation and nitrate assimilation. Root elongation is inhibited and root tips become swollen and discoloured. The fleshy tissue in fruits disintegrate causing disorders like 'heart rot' in sugar beet, 'water core' in turnip and 'browning' of cauliflower.

Typical B toxicity symptoms appear on mature leaves producing marginal or tip chlorosis and necrosis. The critical B toxic content in tissue varies depending on the plant species; however, it ranges from 100 to 1,000 mg per kg dry weight.

20.4.2.5 Manganese

The primary rock minerals containing Mn are pyrolusite (MnO_2) and manganite [MnO (OH)]. Total Mn levels of soil vary between 200 and 3,000 ppm. In biological systems, Mn occurs in oxidation states II, III and IV, with Mn(II) and Mn(IV) being fairly stable and Mn(III) unstable. In plants, Mn(II) is the dominant form, but it can readily undergo oxidation reaction and form Mn(III) and Mn(IV). This property makes it possible for Mn to play a crucial role in redox reactions. The preferred form of uptake by plants is the divalent cation, Mn^{2+}. Under waterlogged condition, Mn availability increases just like Fe. Mn^{2+} can replace other divalent cations like Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} from their active uptake site.

20.4.2.5.1 Uptake and Distribution

Mn uptake is an active process involving metabolic energy. It is relatively immobile in the plants. It is preferentially translocated to meristematic tissues; therefore, young plant organs are rich in Mn. The genes involved in transport of transition metal in plants have been identified which are also responsible for Mn^{2+} transport. The gene families associated with Mn^{2+} transport include cation/H^+ antiporters, natural resistance-associated macrophage protein (NRAMP) transporters, ZIP transporters, cation diffusion facilitator (CDF) transporter family and P-type ATPases. These transporters are responsible for accumulation of Mn into the cell and its release from various organelles. The active sequestration of Mn into endomembrane compartments, particularly the vacuole and endoplasmic reticulum, also takes place by these transporters (reviewed by Pittman 2005).

20.4.2.5.2 Biological Functions of Mn

Mn is essential for plant metabolism and approximately 35 enzymes of a plant cell contain Mn in three oxidation states, II, III and IV. Mn can fulfil two functions in protein – it serves as catalytically active metal and it exerts an activating role on enzymes. Enzymes in which Mn has catalytic role are Mn-containing superoxide dismutase which protects the cell from damaging effects of free radicals, oxalate oxidase and Mn-containing water-splitting system of PS II (Barber 2003). Some of the Mn-activated enzymes are PEP carboxykinase, isocitrate dehydrogenase, malic enzyme and phenylalanine ammonia lyase (PAL). Manganese activation was seen in enzymes of nitrogen metabolism (glutamine synthetase, arginase), gibberellic acid biosynthesis, RNA polymerase activation and fatty acid biosynthesis.

20.4.2.5.3 Symptoms of Deficiency and Excess Mn

Visible symptoms of Mn deficiency first appear on younger leaves in the form of small yellow

spots and interveinal chlorosis. At ultrastructural level, Mn deficiency leads to disorganisation of chloroplast and lamellar system. The cell volume is reduced, cell walls dominate and the inter-epidermal tissue shrinks. 'Grey speck' in oats and 'marsh spot' in cotyledons of pea are the symptoms of Mn deficiency.

Mn toxicity symptoms are characterised by brown spots in older leaves surrounded by chlorotic areas. Excess Mn can also induce deficiency of other mineral nutrients such as Fe, Mg and Ca. The critical toxicity level of Mn varies from 200 (in maize) to 5,300 ppm (in sunflower) (Foy et al. 1988).

20.4.2.6 Molybdenum

Most soils contain Mo between 0.6 and 3.5 ppm. It occurs in soil as molybdate oxyanion, MoO_4^{2-}. A fraction of soil Mo also occurs in organic form.

20.4.2.6.1 Uptake and Distribution

Plants absorb Mo as molybdate ion (MoO_4^{2-}). However, the uptake may be reduced due to the competition by other anions like SO_4^{2-}, whereas ions such as PO_4^{2-} are known to enhance the uptake of Mo. The uptake of Mo in plant cells has been discovered recently (Tejada-Jimenez et al. 2007; Tomatsu et al. 2007; Baxter et al. 2008). Mo is cotransported through various mechanisms such as phosphate uptake system (Heuwinkel et al. 1992), P-type ATPases, heavy metal transporters (Palmgren and Harper 1999), sulphate transporters (Tweedi and Segel 1970) and nonspecific anion transporters (Mendel and Hansch 2002). The chemical properties indicate that it is transported as molybdate ion (MoO_4^{2-}), similar to sulphate (SO_4^{2-}), phosphate (PO_4^{2-}), tungstate (WO_4^{2-}) and vanadate (VO_4^{2-}). A putative sulphate transporter, *AtSultr5;2*, has been identified as molybdate transporter (MOT1) in Arabidopsis (Tomatsu et al. 2007). MOT1 is classified as high-affinity transporter (K_m <21 nM) expressed in both roots and shoots and located in plasma membrane and vesicles. In plants, Mo is localised mainly in the phloem and vascular parenchyma and is readily translocated throughout the system (Gupta 1997). Unlike other elements, Mo can be taken up in excess by the plants without resulting in any toxic effects.

20.4.2.6.2 Biological Functions of Mo

Mo is an essential component (cofactor) of a few important enzymes in higher plants. In these enzymes, Mo has both structural and catalytic functions and is involved directly in redox reactions. These enzymes are nitrate reductase, nitrogenase, xanthine oxidase/dehydrogenase and sulphite reductase. The physiological processes controlled by these enzymes are N assimilation, S metabolism, phytohormone biosynthesis and stress reactions. The final step of abscisic acid biosynthesis is catalysed by Mo-enzyme aldehyde oxidase, while sulphite oxidase protects the plant against toxic levels of sulphite. A novel Mo-enzyme, mitochondrial amidoxime reducing component (mARC), has been reported on the envelope of mammalian mitochondria. Here it is associated with detoxification and catalyses the reduction of N-hydroxylated amidines acting jointly with cytochrome *b*5 and cytochrome *b*5 reductase. The homologues of this new enzyme were found in plants and eubacteria.

20.4.2.6.3 Symptoms of Deficiency and Excess Mo

In Mo-deficient plants, particularly legumes, symptoms of N deficiency are common, producing chlorosis in younger leaves and stunted growth. In some dicotyledonous species, e.g. cauliflower, there is a drastic reduction in size and irregularities in the formation of leaf blade. Under such severe Mo deficiency, only the midrib of leaf is present giving it a whip appearance called 'whiptail'. Local chlorosis and necrosis along the main veins of mature leaves called 'yellow spots' are common in citrus.

Mo toxicity generally does not occur because there is a wide gap between the critical concentrations for deficiency and toxicity levels, which vary by a factor of 10^4 times (0.1–1,000 μg Mo per gram dry weight). However, if at all it is taken up beyond the toxic levels, malformation of leaves and a golden-yellow discoloration of shoot tissue occur.

