M. Naeem · Abid A. Ansari
Sarvajeet Singh Gill *Editors*

Essential Plant Nutrients

Uptake, Use Efficiency, and Management

Springer

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Editors
M. Naeem
Department of Botany
Plant Physiology Section
Aligarh Muslim University
Aligarh, India

Abid A. Ansari
Department of Biology
Faculty of Science
University of Tabuk
Tabuk, Saudi Arabia

Sarvajeet Singh Gill
Centre for Biotechnology
Maharshi Dayanand University
Rohtak, Haryana, India

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Contributors

Faraht Abbas Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Neama Abdalla Plant Biotechnology Department, Genetic Engineering Division, National Research Center, Dokki, Giza, Egypt

Muhammad Adrees Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Tariq Aftab Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh, India

Zaheer Akbar Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Md. Khairul Alam School of Veterinary and Life Sciences, Murdoch University, Perth, WA, Australia

Akbar Ali Department of Botany, Dayalbagh Educational Institute, Agra, India

Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh, India

Arlene Asthana Ali Department of Biotechnology, Jamia Millia Islamia, New Delhi, India

Qasim Ali Department of Botany, Government College University, Faisalabad, Pakistan

Shafaqat Ali Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Abdullah H. Al-Saeedi College of Agricultural and Food Sciences, King Faisal University, Al-Hassa, Saudi Arabia

Tarek Alshaal Soil and Water Department, Faculty of Agriculture, Kafrelsheikh University, Egypt

Claudia Álvarez Universidad Nacional de Colombia, Medellin, Colombia

Megahed Amer Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center, Giza, Egypt

Abid A. Ansari Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Felipe Aquea Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, Chile

Muhammad Saleem Arif Department of Environmental Sciences and Engineering, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Arslan Ashraf Department of Botany, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Ashraf Department of Soil & Environmental Sciences, University College of Agriculture, University of Sargodha, Sargodha, Pakistan

Lohit K. Baishya ICAR Research Complex for NEH Region, Medziphema, Nagaland, India

K.P. Baiyeri Department of Crop Science, University of Nigeria, Nsukka, Nigeria

Kaushik Batabyal Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan

Prem Kumar Dantu Department of Botany, Dayalbagh Educational Institute, Agra, India

Tariq Ahmad Dar Centre for Biodiversity Studies, School of Biosciences and Biotechnology, Baba Ghulam Shah Badshah University, Rajouri, India

Ayman El-Ghamry Soil and Water Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt

Hassan El-Ramady Soil and Water Department, Faculty of Agriculture, Kafrelsheikh University, Egypt

S. Kala Soil Chemistry and Fertility, IISWC, Research Centre, Kota, Rajasthan, India

Tamer Elsakhawy Agriculture Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Giza, Egypt

Masayuki Fujita Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan

Aliaa Gad Agriculture Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Giza, Egypt

Sarvajeet Singh Gill Stress Physiology and Molecular Biology Lab, Centre for Biotechnology, MD University, Rohtak, Haryana, India

Arti Goel Amity Institute of Microbial Biotechnology, Amity University Noida, Noida, Uttar Pradesh, India

Viraj Gunarathne Environmental Chemodynamics Project, National Institute of Fundamental Studies, Kandy, Sri Lanka

Muhammad Zulqarnain Haider Department of Botany, Government College University, Faisalabad, Pakistan

Eman Hamad Agriculture Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Giza, Egypt

Mirza Hasanuzzaman Molecular Biotechnology Group, Center of Molecular Biosciences, Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa, Japan

Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Tanvir ul Hassan Dar Centre for Biodiversity Studies, School of Biosciences and Biotechnology, Baba Ghulam Shah Badshah University, Rajouri, India

Israr Ul Hassan Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dhofar University, Salalah, Oman

Zaid ul Hassan Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Md. Shahadat Hossain Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan

Afzal Hussain Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Cristian Ibañez Laboratorio de Biología Molecular y Bioquímica, Departamento de Biología, Universidad de La Serena, La Serena, Chile

Mohd. Idrees Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dhofar University, Salalah, Oman

Claudio Inostroza-Blancheteau Núcleo de Investigación en Producción Alimentaria (NIPA-UCT), Escuela de Agronomía, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile

Muhammad Iqbal Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

M. Irfan Qureshi Department of Biotechnology, Jamia Millia Islamia, New Delhi, India

K.S. Kartikha Soil Chemistry and Fertility CPCRI, Regional Station, Vittal, Karnataka, India

Katalin Sárdi Department of Crop Production and Soil Science, University of Pannonia, Keszthely, Hungary

Rizwana Kausar Soil and Water Testing Laboratory for Research, Sargodha, Punjab, Pakistan

M. Masroor A. Khan Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh, India

Juan D. Leon Universidad Nacional de Colombia, Medellin, Colombia

Jubayer Al Mahmud Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa, Japan

Department of Agroforestry and Environmental Science, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Manish Mathur Central Arid Zone Research Institute, Jodhpur, Rajasthan, India

Sonia Mayakaduwa Environmental Chemodynamics Project, National Institute of Fundamental Studies, Kandy, Sri Lanka

B.P. Meena Agronomy, IISS, Bhopal, Madhya Pradesh, India

Gyanendranath Mitra Faculty of Agriculture, Department of Agricultural Chemistry, Soil Science and Biochemistry, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, India

Felipe Moraga Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, Chile

Ahmed Mosa Soil and Water Department, Faculty of Agriculture, Mansoura University, Egypt

Muhammad Salman Mubarik Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan

M. Naeem Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh, India

Kamrun Nahar Department of Agricultural Botany, Faculty of Agriculture, Sher--e-Bangla Agricultural University, Dhaka, Bangladesh

Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa, Japan

Bouzid Nedjimi Laboratory of Exploration and Valorization of Steppe Ecosystem, Faculty of Science of Nature and Life, University of Djelfa, Djelfa, Algeria

Hirosuke Oku Molecular Biotechnology Group, Center of Molecular Biosciences, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan

Alaa E.-D. Omara Agriculture Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Giza, Egypt

Nelson W. Osorio Universidad Nacional de Colombia, Medellin, Colombia

Laura Osorno Universidad Nacional de Colombia, Medellin, Colombia

Anisur Rahman Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan

I. Rashmi Soil Chemistry and Fertility, IISWC, Research Centre, Kota, Rajasthan, India

Nasir Rasool Department of Chemistry, Government College University, Faisalabad, Pakistan