20.4.2.7 Chlorine

In nature, chlorine is abundantly present since it is available in various sources such as soil

reserves, irrigation water, rain, fertilisers and air pollution. Therefore, in crop production, chloride toxicity is of major concern than its deficiency. It is present in aqueous form in soil as monovalent anion, Cl^-. It is not adsorbed to soil particles and is mobile thus highly prone to leaching.

20.4.2.7.1 Uptake and Distribution

Chlorine uptake is an active process and uptake occurs very rapidly in considerable amounts. However, its uptake also takes place against an electrochemical gradient by carrier proteins present in plasma membrane. In green tissue, under light Cl^- uptake is enhanced as ATP formation during photosynthetic phosphorylation provides energy source for active uptake. Uptake of chloride through transporters was confirmed after the discovery of chloride channel (CLC) family protein in *A. thaliana* and *Nicotiana tabacum* (Hechenberger et al. 1996, Lurin et al. 1996). Seven CLC genes each have been identified in *A. thaliana* (*AtClCa–AtClCg*; Marmagne et al. 2007) and rice (*Oryza sativa*, *OsClC1–OsClC7*; Diédhiou and Golldack 2005). These CLC proteins are involved in anion transport across plant membranes (reviewed by De Angeli et al. 2009).

Uptake of Cl^- faces competition with NO_3^- and SO_4^{2-}. Besides root, foliar absorption of Cl^- as chlorine gas also occurs. Chloride content in plant tissue is usually in the range of 50–500 μmol per kilogram of dry weight. In the cell, it is preferentially stored in the vacuole.

20.4.2.7.2 Biological Functions of Cl

Cl^- is known to be associated with more than 130 organic compounds in plants. Cl^- being a mobile anion, majority of its function is associated with electrical charge balance. It plays an important role in photosynthesis. In photosystem II (P_{680}) of oxygen evolving complex, there are three extrinsic polypeptides which contain Mn. Cl^- acts as a bridging ligand and stabilises the oxidised state of Mn in these associated polypeptides. H^+-pumping ATPase present on tonoplast is specifically stimulated by Cl^-. In guard cells of some plants, e.g. onion, Cl^- accumulation regulates opening and closing of stomata. Therefore, Cl^- indirectly affects plant growth by stomatal regulation. The seismonastic leaf movement of *Mimosa pudica* is directly controlled by this ion. The 'osmotic motor' for the leaf movement is powered by a plasma membrane H^+-ATPase which drives KCl and water fluxes.

20.4.2.7.3 Symptoms of Deficiency and Excess Cl

Cl^- deficiency symptoms include reduction in leaf surface area, wilting of leaf at margins, interveinal chlorosis of mature leaves and restricted and highly branched root systems. Under severe Cl^- deficiency, curling of the youngest leaf followed by shrivelling and necrosis takes place. In palm trees, besides wilting and premature senescence of leaves, frond fracture and stem cracking are typical Cl^- deficiency symptoms.

Toxicity of Cl^- is a more serious problem in agriculture. Cultivation on salt-affected soils shows Cl^- toxicity symptoms in plants. Typical symptoms of Cl^- toxicity are bronzing of leaf tips and margins, premature yellowing and abscission of leaves. Some crops such as sugar beet, barley, maize, spinach and tomato are highly tolerant, while tobacco, beans, citrus, potatoes and legumes are highly susceptible to Cl^- toxicity.

20.4.2.8 Nickel

Chemically Ni is related to Fe and cobalt. It occurs not only in Ni(II) oxidation states but also in I and III states in biological systems and forms stable complexes with cysteine, citrate and Ni enzymes. Ni forms chelates and can readily replace other heavy metals (like Fe) from physiologically important sites. Ni content of soil is usually less than 100 ppm. It is derived from the weathering of ultrabasic igneous rocks (serpentine mineral).

20.4.2.8.1 Biological Functions of Ni

Nickel was the 17th mineral element to be regarded as essential trace (micronutrient) element for plant growth. It was found to be a metal component in enzymes such as urease, dehydrogenases, hydrogenases and methyl reductases in a large number of bacteria. Earlier reports showed that low Ni concentration stimulates germination and growth of many crop species. The first evidence of Ni as a constituent of urease enzyme in higher plants (jack bean) was provided by Dixon et al. (1975). Later,

Ni requirement for legumes and nonlegumes was also established (Freyermuth et al. 2000). Some functions of Ni are now clearly defined and therefore it was included in the list of essential micronutrients (Eskew et al. 1983, 1984).

Nickel is taken up as divalent cation, Ni^{2+}. It is mobile in xylem and phloem, so after uptake, considerable amount of it is transferred to the seeds and fruits. Ni has an important role in N metabolism. The urease enzyme present in plants is involved in breakdown of urea to CO_2 and ammonia via the ornithine cycle. Accumulation of urea in the absence of urease would be toxic to the plant's cellular machinery. Moreover, recent findings suggest that in addition to urea metabolism, plant ureases have a protective role against phytopathogens (Follmer 2008).

20.4.2.8.2 Symptoms of Deficiency and Excess Ni

In crop plants, Ni toxicity is a major problem rather than deficiency. Under Ni deficiency, accumulation of toxic levels of urea might occur. However, the Ni toxicity symptoms closely related to Fe deficiency symptoms. Acute Ni toxicity gives rise to chlorosis. In cereals, pale yellow stripes along the length of the leaf occur. The whole leaf may turn white and necrosis occurs at leaf margins. In dicots, the Ni toxicity causes chlorotic patches between leaf veins, similar to Mn deficiency.

20.4.3 Beneficial Elements

Summary of beneficial elements regarding plant concentrations, beneficial effect, physiological mechanisms and deficiency or toxicity symptoms of nutrients (Pilon-Smits et al. 2009) is presented in Table 20.6.

20.5 Ion Absorption

The anions and cations freely dissolved in the soil solution are the most readily available forms to be absorbed by the roots, even though per se their concentration may be low. These nutrients reach the vicinity of roots in three ways: (1) diffusion of ions through the soil solution, (2) the passively charged ions carried along as water moves by bulk flow into the roots and (3) extending growth of roots towards the dissolved ions. In both higher and lower plants, ion uptake is characterised by three principles:

1. *Selectivity*: Plants preferentially take up certain mineral elements, while others are discriminated against or almost excluded. In soil solution, some mineral elements may be present in higher concentration, but they are not entirely required for plant growth. So, the plant has mechanism to exclude or selectively take up only those elements required for its growth.
2. *Accumulation*: The concentration of mineral elements can be much higher in the plant cell sap than in the external solution. It has been seen that the ion concentration in the root cell sap is generally much higher than that in the nutrient solution. This is evident in the case of potassium, nitrate and phosphate, which are accumulated at higher concentration in the root cells.
3. *Genotype*: Distinct differences among the plant species exist in terms of ion uptake. This difference depends on the condition of the growing media. For example, in alga species, *Nitella* grown in pond water had higher concentration of potassium, sodium, calcium and chloride in the cell sap, while in *Valonia* grown in highly saline sea water, only potassium remained at higher concentration in the cell sap, whereas the sodium and calcium concentrations remain at a lower level than in sea water.