Zed Rengel Soil Science and Plant Nutrition, School of Earth and Environment, The University of Western Australia, Crawley, WA, Australia

Marjorie Reyes-Díaz Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Temuco, Chile

Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile

Muhammad Riaz Department of Environmental Sciences and Engineering, Government College University Faisalabad, Faisalabad, Pakistan

Muhammad Rizwan Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan

Dibyendu Sarkar Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Sher Muhammad Shahzad Department of Soil & Environmental Sciences, University College of Agriculture, University of Sargodha, Sargodha, Pakistan

Tarek Shalaby Horticulture Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Shaikh, Egypt

College of Agricultural and Food Sciences, King Faisal University, Al-Hassa, Saudi Arabia

K.C. Shinogi Agriculture Extension, IISS, Bhopal, India

Abhay Shirale Soil Chemistry and Fertility, IISS, Bhopal, Madhya Pradesh, India

Minu Singh Department of Biotechnology, Jamia Millia Islamia, New Delhi, India

Teg Bahadur Singh Department of Botany, Dayalbagh Educational Institute, Agra, India

Moin Uddin Botany Section, Women's College, Aligarh Muslim University, Aligarh, India

F.D. Ugese Department of Crop Production, University of Agriculture, Makurdi, Nigeria

Meththika Vithanage Environmental Chemodynamics Project, National Institute of Fundamental Studies, Kandy, Sri Lanka

Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Tahira Yasmeen Department of Environmental Sciences and Engineering, Government College University Faisalabad, Faisalabad, Pakistan

About the Editors

M. Naeem is assistant professor in the Department of Botany at Aligarh Muslim University, India. For more than a decade, he has devoted his research to improving the yield and quality of commercially important medicinal and aromatic plants (MAPs). His research focuses on escalating the production of MAPs and their active principles using a novel and safe technique involving radiation-processed polysaccharides as well as the application of potent PGRs. To date, he has successfully run three major research projects as the principal investigator, two of which were sanctioned by the Department of Science & Technology, New Delhi. He also completed another fascinating research project on *Catharanthus roseus* awarded by CSTUP, Lucknow. Dr. Naeem has published more than 80 research papers in reputable national and international journals as well as six books. He has also participated in various national and international conferences and acquired life memberships to various scientific bodies in India and abroad. Based on his research contributions, Dr. Naeem has been awarded a research associateship from the Council of Scientific and Industrial Research, New Delhi; a Young Scientist Award (2011) from the State Government of Uttar Pradesh; a Fast Track Young Scientist Award from the Department of Science & Technology, India; a Young Scientist of the Year Award (2015) from the Scientific and Environmental Research Institute, Kolkata; and a Rashtriya Gaurav Award (2016) from the International Friendship Society, New Delhi.

Abid A. Ansari is assistant professor in the Department of Biology, Faculty of Science, University of Tabuk, Saudi Arabia. Dr. Ansari's research work is concerned with phytoremediation and eutrophication. Dr. Ansari has to his credit a number of research articles of national and international repute, ten edited books, and a number of book chapters on varied aspects of his field of research. He has been awarded Scientist of the Year 2014 and Environmentalist of the Year 2011 by the National Environmental Science Academy, India, and Research Excellence 2016 by the University of Tabuk. He has also participated in various national and international conferences and acquired memberships to various scientific bodies, including the Saudi Biological Society, the International Phytotechnology Society, and the Society for Ecological Restoration.

Sarvajeet Singh Gill is assistant professor at the Centre for Biotechnology, MD University, Rohtak, India. He has made significant contributions towards abiotic stress tolerance in crop plants.

Dr. Sarvajeet Singh Gill's research includes abiotic stress tolerance in crop plants, reactive oxygen species signalling and antioxidant machinery, gene expression, helicases, crop improvement, transgenics, nitrogen and sulphur metabolism, and plant fungal symbiotic interactions. Together with Dr. Narendra Tuteja at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, he worked on plant helicases and discovered a novel function of plant MCM6, PDH45 and p68 in salinity stress tolerance that will help improve crop production at suboptimal conditions. A recipient of the Junior Scientist of the Year Award 2008 from the National Environmental Science Academy, Sarvajeet Gill has edited several books and has a number of research papers, review articles, and book chapters to his name.

Part I
Concepts of Plant Nutrients Uptake

Chapter 1
Essential Plant Nutrients and Recent Concepts about their Uptake

Gyanendranath Mitra

Abstract Plants acquire a number of mineral nutrients essential for their metabolism and growth from soil or any other rooting medium. The nutrients have to get through the plasma membrane of root hair cells for use in plant metabolism. According to recent concepts this process is strictly regulated by large groups of genes, which are specific for each nutrient. These genes produce m-RNA transcripts which translate sets of transporter proteins specific for each nutrient. The transporter proteins are lodged inside minute pores located on the plasma membrane. They regulate passage of each nutrient into the cytoplasm. A large number of metabolic enzymes are up- or down-regulated in response to deficiency or sufficiency of plant nutrients. Amino acids, plant growth regulators, intermediate metabolites, and the nutrients themselves are involved in the induction or repression of transporter encoding genes as well as post-translation modification of transporter proteins.

Keywords Nutrient uptake • Plant growth • Nutrient transporters • Abiotic stress

1.1 Essential Plant Nutrients

Plants take up several mineral elements in their ionic forms from the soil or any other growth medium for their metabolism and growth. Some of these elements are called essential since absence or low concentrations of them interfere with plant metabolism and growth and show characteristic deficiency symptoms, which can be corrected by their application. So far 18 elements, C, H, O, N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mn, B, Cl_2, Mo, Co and Ni, have been considered essential for plant nutrition (NRCCA 2010). They are further classified into Macro- and micronutrients.

This article is a brief account on the subject and mostly based on the information given in the book *Regulation of Nutrient Uptake by Plants: A Biochemical and Molecular Approach* by Gyanendranath Mitra, Springer, 2015, with updates from recent research publications.

G. Mitra (✉)
Faculty of Agriculture, Department of Agricultural Chemistry, Soil Science and Biochemistry, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, India
e-mail: gnmitra@gmail.com

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1. **The Macronutrients**, applied in larger quantities to the plants, consist of
   (a) Structural elements: C, H, and O
   (b) Primary nutrients: N, P and K
   (c) Secondary nutrients: S, Ca and Mg.
2. **The Micronutrients**, applied in small quantities to plants, consist of: Zn, Fe, Mn, Cu, B, Mo, Cl^{-}, Co and Ni.

1.1.1 Beneficial Plant Nutrients

They are not essentially required for all the plants. Some of them are essential for some of the plants but others are beneficial to a few plants, and animals who consume these plants. These include Na, Co, Si, Se and V.

Sodium (Na^{+}) is essential for *halophytes*, which accumulate salt in vacuoles to maintain turgor and growth. A few of the C4 plants (except corn and sorghum) need Na^{+} essentially for specific functions, such as in the concentration of CO_2. Other beneficial effects of Na^{+} include greener and glossy leaves due to increased cuticular wax formation and improvement of taste and texture of crops (Brownell and Crossland 1972). Silicon strengthens the stem and provides protection to plants from biotic and abiotic stress. Cobalt is involved in nitrogen fixation by root nodule bacteria and other diazotrophs. Consumption of Selenium rich crop plants such as cabbage, mustard, onion and broccoli provides protection to human beings against cancer and heart disease. The importance of V is due to the discovery in 1980 that it can act as an insulin-mimetic agent.

1.1.2 Non-essential Plant Nutrients

Plants often survive in hostile ionic environment in mineral-rich soils. In their ionic form,

$$Al^{3+}, Au^{+}, Cd^{2+}, Cu^{+}, Cu^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+},$$
$$Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, W^{6+} \text{ and } Zn^{2+}$$

become toxic to plants at different threshold concentrations. Among them, Zn, Fe, Mn, Cu, B, Mo, Co and Ni are micronutrients and essential for plant growth at low concentrations but become toxic beyond a threshold concentration.

Elements such as Cr, V, W, As, Ag, Hg, Sb, Cd, Pb and U with no known function in plant metabolism have been found to be taken up by plants (Godbold and Hüttermann 1985; Breckle 1991; Nies 1999; Schützendübel and Polle 2002). If the soil or growth medium is rich in one or more of the non-essential elements, these elements are likely to be taken up by plants to a tolerable, or sometimes toxic concentrations. These ele-

ments may be of geological origin or accumulated in the soil due to anthropogenic causes. Soils around mining sites or nearer to solid waste dumps from mineral-based industries may contain elements toxic to plants. Use of untreated effluents from industries, use of sewage and sludge from urban centres and use of polluted water as source of irrigation and atmospheric deposits of radioactive isotopes from any overground nuclear activities on soils and plants are other sources of non-essential elements. When food crops are grown on these soils the non-essential elements are likely to be taken up by the plants and may enter into the food chain of man and animals (Greger 2004; Kabta-Pendias and Szdeke 2015). Higher plants have built-in cellular mechanisms for metal detoxification and tolerance to protect them from uptake of these toxic nutrients, such as (1) restriction of metal movement to roots by mycorrhizal association, (2) binding the metals to cell wall and to root exudates, (3) reduce influx across plasma membrane, (4) active efflux into apoplast, (5) scavenging by root border cells, (6) chelation in cytosols by various legands, and (7) transport of accumulated metals to the vacuole. Further an elaborate membrane transport system regulates movement of metal ions across plasma membrane (Mitra 2015).

1.1.3 Radioactive Nuclides

Radioactive nuclides are a part of the terrestrial environment emanating from radioactive substances present in the earth's crust and from cosmic rays. Recently there has been enrichment of specific nuclides in the environment due to (1) manufacture and testing of nuclear weapons, (2) extensive construction of nuclear power plants, (3) commercial fuel reprocessing (4) nuclear waste disposal, (5) Uranium mining and enrichment and (6) nuclear accidents. (Major accidents happened in Chernobyl in USSR, 26th April, 1986, caused by explosion in nuclear power plant due to operational error and in Daiichi Fukushima, Japan, on 11th March, 2011, due to meltdown of nuclear power plant damaged by Tsunami).

The radioactive nuclides released by nuclear weapon tests include

$$^{140}Ba, ^{14}C, ^{141}Ce, ^{144}Ce, ^{137}Cs, ^{55}Fe, ^{3}H, ^{131}I, ^{54}Mn,$$
$$^{239}Pu, ^{241}Pu, ^{103}Ru, ^{106}Ru, ^{125}Sb, ^{89}Sr, ^{91}Y, ^{96}Zr.$$

Some of these and/or their daughter nuclides are released in other operations as described above. The four most harmful radio-nuclides released due to Chernobyl disaster were ^{131}I ($t_{1/2}$ = 8.02 days, causes thyroid cancer), ^{134}Cs ($t_{1/2}$ = 2.07 years, accumulates in heart), ^{137}Cs ($t_{1/2}$ = 30.2 years) and ^{90}Sr ($t_{1/2}$ = 28.8 years, accumulates in bones). The radioactive nuclides monitored from Fukushima Daiichi explosions were ^{131}I and ^{137}Cs. The regulatory levels fixed by Japan were 2 Bq/g for ^{131}I and 0.5 Bq/g for ^{137}Cs. There were soil contaminations with these two nuclides. Soils of a large area of eastern and north-eastern Japan were contaminated with ^{137}Cs. Chernobyl accident data have shown that ^{137}Cs adsorbed on the top soil layer can remain there for long years making the soil unfit for crop production (Yasunari et al. 2011).

Soon after the Chernobyl disaster four square kilometres of pine forest directly downwind of the reactor turned red and died. The radiation level caused by Chernobyl disaster is still very high and 30 km around the factory has been declared as 'Zone of alienation'. It may take 20,000 years to become fit for human habitation. The area however has reverted to become a natural forest and overrun by wildlife due to lack of competition from humans for space and resources. This indicates that plants and animals can survive in a relatively high radiation zone. A study was conducted on progeny of *Arabidopsis* plant collected from Zone of alienation with different levels of contamination. The study indicated a significantly higher resistance of progeny *Arabidopsis* plants to mutagens. There was increased expression of radical scavenging genes *CAT1* and *FSD3* and DNA repair genes *RAD1 and RAD51-like* in these plants (Kavalchuk et al. 2004).