The accumulation of ions in plant tissues in quantities more than the circumambient solution indicates that the ions diffuse through the cell against a concentration gradient. This type of ion uptake requires expenditure of metabolic energy, and therefore, it is dependent on the metabolic status of absorbing cells or tissues in plants. This phenomenon of ion or salt absorption called active absorption, and is most common in rapidly growing tissues such as meristematic cells, and it decreases with the increase in maturity of cells. There are some evidences which prove that the active absorption of solute requires metabolic

Table 20.6 Plant concentrations, beneficial effect, physiological mechanisms and deficiency or toxicity symptoms of nutrients categorised as beneficial elements

Element	Form	Plant concentration	Beneficial effects	Mechanisms hypothesised	Symptoms	Plant species/hyperaccumulators
Aluminium	Al^{3+}, $Al(OH)^{2+}$ $Al(OH)_2^{+}$	<0.1 % non-accumulators ≥0.1 % Al accumulators	Increases root and shoot growth Resistance to herbivore	Increases antioxidant activity Increases P availability Decreases Fe toxicity	Toxicity: inhibition of root growth, alteration in root architecture, disruption of root elongation, symptoms similar to P deficiency	*Miscanthus sinensis* (maiden grass), *Camellia sinensis* (tea, *Melastoma malabathricum*
Cobalt	Co^{2+} Co^{3+}	10^{-6} – 0.001 % non-accumulators ≥0.1 % Co accumulators	Increases growth, nodule number and weight, plant nutrient levels, as well as seedpod yield and seed quality in legumes Retardation of leaf senescence Enhancement of drought resistance in seeds Resistance to herbivore	Cobalamin (vit B_{12})-dependent enzyme systems in *Rhizobium*: methionine synthase, ribonucleotide reductase, methylmalonyl-coenzyme A mutase, inhibition of ethylene biosynthesis	Deficiency: affects nodule development, yellowing of leaves similar to N deficiency symptoms, lower plant N content, accumulation of Co in root nodules Toxicity: symptom resembles Mn deficiency	Families of Lamiaceae, Scrophulariaceae, Asteraceae and Fabaceae examples: *Pisum sativum* (Pea), *Lotus japonicus* (Lotus) *Crotalaria cobalticola* (indicator plant)
Sodium	Na^{+}	<0.05 % non-halophytes >0.25 % in halophytes	Increases plant growth, leaf area, number of stomata per unit leaf area Essential for C_4 and CAM plants Can facilitate nitrate uptake Improves water balance of plants	Cell expansion Stomatal regulation by substituting K and maintaining higher relative water content Regeneration of phosphoenolpyruvate	Enzymes activated by K are inhibited, less starch content in tissue, accumulation of sucrose	*Atriplex vesicaria*, *Zea mays* (maize), *Sorghum bicolor* (sorghum), members of Chenopodiaceae

Selenium	Se_2^-, SeO_3^{2-}, **SeO_4^{2-}**	≤0.01 % non –Se accumulators 0.01– 0.1 % Se accumulators ≥0.1 % Se hyperaccumulators	Increases plant growth Resistance to pathogen and herbivore attack Structural component of specific selenoproteins and seleno-tRNAs	Increases antioxidant activity Prevents P toxicity Replaces SO_4^{2-} and forms amino acids selenocysteine (SeCys) and selenomethionine (SeMet) Volatile Se (dimethylselenide) deters herbivores, Accumulated Se is toxic to herbivores and pathogens	Toxic levels in plants lead to disorders in animals feeding on them; acute toxicity leading to death, chronic blindness and paralyses, chronic alkali disease leading to lameness Deficiency of Se in produces symptoms of P toxicity in plants	*Astragalus bisulcatus* (milkvetch), species of *Xylorrhiza*, *Stanleyea*, *Brassica oleracea* (broccoli) Ryegrass, lettuce, potato, duckweed
Silicon	$Si(OH)_4$	<0.5 % most species 10–15 % horsetails	Maintains plant sturdiness thus preventing lodging, leaf erectness for better light interception Resistance to pathogen and herbivore attack Resistance to abiotic stress (heavy metals, salinity, drought, UV radiation, extreme temperature)	Strengthens cell walls Activation or synthesis of stress-related molecules Systemic acquired resistance, SAR-like bioactivity Regulation of metal transport (Mn and Fe) Increases antioxidant activity Sodium exclusion from roots	In animals, fed on high Si content feed leads to abrasion of rumen wall, secondary deposition in kidney	*Equisetum arvense* (horsetail millet), *Oryza sativa* (paddy rice), *Saccharum officinarum* (sugarcane)

energy: (1) higher rate of respiration increases salt accumulation inside the cell, (2) respiratory inhibitors check the process of salt uptake and (3) by decreasing oxygen content in the medium, the salt absorption also decreases. These evidences suggest that absorption of salt is directly coupled with respiratory rate and energy level in the plant body.

20.5.1 Pathway of Solute from External Solution into the Cells

The water and dissolved ions in the external solution move into the xylem cells of roots via three possible pathways: (1) through the cell walls or apoplast of epidermal and cortical cells; (2) through the cytoplasmic or symplast system, moving from cell to cell; and (3) from vacuole to vacuole of the living root cells where the cytosol of each cell forms part of the pathway. In the following paragraphs, this pathway is discussed in detail.

Mineral salts dissolved in water are absorbed by roots and taken up through the xylem vessel from where it is distributed to all parts of the plant body. The dissolved cations and anions along with water moves from cell to cell through spaces between cell wall by diffusion, called *apoplastic pathway*. On the other hand, ions entering cell wall of the epidermis move across the cell wall of cortex, cytoplasm of endodermis and cell walls of pericycle and finally reach the stele. This pathway involving the living (cytoplasm) part of the cell is referred to as *symplastic pathway*. In this pathway, nutrient elements entering the cytoplasm of the epidermis move across the cytoplasm of the cortex and endodermis of pericycle through plasmodesmata and finally reach the xylem vessels.

After the nutrients are absorbed by roots, it is translocated to other plant parts by the transpiration stream moving through the xylem. As water is continuously lost by aerial parts of the plant called *transpiration*, it creates a transpirational pull. This driving force keeps the water moving up along the plant with mineral salts in the xylem vessel. This transpirational pull is so strong that water and nutrients move in upward direction to several feet in the tallest trees growing in nature. Stout and Hoagland have proved that mineral nutrients absorbed by the roots are translocated through the xylem vessel.