According to World Nuclear Association (2015) the human environment has always been radioactive and accounts for 85% of annual radiation dose, 2.4 mSv/year. The radiation dose received from all nuclear activities accounts for less than 1%.

1.2 Recent Concepts about Nutrient Uptake by Plants

Globally arable soils are deficient in one or more of plant nutrients. The concentration of plant nutrients in soil solution depends on characteristic of the soil, local climatic conditions, nutrient removal due to increased intensity of cropping and management practices such as excessive or less fertiliser use, inadequate irrigation and drainage. The agronomic field operations also change the nutrient profile of the soil.

The requirements of plants for nutrients change with their growth stages, which do not often match with nutrients available in soil solutions. The nutrients available may be in excess or deficient. It has recently been found that plants adopt special mechanisms to acquire nutrients to meet their needs irrespective of their concentration in soil solution.

Plants take up mineral nutrients for their metabolism and growth. As a first step the mineral nutrients need to be transported across plasma membrane of root hair cells into cytoplasm for use in plant metabolism. Recent research indicates that this process is under strict genetic control. There are different groups of genes for each nutrient, which encode transporter proteins whose functions include acquiring specific nutrient from the soil solution and transporting them across plasma membrane of root hair cells for use in plant metabolism. There are different sets of genes, which are induced due to deficiency or sufficiency of a plant nutrient. They produce mRNA transcripts for translation of transporter proteins. Induction or repression of these genes is caused by amino acids, plant growth regulators, intermediate metabolites or nutrients themselves (Orsel et al. 2002; Hammond et al. 2004; Rodriguez-Navarro and Rubio 2006; Miller et al. 2008).

A large number of genes, which are involved in encoding transporter proteins for uptake of different nutrients, have been identified for a number of plants. The amino acid sequence and structure of corresponding transporter proteins and their mechanism of action have been reported.

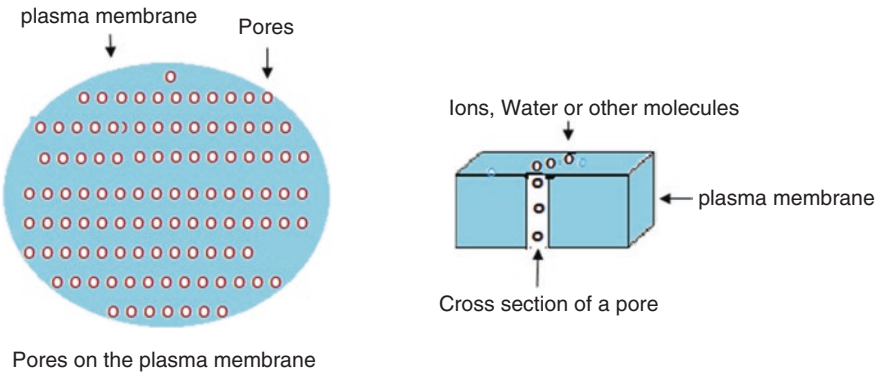

Fig. 1.1 Pores on plasma membrane and cross section of a pore (adapted from Mitra 2015)

The transporter proteins have been classified as (1) Chanel proteins and (2) Transporters.

1.2.1 Ion Channels and Transporters

There are a large number of pores on the plasma membrane of cells, which allow passage of specific nutrient ions or molecules and do not allow any others to pass through them. Such selectivity is ensured by large molecules of intrinsic trans-membrane transporter proteins with fixed topology, which are lodged across the pores of the plasma membrane (Fig. 1.1). They are too large to move across the membrane. The transporter proteins consist of

1. Channel proteins and
2. Transporters (Carriers) proteins.

1.2.1.1 Channel Proteins

Channel proteins are large molecules with multiple trans-membrane α-helices. They alternate between open and closed conformations (gating). There is conformational change of the channel protein due to any one of the extrinsic factors, such as (1) changes in membrane potential (2) binding of a small regulatory molecule or (3) membrane stretch (e.g. via link to the cytoskeleton) (Dubyak 2004; Rainer 2012). These factors determine if the channel is in a gated state (open for transport) or closed state (incapable of ion transport). The extrinsic factors control the accessibility of ions to the pore domain, which acts as a pathway for movement of ions from one side of the membrane to the other side. Since there are no energetic interactions, between channel protein and the transported ion, the rate of transport of ion is fast. There is probably no binding site within the pore to restrict their movement. Even if

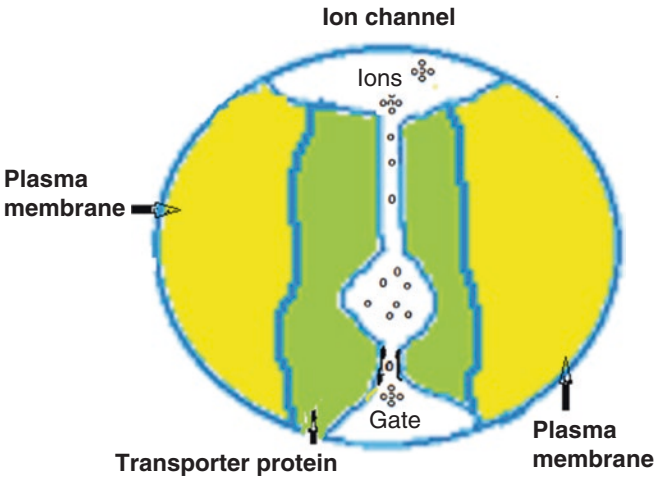

Fig. 1.2 Ion channel, with transporter proteins lining the pore of plasma membrane (adapted from Mitra 2015)

they exist they are shallow and separated by small free energy barriers (Roux et al. 2011).

All channels mediate passive transport of ions down their chemical or electrochemical gradient across the membrane due to difference in concentrations of ions on each side of the membrane as well as any electrical potential across the membrane (Fig. 1.2).

1.2.1.2 Ion Transporters (Carriers)

Transporter proteins are 'vectoral' enzymes (Dubyak 2004). Their functioning involves (1) a selective recognition/binding of the ion to be transported, (2) conformational changes in carrier protein due to binding of the ion and (3) physical movement of the ion across the membrane caused by such conformational changes. Ion transporters can catalyse movement of ions against their electro-chemical gradient (not ion channels) deriving energy from ATP hydrolysis. There are three types of ion transporters:

1. **Uniporters**: They transport one type of ion across the membrane, e.g.: P-type ATPases, $Ca2^{+}$-ATPase.
2. **Symporters (co-transporters)**: They transport more than one type of ion across the plasma membrane, e.g.: NRTs ($2H^{+}/NO_3^{-}$ co-transport), TaHKT1 (K/Na co-transporter).
3. **Anti-porters (Exchangers)**: There is exchange of one ion for the other, which moves in opposite directions, e.g.: CHX (K^{+}/H^{+} anti-porter), CAX (Ca^{2+}/H^{+} anti-porter) (Fig. 1.3).

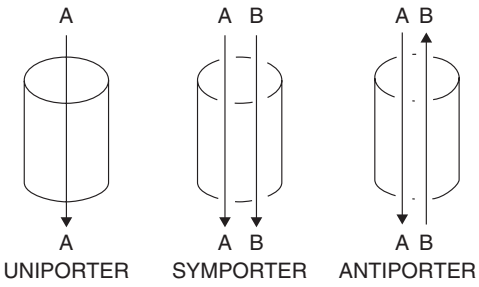

Fig. 1.3 Types of transporters: Uniporters, Symporters and Antiporters (adapted from Mitra 2015)

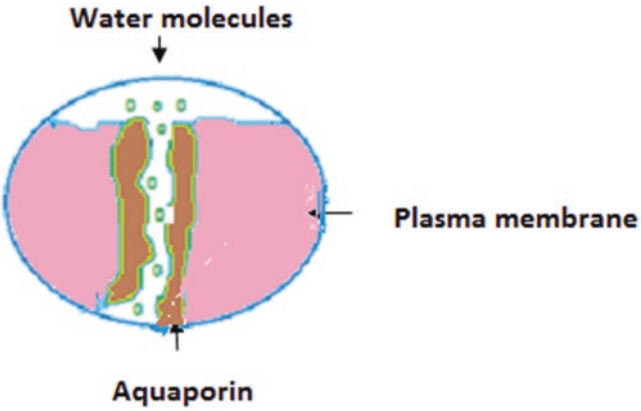

Fig. 1.4 Water channel, with aquaporins lining the walls of the pore across plasma membrane, (adapted from Mitra 2015)

1.3 Mechanism of Uptake of Water

Water is taken up into cells through water channels. Aquaporins, a large protein family found in both eukaryotes and prokaryotes, control movement of water through the narrow channels located on the plasma membrane. Molecules with proper orientation are selectively allowed to pass through the channel. Ions carrying charges such as (H^+) and (H_3O^+) are prevented from passage. Smaller uncharged molecules such as glycerol and urea are allowed passage through the channel. Glycerol molecules, which are larger than water molecules, appear to move in a single file through the amphipathic channel where NPA motifs play a critical role (Chaumont et al. 2000) (Fig. 1.4).

Plant genomes contain a large number of aquaporin (AQP) genes to cope up with adverse water regimes encountered by them during their growth period. *Arabidopsis* has 38 AQP genes of 2–3 kb size (Johanson et al. 2001; Quigley et al. 2002), maize 33 (Chaumont et al. 2000), barley 23 (Katsuhara et al. 2002), rice 34 (Nguyen et al. 2013), wheat 35 (Forrest and Bhave 2008) and soybean 66 (Zhang et al. 2013) AQP genes.

The proteins coded by AQP genes belong to a major intrinsic protein (MIP) family. MIPs are classified into five sub-families such as PIP (plasma membrane intrin-

sic protein), TIP (tonoplast intrinsic protein), NIP (Nod 26-like intrinsic protein), SIP (small basic intrinsic protein), and XIP. (Recently, XIP genes have been reported to be involved in the transport of a wide range of hydrophobic solutes (Venkatesh et al. 2015)). The members of the families are not confined to the location as indicated by their names. They may be found elsewhere.

1.4 Primary Nutrient (N, P and K)

The primary nutrients, Nitrogen (N), Phosphorus (P) and Potassium (K), are required in relatively large quantities by plants for their metabolism and growth. Arable soils do not contain sufficient reserve of these nutrients to meet the requirements of crops grown on the same patch annually. Crop removal of these nutrients needs to be replenished every year by adding required doses of fertilisers containing these nutrients to harvest optimum yields of crops. There is significant reduction of crop yield due to deficiency of any of these nutrients.

1.4.1 Uptake of Nitrogen (N)

Surface soils generally contain 0.03–0.4% of total N, 95% of which is in the organic form. Plants contain about 1–6% of N of their dry weight. Nitrogen is primarily taken up by plants as NO_3^- or NH_4^+ ions. Unfertilised soils may contain NO_3^- at a concentration of <1 mM but application of fertilisers may raise it >70 mM.

Concentration of N is more or less constant within cytoplasm. Nitrate concentration in cytoplasm is limited to about 2–5 mM and 5–75 mM inside vacuole (Miller and Smith 1996). NH_4^+ is toxic and is not allowed to accumulate within the plants.

Nitrogen is a constituent of amino acids, proteins, purine and pyrimidine rings of nucleic acids, chlorophyll and enzymes. All of these compounds are involved in plant metabolism and growth. Adequate nitrogen supply results in vigorous growth of plants and the leaves turn dark green due to high photosynthetic activity. Deficiency of N causes impaired photosynthesis and degradation of chloroplasts. This appears first as yellowing of older leaves while growing leaves remain green.

1.4.1.1 Mechanism of N Uptake

Plants take up N primarily as NO_3^- and NH_4^+. Acquisition, uptake, transport and redistribution of both of them are under strict genetic control (Siddiqi et al. 1990; King et al. 1993). The primary event of NO_3^- uptake is its transport through plasma membrane of root epidermal and cortical cells. This is carried out by a favourable H^+ (proton) electrochemical gradient maintained by the plasma membrane (PM) H^+-ATPases (Proton pumps) (Miller and Smith 1996; Quaggiotti et al. 2003; Sperandio et al. 2014). PM H^+-ATPase activity maintains membrane potential ($\Delta\Psi$) and proton motive

force (Δp) necessary for ion transport. Both for high and low affinity transport system NO_3^- uptake takes place by symport of $2H^+/NO_3^-$ (Crawford and Glass 1998).