Nutrient uptake by roots is a well-regulated process and is also specific and selective. Subsequent translocation of ions across cortex to reach the stele (xylem loading) is also determined by several interrelated factors. The pathway of ion or solute movement across the root faces some special constraints because of the anatomy of roots. All plant cells are separated by cell walls, and ions can move through channels in the cell wall spaces without ever entering a living cell. This continuum of cell wall, which allows extracellular ion movement, is called the free space or apoplast. Besides cell wall forming a continuous phase, the cytoplasms of neighbouring cells are also connected to each other and form a continuum, collectively termed as the *symplast*. The cytoplasmic bridges interconnecting the neighbouring cytoplasm are called *plasmodesmata*. These are cylindrical pores of 20–60 nm in diameter. Each plasmodesmata (singular) is lined with a plasma membrane and contains a narrow tubule, called *desmotubule* which is the continuation of endoplasmic reticulum. The cells near the root tips where most nutrient absorption occurs has a high density of plasmodesmata.

The anatomy of root cell and the pathway of solute or ion movement in root cells are depicted in Fig. 20.7. The apoplast forms a continuous phase from the root surface through the cortex. There is a layer of specialised cells called endodermis at the boundary between vascular cylinder and the cortex. The endodermis contains a layer or strip of suberised cells known as Casparian strip, which functions as a barrier to the entry of water and mineral ions into the stele through the apoplast. This Casparian strip has hydrophobic properties and completely surrounds each endodermis cell. When a solute enters the root, it may enter the symplast immediately by crossing an epidermal cell plasma membrane, or it may diffuse between the epidermal cells through the cell

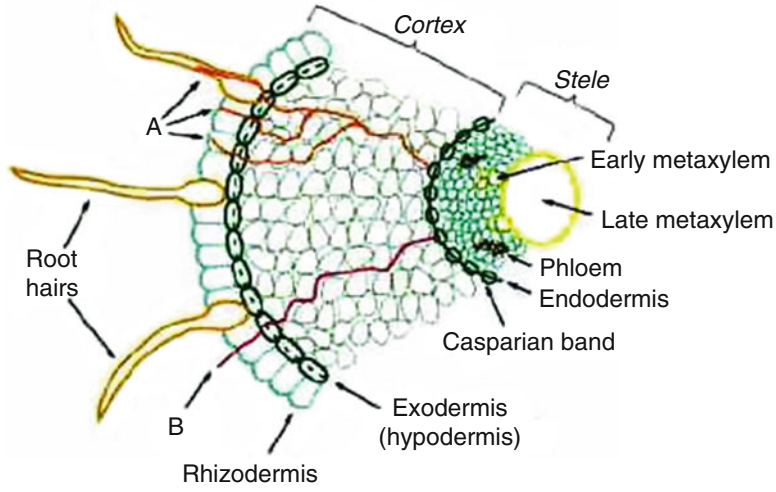

Fig. 20.7 Pathway of ion transport across the root (*A*) symplastic (*B*) apoplastic

walls. From the apoplast of cortex, ions/solutes may cross the plasma membrane of cortical cell, thus taking the symplastic route, or may radially diffuse all the way to endodermis through the apoplastic route. In either case, the ion/solute must first enter the symplast before entering into the stele due to the presence of Casparian strip.

Once ions enter the symplast of root at epidermis or cortex, they are loaded into the tracheid or vessel element of stele to be translocated to the shoot. Since xylem treachery elements are dead cells, the ions exit symplast by crossing a plasma membrane for the second time. 'Xylem loading', as the name suggests, is the movement of ions from symplast into the conducting cells of xylem. Here again, the Casparian strip prevents back-diffusion of ions through apoplast. Though this strip acts as a barrier to water and solute movement, the major advantage is that it helps the plant to maintain an ionic concentration in xylem higher than the soil water surrounding roots.

There is a possibility that ions could enter tracheid and vessel element of xylem by passive diffusion. But in this case, the movement of ions from root surface to the xylem would take only a single step requiring metabolic energy. This single energy-dependent step of ion uptake occurs at the plasma membrane surfaces of root epidermal, cortical and endodermal cells. According to the passive diffusion model, ions move passively into the stele via symplast through a concentration gradient and then leak out of the living cells of stele (possibly because of lower oxygen availability interior of root) into the nonliving conducting cells of xylem.

The electrochemical potential of various ions across the roots could be measured by using ion-specific microelectrodes. From various studies, it was indicated that K^+, Cl^-, Na^+, SO_4^{2-} and NO_3^- were all taken up actively by epidermal and cortical cells and maintained in xylem against an electrochemical potential gradient when compared with the external medium. However, none of these ions is at a higher electrochemical potential in xylem than in cortex or living portion of stele. Therefore, the final movement of ions into xylem could be due to passive diffusion. However, other studies have shown that this final step of xylem loading may also involve active process within the stele. By using treatments with inhibitors and other plant hormones, investigators have shown that ion uptake by cortex and ion loading in xylem operates independently. Treatments with protein synthesis inhibitor like cycloheximide or with cytokinin (benzyladenine) inhibit xylem loading without affecting uptake by cortex. This result indicated that efflux from the stelar cells is regulated independently from uptake by cortical cells.

Recent biochemical studies have indicated that the xylem parenchyma cells have a role in xylem loading. The plasma membranes of xylem

parenchyma cells contain H^+ pumps, water channels and a variety of ion channels specialised for influx or efflux. Absorption of ions by roots is pronounced in the root hair as compared to meristem or elongation zones. Presence of root hairs greatly increases the surface area available for ion absorption.

20.5.2 Mechanisms of Ion Absorption

According to Fick's law, the movement of molecules or ions by diffusion always takes place spontaneously from a place of high concentration to a low concentration, that is, down a concentration or chemical gradient until equilibrium is reached. This movement of molecules by diffusion without the involvement of metabolic energy is termed as passive transport. Once the equilibrium is reached, no further net movement of solute will occur without the application of a driving force. On the other hand, movement of substances after the equilibrium is reached or against a concentration gradient by involving metabolic energy termed as active transport.

20.5.3 Passive Absorption

The concept of passive absorption is based on 'outer space' also called as 'free space' or 'diffusion space'. If only a portion of tissue volume is open to free diffusion, the ions will move freely in or out of the tissue. After sometime, the part of tissue undergoing free diffusion will reach equilibrium with external solution resulting in same ion concentration within the tissue as that of the external solution. The part of a cell or tissue that allows free diffusion of ions is called free space or outer space. Usually the volume of root tissue accessible for free space is only a small fraction ~5 % of total root volume. The extent of solute flux into free space for a given volume of free space depends on several factors such as transpiration rate, solute concentration and root hair formation. However, the presence of such a free space enables cortex cells to take up solutes directly from the external solution.

The cell walls contain negative charge because of polygalacturonic acid [carboxylic groups (R. $CHOO^-$)] present in the middle lamella. These negative charges in apoplasm act as cation exchangers resulting in accumulation of cations, whereas the anions are repelled. Therefore, the entry of charged solutes is restricted in the free space. Hope and Stevens (1952) introduced the term apparent free space (AFS) to describe 'free space'. AFS comprises of water free space (WFS) which is freely accessible to ions, charged and uncharged molecules. Another term, Donnan free space (DFS), was introduced where cation exchange and anion repulsion take place (Fig. 20.8). The various theories of passive absorption are discussed below:

20.5.3.1 Mass Flow Hypothesis

According to this theory, ions are absorbed by root along with mass flow of water under the influence of transpirational pull. In detopped tomato plants, an increase in transpiration increased salt absorption by replacing ions once they were released into the xylem duct. The dilution thus caused enhanced ion absorption. This resulted in accumulation of a small proportion of total salt uptake by plants through passive absorption. However, this salt accumulation by passive process could be explained as follows: (1) free diffusion of ions along concentration gradient into the apparent free space of a tissue, (2) accumulation of ions against concentration gradient due to ion exchange or Donnan equilibrium and (3) mass flow of ions through roots due to transpirational 'pull' may also occur. All these processes do not require expenditure of metabolic energy.

20.5.3.2 Ion Exchange Theory

Both cations and anions have a tendency to get adsorbed on the surfaces of cell walls or membranes of cells and exchange with ions present in soil solution. During the absorption of a positively charged ion such as K^+, either a positively charged ion such as H^+ is displaced from the cell (ion exchange) or a negatively charged ion enters the cell. Similarly, anions can exchange with free

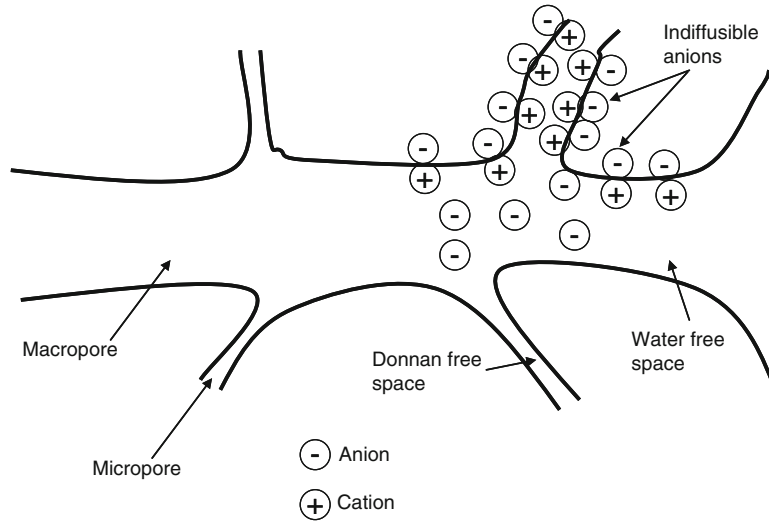

Fig. 20.8 Schematic representation of the pore system of the apparent free space (Source: Marschner 1995)

hydroxyl (OH^-) ions. This process of exchange between the adsorbed ions and ions in the solution is known as ion exchange. Sometimes the uptake of nutrient cations from a soil solution into the roots exceeds the anion uptake and vice versa. In each case, the neutrality is maintained. In a case where anion uptake exceeds cation uptake, the OH^- and bicarbonate (HCO_3^-) ions are transported outwardly from inside the cells into the free space. Likewise, if cation absorption exceeds anion uptake, cells exchange some hydrogen ions. The ionic exchange process is explained as follows:

1. *Carbonic acid exchange theory*

   The roots respire continuously giving out CO_2 in the rhizosphere. This combines with water and forms carbonic acid (H_2CO_3), which dissociates into H^+ and HCO_3^- ions. A zone of carbonic acid is developed in root tips because of high respiratory activity. The H^+ ions exchange with cations absorbed on the clay micelle, and the cations come into soil solution where it diffuses onto the root surface.

2. *Contact exchange theory*

   A similar ion exchange takes place between root and soil colloids at the point where they are in direct contact with each other without being first dissociated in the soil solution.

3. *Donnan equilibrium*

   This theory takes into account the effect of fixed or nondiffusible ions. The cell contents are separated from external solution by a differentially permeable membrane. This membrane is impermeable to anion concentration present in cell. A negative nondiffusing charge on one side will create a potential gradient across the membrane through which the ions will diffuse. This results in diffusion of equal number of cation and anion through the membrane until electrochemical equilibrium is reached. However, because of the 'fixed' negative (anion) charge on the inner side of the membrane, additional cations will be required to balance this charge. Therefore, the cation concentration will be greater in internal solution (inside the cell) than the external solution. Also, the concentration of anions in external solution will be less than that of the external one because of the excess of negative charges due to 'fixed' anions. Therefore, Donnan equilibrium, a term proposed by F.G. Donnan, is attained if the product of anions and cations in the internal solution becomes equal to the product of anions and cations in the external solution.

$$\text{Donnan equilibrium} = \frac{(\text{Positive ions inside})}{(\text{Positive ions outside})} = \frac{(\text{Negative ions inside})}{(\text{Negative ions outside})}$$

20.5.3.3 Facilitated Diffusion

The passive transport can also take place by carrier proteins localised in the plasma membrane where it can transport a much wider range of possible substances. This passive transport mediated by a carrier is called facilitated diffusion. However, it resembles diffusion only in that it transports substances down their gradient of electrochemical potential without any additional input of energy.

20.5.4 Active Absorption

As discussed in the previous section, active absorption involves transport of solutes against the concentration gradient. Carriers, pumps and channels located in the membrane are involved in active transport of ions/solutes (Fig. 20.9). It involves electrogenic and cytochrome pumps to be discussed in the following sections. Active transport is further divided into two categories: primary active transport and secondary active transport.

20.5.4.1 Primary Active Transport

When the transport of ions is directly coupled to expenditure of metabolic energy, such as ATP hydrolysis, an oxidation-reduction reaction (the electron transport chain of mitochondria and chloroplast), or absorption of light by the carrier protein (in halobacteria, bacteriorhodopsin), it is termed as primary active transport. Membrane proteins involved in primary active transport are called pumps. Most pumps transport energy ions, such as H^+ or Ca^{2+}.