1.4.1.2 Nitrate Transport Genes

There are four families of genes involved in transport of NO_3^- across plasma membrane in *Arabidopsis* such as (1) NRT1 (Nitrate Transporter1/Peptide Transporter family, 53 members), (2) NRT2 (7 members), (3) CLC (Chloride channel, 7 members) and (4) SLAC1/SLAH (Slow Anion Channel-Associated 1 homologues, 5 members) (Krapp et al. 2014). The four families have a total of 73 genes out of which 60 are from NRT1/PTR and NRT2 families. Out of 35 genes characterised 24 are nitrate transporters (Krapp et al. 2014).

1.4.1.3 Nitrate Transporters (NRTs)

There are three different nitrate transport systems in plants. When the external NO_3^- concentration is high (1–50 mM), an essentially unregulated and constitutively expressed Low Affinity Transport system (LATS) operates (Crawford and Glass 1998). A High Affinity Transport (HATS) system operates, when external NO_3^- concentration is low (<0.2 mM). Some of them are constitutively expressed (cHATS) and others induced by NO_3^- (iHATS) (Fig. 1.5).

The NRT1 genes encode low affinity transporters (LATS), when the NO_3^- concentration in the soil is high (>1 mM, Orsel et al. 2002). The NRT2 genes encode high affinity nitrate transporters at low NO_3^- concentration (<0.2 mM). Some of the NRT2 genes are inductive (iHATS) and others constitutive (cHATs). AtNRT1;1 (CHL1) is a dual affinity nitrate transporter, switched off and on by phosphorylation/dephosphorylation of threonine T101 in its polypeptide chain (Liu et al. 1999). The CBL (calcineurin B-like) inter-

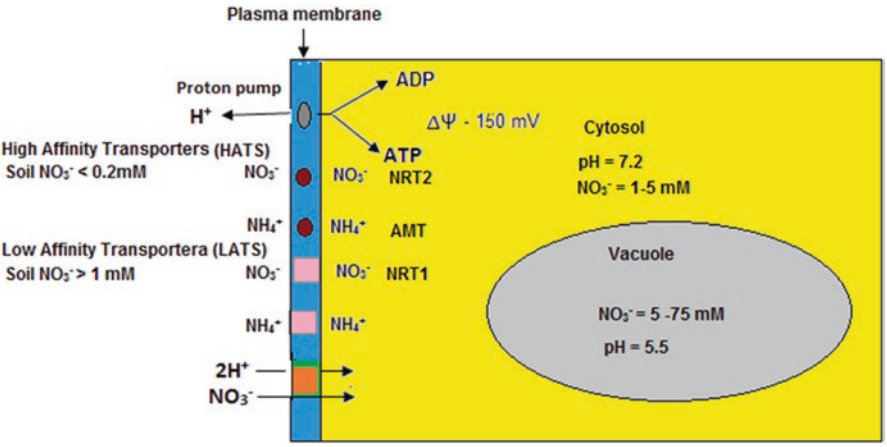

Fig. 1.5 Mechanism of nitrogen uptake by plants (adapted from Mitra 2015)

acting protein kinase, CIPK23 (SnRK3;23), phosphorylates T101 under low nitrate conditions, allowing NRT1;1 to act as a high affinity nitrate transporter (Ho et al. 2009). Dephosphorylated NRT1;1 is a low affinity nitrate transporter.

Nitrate transport in *Arabidopsis* is carried out by two transporters from NRT1 family, AtNRT1;1 and AtNRT1;2, and two from NRT2 family, AtNRT2;1 and AtNRT2;2. When external NO_3^- concentration is low, NRT2;1 proteins localised on the plasma membrane constitute the major component of HATs (72%) activity (Li 2006). It requires a second protein NAR2 for its stability. AtNRT2;1 and AtNAR2;1 form a tetramer with two subunits each, which constitute the active NO_3^- transporter (Yong et al. 2010).

In higher plants *NRT2* genes isolated so far are preferentially expressed in the roots (Tsay et al. 2007). OsNRT1 expressed in epidermal cells of rice roots is a homologue of *Arabidopsis* AtNRT1;1(CHL1) (Lin et al. 2000). Four HATs OsNRT2;1, OsNRT2;2, OsNRT2;3 and OsNRT2;4 and two NAR proteins OsNAR2;1 and OsNAR2;2 have been isolated from rice (Feng et al. 2011; Sperandio et al. 2014). In maize Zm NRT2;1 is involved in influx activity and ZmNRT2;2 in xylem loading process (Trevisan et al. 2008).

OPTs and PTRs: Some members of NRT1 are peptide transporters called OPTs (oligopeptide transporters) involved in transport of tetra- and penta-peptides. Some of the OPTs transport glutathione, glutathione conjugates, phytochelatins and metals (Tsay et al. 2007). PTRs are di- and tri-peptide transporters. Seven families of NRT1/PTRs have been identified. They transport nitrate, di- and tri-peptides, auxins and carboxylates (Fan et al. 2014). During seed germination of barley, HvPTR1 expressed in plasma membrane of scutellar epithelial cells, transport peptides produced by hydrolysis of endosperm storage proteins to the developing embryo.

1.4.1.4 Ammonium Transporters

The AMT1 family of high affinity NH_4^+ transporters contains five members in *Arabidopsis*, of which AtAMT1;1, AtAMT1;2 and AtAMT1;3 have been studied in detail. In rice four NH_4^+ transporter genes have been identified (Suenaga et al. 2003). OsAMT1;1 is expressed in roots and shoots. OsAMT1;2 is root specific and induced by NH_4^+. OsAMT1;3 is root specific and depressed by nitrogen application (Sonoda et al. 2003). Two rhizodermis localised transporters ZmAMT1;1 and ZmAMT1;3 have been identified from maize.