The pumps can be further characterised as either electrogenic or electroneutral. Electrogenic transport refers to ion transport involving the net movement of charge across the membrane,

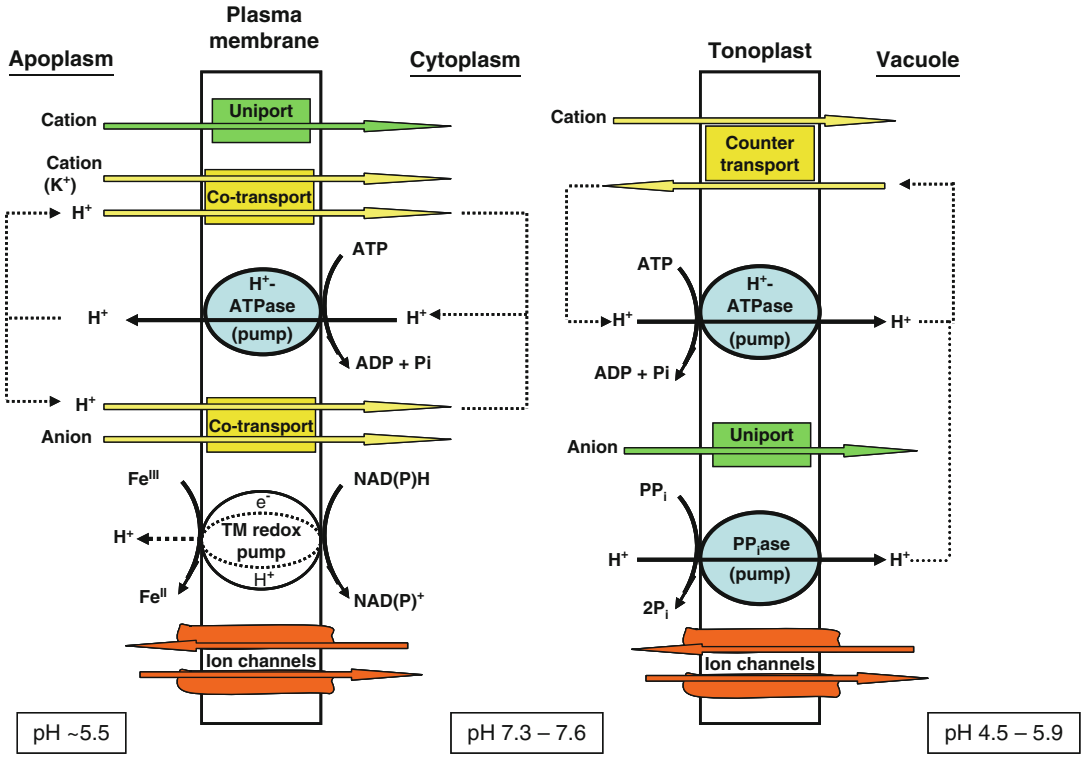

Fig. 20.9 Model for the localisation and functioning of electrogenic proton pumps (H+-ATPase, PPiase), transmembrane redox pump (NAD(P) oxidase), ion channels and transport of cations and anions across the plasma membrane and tonoplast (Source: Marschner 1995)

whereas in electroneutral transport, no net movement of charge is involved. The pumps are driven by energy released during hydrolysis of ATP carried out by ATPase located at plasma membrane and tonoplast. This enzyme is considered as '*master enzyme*' because of its key role in regulation of cytoplasmic pH and the driving force for cation and anion uptake. For example, the Na^+/K^+-ATPase of animal cell pumps out three Na^+ for every two K^+ ions taken in, resulting in net outward movement with one positive charge. The Na^+/K^+-ATPase is therefore an electrogenic ion pump. In contrast, H^+/K^+-ATPase of the animal gastric mucosa pumps one H^+ out of the cell for every one K^+ taken in, so there is no net movement of charge across the membrane. Therefore, the H^+/K^+-ATPase is an electroneutral pump.

In general, H^+ is the principal ion which is electrogenically pumped across the cell membranes of plants, fungi and bacteria. The plant H^+-ATPases exhibit a regulatory role in creating the electrochemical potential gradient across the plasma membrane, while the vacuolar H^+-ATPase (V-ATPase) and the H^+ pyrophosphatase (H^+-ppase) pump proton into the lumen of vacuole and Golgi cisternae.

20.5.4.2 Secondary Active Transport

The other important mechanism by which solutes can be transported across membrane against the gradient of electrochemical potential is coupling of 'uphill' transport of one ion/solute to the 'downhill' transport of another. Carrier proteins located in the membranes carry out such cotransport of ions/solutes, which is also called 'secondary active transport'. These carriers are indirectly driven by the action of pumps. In carrier-mediated ion transport, the substance being transported is initially bound to a specific site on the carrier protein. This binding causes a conformational change in the protein, which exposes the substance to solution on the other side of the membrane. Transport completes when substance dissociates from the carrier's binding site. Rate of transport by a carrier is about 10^6 times slower than transport through a channel.

Secondary active transport utilises the energy stored in the proton-motive force (PMF) or Δp, created when protons are extruded from the cytosol by the electrogenic H^+-ATPases. This takes place both at the plasma membrane and at the vacuolar membrane where a membrane potential and pH gradient develop at the expense of ATP hydrolysis. The PMF represents stored free energy or the H^+ gradient that is used to drive the transport of other substances against their chemical potential gradient. The secondary active transport may be either symport (two ions/solutes moving in the same direction) or antiport (downhill proton movement drives active (uphill) transport of solute in the opposite direction). Transport by carriers may be 100–1,000 ions or molecules per second.

The presence of ion channels in plant cell membranes has also been established. These ion channels have a unique ability to regulate or 'gate' ion flux subject to the physical and chemical environment of the channel protein. Transport through the channel may or may not involve transient binding of solute to the channel protein. In any case, as long as the channel pore is open, solute that can penetrate the pore diffuses through it extremely rapidly. Ion transport through channels is always passive. The pore size and density of charges on interior lining determine the transport specificity; channel transport may be mainly limited to ions or water. The region in the channel that determines specificity is called 'selectivity filter'. However, ion channels remain closed most of the time and their number per cell is also meagre.

A class of relatively abundant membrane proteins that form water channels, are named as 'aquaporins'. Aquaporins are common to plant and animal membranes; their expression and activity in response to water availability are regulated by several mechanisms including protein phosphorylation.

20.5.4.3 Cytochrome Pump

H. Lundegardh in 1954 proposed this theory when he observed a quantitative relationship between anion absorption and respiration, whereas no such correlation with cation absorption was found. It was also noticed that salt respiration and anion absorption were inhibited by

cyanide or even carbon monoxide. He, therefore, suggested that anions could be transported across the membrane by cytochrome system and absorption of anion is independent of cation. Energy is supplied by direct oxidation of respiratory intermediates. The rate of respiration solely determined by anion absorption is called 'anion respiration' or 'salt respiration'. The respiration rate (excluding anion respiration) observed in distilled water is called 'ground respiration'.

$$\text{Total respiration}\left(R_t\right) = \text{Ground respiration}\left(R_g\right) + \text{Salt or anion respiration}\left(R_a\right)$$

20.5.5 Passive Absorption

The absorption of minerals without expenditure of metabolic energy is termed 'passive absorption'. Passive ion absorption in the root system was demonstrated by Briggs and Robertson (1957). The distinguishing characteristics of passive absorption include:

1. Mineral salt absorption not affected by temperature or metabolic inhibitors
2. Rapid uptake of ions when plants are transferred from a medium of low to high concentration

20.6 Biofertilisers

Biofertilisers are natural fertilisers consisting of microbial inoculants of bacteria, fungi and algae, either alone or in combination resulting in enhanced growth of plants mediated by increased availability of nutrients. Biofertilisers can be applied to seed, plant surfaces or soil, where they colonise the rhizosphere or the interior of plant and increase the supply of primary nutrients to the host plant. These microorganisms, either through symbiotic or non-symbiotic association, help the plants to fix N from atmosphere, solubilise and mobilise fixed P; translocate minor elements like Zn and Cu to the plants; produce plant growth-promoting hormones, vitamins and amino acids; and control plant pathogenic fungi. Thus, biofertilisers are expected to reduce the use of chemical fertilisers and pesticides. Besides microorganisms, biofertilisers include organic fertilisers such as farmyard manure (FYM) obtained by decomposing farm wastes.

The use of biofertilisers is both economical and environment friendly. These microorganisms are abundant in the natural environment. Use of biofertilisers leads to enhanced crop productivity and reduction in the usage of chemical fertilisers resulting in improvement of soil texture and mitigating other harmful environmental effects. As a matter of fact, the system of integrated nutrient management (INM) subscribes to the use of a combination of inorganic and organic/biofertilisers. Biofertilisers include the following groups:

1. Symbiotic N fixers, e.g. *Rhizobium* spp. for legumes
2. Non-symbiotic free-living N fixers, e.g. *Azotobacter*, *Azospirillum*
3. Algae biofertilisers (blue-green algae (BGA) in association with *Azolla*)
4. Phosphate solubilising bacteria (PSBs)
5. Mycorrhiza
6. Organic fertilisers

20.6.1 Symbiotic Nitrogen Fixers and Algal Biofertilisers

Bacteria such as *Rhizobium* spp. exhibit symbiotic relationship with host plants especially legumes. Blue-green algae (BGA) or cyanobacteria have been used in agriculture since long. The bacterial biofertilisers fix about 50–150 kg N/ha or more per year. The root-nodulating bacteria, such as *Rhizobium*, are common in nature. However, there are a few other genera that produce stem nodules. For example, *Azorhizobium* spp. produce stem nodules on *Sesbania rostrata*. Rhizobium is classified based on the specific legume species they nodulate. For example, *R. leguminosarum* nodulates pea (*Pisum sativum*), khesari (*Lathyrus sativus*) and lentil (*Lens culinaris*); *R. phaseoli* produces nodules on French bean (*Phaseolus vulgaris*) and bean (*Phaseolus*

multiflorus); *R. meliloti* nodulates lucerne (*Medicago sativa*) and fenugreek (*Trifolium foenumgraecum*); *R. trifoli* nodulates clover (*Trifolium* sp); and *R. lupini* nodulates white lupins (*Lupinus alba*).

Cyanobacteria or BGA are photosynthetic prokaryotic organisms that can fix atmospheric N in association with a fresh water fern called *Azolla*. Cyanobacteria belong to the genera *Nostoc, Anabaena, Plectonema, Aulosira* and *Tolypothrix. Azolla* cultures are inoculated in paddy fields under both aerobic and lowland conditions. However, the effectiveness of the inoculum is higher under lowland condition, wherein about 20–30 kg N is fixed per ha per crop season. The enzyme nitrogenase is present in cyanobacteria and N fixation occurs in specialised cells called 'heterocysts'. These heterocysts have *nif* gene (involved in N fixation) and also act as oxygen-proof compartment, protecting nitrogenase from oxygen inactivation. *Azolla* owes its N-fixing capacity to *Anabaena,* while the fern supplies food material to the alga. In addition to N fixation, BGA decomposition contributes to the ecosystem biomass, thereby enhancing soil physical properties.

20.6.2 Non-symbiotic Free-Living N Fixers

Azotobacter and *Azospirillum* are free-living bacterial genera which fix atmospheric N. *Azotobacter* can be used to inoculate crops like maize, wheat, mustard, cotton, potato and other vegetable crops. By utilising soil organic matter, it fixes about 30 kg N per ha per year. *Azospirillum* inoculants are recommended especially for cultivation of wheat, maize, sugarcane, millets and sorghum.

20.6.3 Phosphate Solubilising Microorganisms

Phosphate solubilising microorganisms capable of solubilising Pi from insoluble sources enhance P bioavailability. These microorganisms belong to the bacterial genera *Thiobacillus*, *Bacillus*, etc. These bacteria, known as phosphate solubilising bacteria (PSBs), produce substances like pseudobactin (also called 'siderophores') that chelate Fe.

Availability of P is low both in alkaline and acidic soils since it is fixed as insoluble oxides. The inoculants of PSBs when applied to either soil or seed can be useful to mobilise fixed P. Rock phosphate when used with PSBs can reduce the phosphatic fertiliser requirement by 50 %. Mere seed inoculation with PSBs results in crop yield responses equivalent to that obtained by application of 30 kg P_2O_5 per ha in the form of chemical fertilisers.

20.6.4 Mycorrhizae

Mycorrhizae (*mycor* fungus; *rhiza* root) are a symbiotic (intimate) and mutualistic (mutually beneficial) association between a nonpathogenic or weakly pathogenic fungus and living root cells of plant, particularly cortical and epidermal cells. The fungi receive organic nutrients from the plant and, in turn, improve the mineral element and water-absorbing properties of roots. The roots of most soil grown plants are mycorrhizal. About 83 % of dicot and 79 % of monocot plants and all gymnosperms are mycorrhizal worldwide. Generally, the fungus infects tender young roots because, on older parts, the epidermis and cortex are lost and a protective layer of suberin develops in cork cells. Root hair production either slows or ceases upon infection, so mycorrhizae often have few such hairs. This reduction in root hairs greatly decreases the absorbing surface area; however, the slender fungal hyphae extending from mycorrhizae explore greater soil volume.

Two main groups of mycorrhiza are present in nature: (1) ectomycorrhiza and (2) endomycorrhiza. However, there is a rare group with intermediate properties called the ectendotrophic, which is also sometimes found. In ectomycorrhizae (ECM), the fungal hyphae form a mantle outside the root and also inside the root in the intercellular spaces of epidermis and cortex. No intracellular penetration into epidermal or corti-

cal cells occurs, but an extensive network called the Hartig net is formed between these cells. Ectomycorrhizae are commonly found on the roots of trees including members of Pinaceae family (pine, fir, spruce, larch, hemlock), Fagaceae (oak, beech, chestnut), Betulaceae (Birch, alder), Salicaceae (willow, poplar) and a few other families.

In endomycorrhiza, fungi live inside the cortical cells and also grow in the intercellular spaces. Endomycorrhiza consists of three subgroups, namely, the vesicular arbuscular mycorrhizae (VAM) the most common, the ericoid and the orchidaceous mycorrhizae. The VAM belongs to the family Endogonacae. They produce a branched haustorial structures called arbuscules between the cortical cells and a mycelium that extends out in the soil. These extraradical mycelium or hyphae are involved in absorbing mineral salts and water. The arbuscules are short lived, about 10–12 days, and are the main sites of solute exchange with the host. There are mainly four genera of VAM fungi, namely, *Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis*. Out of these genera, *Glomus* is present abundantly in soil. It has been found that not all endomycorrhizal fungi produce vesicles as lipid-rich storage organs, so the VAM has been renamed as AM (arbuscular mycorrhiza).