1.4.1.5 Regulation of Nitrate and Ammonium Transporters

Some of the genes encoding nitrate transporters are subjected to transcriptional regulation through inductive effects of NO_3^-, while both encoding NO_3^- and NH_4^+ transporters are subject to down-regulation by glutamine (Anthony et al. 2002). Nitrate and glutamine concentration constitute an intricate N regulatory network at the root tip that is responsible for orchestrating changes in root growth rate and root architecture.

Nitrate stimulates primary root growth, both directly and by antagonising inhibitory effect of glutamine, which stimulates root branching (Walch-Liu and Forde 2008).

Ammonium transporters are oligomeric proteins. They undergo conformational coupling among monomers for ammonium uptake. This provides a mechanism for tight regulation of ammonium transporters. Rapid shut off mechanism is required to prevent toxic accumulation of NH_4^+. Application of higher levels of NH_4^+ blocks NO_3^- uptake by roots.

1.4.1.6 Biotechnological Approach to Increase N-Use Efficiency

The current biotechnological approach to improve NUE includes manipulation of genes involved in (1) N-uptake, (2) N-assimilation and (3) N-translocation to the edible/useful parts of the crops.

No correlations have yet been observed between over-expression of nitrate transporters and NUE. Over-expression of either the NR or the NiR gene in plants increases mRNA levels, and often affects N uptake but does not seem to increase the yield or growth of the plants regardless of the nitrogen source available.

Egami et al. (2012) introduced fungal glutamate dehydrogenase (*gdh A*) gene encoding NADP(H) dependent glutamate dehydrogenase from *A. nidulans* into potato. The GDH potato had higher photosynthetic rate irrespective of N-supply and resulted in higher tuber yield and NUE. A similar claim was made by (Naohiro) Aoki et al. (2009) of the same group for rice and potato (Mitra 2015).

Shrawat et al. (2008) introduced a barley AlaAT (alanine aminotransferase) cDNA driven by a rice tissue-specific promoter (OsAnt1) into rice plants. The transgenic plants had significant increases in the biomass and grain yield as compared to control plants when plants were well supplied with nitrogen. Significant progress is yet to be made in improving NUE through genetic manipulation.

1.4.2 Uptake of Phosphate (Pi)

Total P in surface soils varies from 0.005 to 0.15%, 50% of it in organic form. Pi (phosphate) content of plants is in the range of 0.05–0.5% of their dry weight. Phosphorus is primarily taken up by plants in the forms of phosphate ions. The forms of phosphate ions available to plants are HPO_4^{2-}, $H_2PO_4^-$ and PO_4^{3-} based on the pH of rhizosphere. At pH 7.2 $H_2PO_4^- \approx HPO_4^{2-}$, above pH 7.2 $HPO_4^{2-} > H_2PO_4^-$, but below pH 7.2, $H_2PO_4^-$ dominates and is more than HPO_4^{2-}. The concentrations of phosphate ions, HPO_4^{2-}, $H_2PO_4^-$, in soil solution are very low (0.1–10 μM). Plant uptake of HPO_4^{2-} is much slower than $H_2PO_4^-$ (Mitra 2015). Cellular Pi content is in the range of 2–20 mM (Bieleski 1973; Schachtman et al. 1998).

Phosphorus is a constituent of high energy compounds such as nucleoside triphosphates (ATP, GTP, CTP, UTP), phosphoenolpyruvate, creatinine phosphate, etc., which supply energy to drive endergonic metabolic reactions. Energy storage and energy transfer are the major biochemical functions of the high energy phosphorylated compounds.

A common symptom of Pi deficiency in plants is dark green or purple shoot due to anthocyanin accumulation. Phosphate deficiency causes induction of enzymes involved in synthesis of anthocyanins (Vance et al. 2003; Fang et al. 2009), which protects nucleic acids from UV damage and chloroplast from photo-inhibitory damage (Zeng et al. 2010).

Phosphate deficiency results in starch accumulation in the cells. Low cellular Pi removes allosteric inhibition of the enzyme ADP Glc-pyrophosphorylase involved in starch biosynthesis in cells. Pi deficiency up-regulates some of the glycolytic bypass enzymes such as pyrophosphate (PPi)-dependent phosphofructokinase, PPi-phosphoenol pyruvic kinase, pyruvate phosphate dikinase and the tonoplast H $^{+}$-pyrophosphatase (Plaxton and Podesta 2006). Glycolysis appears to be bypassed by avoiding those reactions requiring P. Under severe Pi deficiency a large decline of ATP and ADP (up to 80%) and other nucleoside phosphates occurs. Plants respond by adopting alternate metabolic pathways (Plaxton and Tran 2011). Moderate Pi deficiency causes significant reduction in glutamine synthetase and nitrate reductase enzymes and affects amino acid metabolism and N-assimilation (Calderon-Vazquez et al. 2008).

Mechanisms of Pi Uptake by Plants: Plants respond to Pi deficiency through (1) morphological adaptations, (2) metabolic changes and (3) genetic responses.

1. ***Morphological Adaptation of Plants due to Pi-Deficiency***: Under conditions of Pi-deficiency plants adapt themselves suitably through modification of their roots and shoots so as to acquire more Pi from soil and use them frugally to support plant growth. Rhizosphere is a critical region around roots, where intense interactions among plant roots, soil and microorganisms take place. Deficiency of Pi has profound effects on root growth and its architecture. These are modified suitably to explore a larger volume of soil so as to absorb more P to meet the P-demand of plants. Pi-deficiency causes delayed leaf development, reduction in number of leaves and leaf expansion, decreased photosynthetic capacity, stunted growth (reduced auxiliary shoot emergence and elongation), impaired flower development and an increased root/shoot ratio of the plants (Vance 2010). Pi from lower and older leaves translocates to newer leaves. Enhanced uptake of Pi by roots and translocation to shoots results in excess Pi accumulation in older leaves and may cause chlorosis and necrosis of leaf tips due to Pi-toxicity.
2. ***Metabolic Changes***: Plant roots exude a variety of organic compounds under normal conditions of growth. These include: sugars, organic acids, amino acids, growth hormones, phenolics, proteins etc., which affect rhizosphere chemistry and alter plant-microbe interaction, allelopathy and nutrient acquisition by plants.