The host plants secrete root exudates containing flavanoids (e.g. β-estradiol), which attract these fungi, and the root infection and colonisation occur. In mycorrhizal roots, for fungal growth, a substantial proportion of carbons fixed during photosynthesis are required. In AM plants, root respiration may be 20–30 % higher than in non-mycorrhizal plants, and 87 % of higher respiration is attributed to the fungus. The mycorrhizal colonisation affects root and shoot growth differently. Its effect is more pronounced in nutrient-poor soils rather than nutrient-rich soils. Mycorrhizal colonisation helps the plant to acquire nutrients which are less mobile in soil, particularly P and also N, Zn, Cu and S. Besides these, the mycorrhizal association also helps in increasing the hormonal (IAA, cytokinin, ABA) content of plants, improves plant-water relations (increase drought tolerance capacity) and suppresses soil-borne fungal and bacterial root pathogens (e.g. *Pseudomonas syringae* in tomato plant).

20.6.5 Organic Fertilisers

Organic fertilisers are the plant and animal wastes which after decomposition release nutrients essential for plant growth. These include farmyard manures (decomposed mixture of dung, urine and litter of farm animals), compost (rotted wastes of farm like sugarcane trash, paddy straw, etc.), sewage and sludge, vermicompost (decomposition of organic matter by earthworms), green manures (undecomposed plant material) and other livestock manures (poultry, sheep and goat sweepings). These organic manures contain small percentage of nutrient elements and they are applied in large quantities. The practice of growing crops with only organic fertilisers and without the use of chemical pesticide or herbicide is called organic farming. Besides supplying nutrients, the organic fertilisers improve soil physical properties, increase availability of other nutrients and control plant parasitic nematodes and fungi.

20.6.6 Advantages and Limitation of Biofertilisers

The relevance of biofertilisers is increasing rapidly. This is because it has been realised that application of chemical fertilisers causes serious harmful effects on the environment and human health. Further, they are costly and in short supply. Therefore, the advantages of using biofertilisers in general are as follows:

1. They add nutrients to the soil and/or increase their availability to the crops.
2. They secrete certain growth-promoting substances such as indole acetic acid (IAA).
3. Under certain conditions, they exhibit antifungal activities, thereby protecting the plants from pathogenic fungi.

4. They are harmless and eco-friendly low-cost agro-input supplementary to chemical fertilisers.
5. They improve soil structure (porosity) and water-holding capacity.
6. They enhance seed germination.
7. They increase soil fertility and fertiliser use efficiency and ultimately increase yield by 15–20 % in general.

However, the limitation in the use of biofertilisers lies in the fact that its acceptability by the farming community is low, the reason being organic fertilisers do not produce quick and spectacular responses. Moreover, the quantity of nutrients provided by biofertilisers is not sufficient to adequately meet the crop demand for high yields.

20.7 Hydroponics: History and Scope in Indian Agriculture

Hydroponics (Greek words 'hydro' water; 'ponos' labour) is a method of growing plants using mineral nutrient solutions without soil. It is also called "controlled environment agriculture" (CEA) since raising plants hydroponically requires control of environmental factors such as light intensity and duration, temperature, humidity, pH of the solution/medium and mineral nutrients.

The study of crop nutrition began thousands of years ago. The classic work on growing terrestrial plants without soil was published by Sir Francis Bacon in 1627, the book named 'Sylva Sylvarum'. After Bacon's work, water culture became a popular research technique. John Woodward (1699) published his work on water culture experiments with spearmint where he mentioned that 'plants grew better in less pure water sources than plants in distilled water'. German botanists, Julius von Sachs and Wilhelm Knop (1859–1865), developed the techniques of soilless cultivation. It was Professor William Frederick Gericke (1937) who finally introduced the term hydroponics and wrote the book named *Complete Guide to Soilless Gardening*. Two other plant nutritionists, Dennis R. Hoagland and Daniel I. Arnon, at the University of California, wrote a classic in 1938 in agricultural bulletin 'The Water Culture Method for Growing Plants without Soil'. They developed several formulations for mineral nutrient solutions, known as Hoagland solutions and the modified Hoagland solutions that are still in use today. This technique was used to establish the criteria of essentiality for different nutrient elements.

There are primarily two types of hydroponics, viz., (1) solution culture and (2) medium culture. In solution culture, there is no solid medium for the roots to support, just the nutrient solution, while the medium culture has a solid medium for the roots and is named after the type of medium being used. Again, solution culture is divided into three main methods of growing plants:

(a) Static solution culture – plants are grown in containers such as plastic buckets, tubs or tanks with nutrient solution. The solution may or may not be aerated, but usually gentle aeration is required.
(b) Continuous flow solution culture – the nutrient solution constantly flowing past the roots, e.g. nutrient film technique.
(c) Aeroponics – no substrate is required, and the roots are suspended in the air in a closed chamber and are sprayed intermittently with fine mist or aerosol of nutrient solution. Aeroponics is widely used in laboratory studies of plant physiology. National Aeronautics and Space Administration (NASA) has done extensive hydroponic research for their 'Controlled Ecological Life Support System' (CELSS). Special attention has been given to this technique since a mist is easier to handle in a zero-gravity environment as compared to a liquid.

In medium culture, there are variations for each media, i.e. passive subirrigation, flood and drain subirrigation, top irrigation and deep-water culture. The media used for growing plants may be expanded clay, rock wool, coir, perlite, vermiculite, sand, gravel/quartz and brick shards.

Plant nutrients used in hydroponics are dissolved in water and are mostly in inorganic and ionic forms. All the 17 elements that are essential

for plant growth are supplied using different chemical combinations. Chelating agents such as EDTA are used to keep Fe in soluble form. The pH of nutrient solution ranges from 5.6 to 6.0 depending on the crop. Once the plants are grown in nutrient solution, the composition of solution is altered due to depletion of specific nutrients more rapidly than others, absorption of water from the solution and alteration of pH by excretion of either acidity or alkalinity. Utmost care should be taken while deciding the optimum salt concentration to be used in solution, so as to avoid toxicity, early nutrient depletion or pH changes which may together attribute to inadvertent effects on plant growth and development.

In India, hydroponics was not a popular practice until 1946. The Government of Bengal initiated Experimental Farm at Kalimpong, Darjeeling. After a careful appraisal of salient problems, the Bengal System of Hydroponics was developed in 1946–1947 representing the efforts to meet Indian requirements. Recently, in 2008, a few farmers in southern districts of Gujarat adopted this technology for cultivating varieties of exotic hybrid tea roses. Further, the hydroponic system was installed on 12 ha of land in Kuch village of Navsari district. Exotic crops such as strawberry, green garlic and tomatoes are also cultivated using this technology. The second biggest hydroponics project in Gujarat is 'Landmark Agrotech'. In India, wastelands with poor-quality soil and sufficient water can be brought under hydroponics for sustainable land use and increase crop productivity.

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