Excretion of organic acids in response to Pi deficiency lowers pH in the rhizosphere by 2–3 units than the bulk of the soil. This may increase dissolution of sparingly soluble soil-P (Marschner 1995). While the protons excreted through organic acids lower the pH, the carboxylate anions react with Fe^{3+}, Al^{3+} and Ca^{2+} present in insoluble compounds of Pi-containing minerals. They form chelates with the cations and release Pi for uptake by the plants. This results in an increase of soil solution Pi concentration by about 1000-fold (Plaxton and Tran 2011). Under conditions

of Pi-deficiency, plants recycle P from older tissues to new tissues. Plants also remobilize from non-essential uses to essential uses.

Genetic Response to Phosphate Deficiency: Phosphate deficiency results in coordinated induction of hundreds of genes encoding enzymes, which maximise capacity of plants to acquire phosphate more efficiently from external sources and reprioritize internal use of phosphorus (Plaxton and Tran 2011).

Phosphate Transporters: Plants have both low and high affinity transport systems encoded by corresponding genes. The low affinity transport systems are constitutive and operate at higher Pi concentration. High affinity phosphate transporters are located primarily in plasma membrane of root hairs and operate at low Pi concentration. The high affinity transporters are induced when Pi is deficient.

Mechanism of Phosphate Transport: The transporters, which are $H_2PO_4^-/H^+$ symporters, move Pi against the steep concentration gradient of about 10,000-fold or higher (concentration of Pi in soil solution ≈ 0.1–10 μM and cellular concentration inside roots ≈ 2–20 mM) through active transport with energy derived from ATP. The movement from root surface to xylem is symplastic and is at a rate of about 2 mM h^{-1} (Bieleski 1973). Transport of Pi to above ground parts is through xylem flow and to cells in tissues through symplastic transport. Movements of Pi through plasma membrane into cells and into vacuole within cells are carried out by $H_2PO_4^-/H^+$ symporters with energy derived from ATP.

Genes Involved in Pi Acquisition and Transportation: Genes of four transporter families, PHT1, PHT2, PHT3 and PHT4, are found in *Arabidopsis*. Members of PHT1 gene family are expressed in root epidermal cell and the encoded transporters are located on the plasma membrane (Lin et al. 2009). They are high affinity $H_2PO_4^-/H^+$ symporters and function to acquire Pi from the rhizosphere.

Early Genes and Late Genes: Genes that respond to P deficiency can be grouped into 'Early genes' that respond rapidly and often non-specifically to Pi deficiency, or 'Late genes' that impact on the morphology, physiology or metabolism of plants upon prolonged Pi deficiency (Vance et al. 2003, Hammond et al. 2004).

PHO Regulon Genes: There is a Pi-starvation-inducible rescue system in plants with their promoter region, the PHO regulon genes, under a common regulatory system (Goldstein et al. 1988). The Pi-responsive genes, TPSI1 from tomato and Mt4 from *Medicago truncatula*, have *cis*-regulatory elements 'GCACG (G/T)' in their binding sites. The AtPHR1 (phosphate starvation response 1) gene from *Arabidopsis* has a motif, a *cis*-element 'GNATATNC' (P1BS, PHR1 specific binding sequence, *cis*-element 'GNATATNC'), which is shared by several Pi-responsive genes.

Late Genetic Response to Pi Deficiency: PHR1 is involved in coordinated regulation of many 'late' Pi starvation genes, such as RNases, phosphatases, TPSI/Mt4 family (Franco-Zorrilla et al. 2004, Hammond et al. 2004) and OPSI1 (Wasaki et al. 2006), which have PHR1 binding sites. PHR1 binds as a dimer to the promoter of 'late' Pi-starvation genes. Most of the Pi taken up by roots is subsequently transported through xylem to shoots.

Sugar Signalling: Müller et al. (2007) have identified 149 transcripts of Pi induced genes, which are regulated by the interaction between Pi deficiency and sucrose availability. Many of these genes encode proteins involved in carbohydrate metabolism and P re-mobilisation.

Micro RNA (miRNA): MicroRNAs (miRNAs) containing 19–25 nucleotides are found in all animals and plants but not in fungi. They are post-transcriptional regulators encoded by specific genes, several at a time or by some portions of the introns of genes, whose m-RNA they regulate. They either completely destroy the m-RNA if their sequences exactly match (usually in plants) or repress the translation of m-RNA if there is a partial match. In the later case several of them simultaneously bind to the UTR (un-translated) region of m-RNA. In plants they may target the coding region itself (He and Hannon 2004).

miRNA and Phosphate Deficiency: Phosphate deficiency causes up-regulation of miR399, which decreases rapidly on Pi addition (Fujii et al. 2005, Bari et al. 2006). Over-expression of *Arabidopsis* miR399 in tomato results in increased accumulation of Pi. There is also augmented excretion of acid phosphatases and protons by roots, which facilitates Pi acquisition from soil (Gao et al. 2010). Homologues of miR399 have been found in rice, tomato, common bean (*Phaseolus vulgaris)* and *Medicago truncatula* (Kuo and Chiou 2011).

Methods Adapted to Improve Phosphate Use Efficiency: Phosphate (Pi) use efficiency (PUE) of crops is generally low (15–20%) due to various soil and plant related factors. Some of the methods adopted to improve PUE are as follows: (1) Growing suitable plant associations with high and low Pi-uptake capacities, (2) facilitation of Pi availability by one crop to the other through rhizosphere acidification, (3) manipulating expression of genes enabling growth in low-P environments.

1.4.3 Uptake of Potassium (K)

Potassium content of soils is in the range of 0.5–2.5%. Plants contain about 2–10% of K of their dry weight. Cytoplasmic concentration of K^+ is maintained at approximately 100 mM, although vacuole may contain 20–200 mM of K^+ (Gierth and Mäser 2007). Apoplastic concentration of K^+ may vary between 10 and 200 mM and may increase up to 500 mM (White and Karley 2010, Wang et al. 2013).

Potassium activates about 60 enzymes involved in various metabolic processes, such as photosynthesis, protein synthesis, oxidative metabolism etc., and improves quality and stress tolerance of crops. It is also involved in osmo-regulation, turgor driven movements and maintenance of the plasma membrane potential. Within the cytosol, K^+ ion neutralises the soluble and insoluble macromolecular anions and stabilises pH at about 7.2, which is optimal for most enzymatic reactions (Marschner 1995).