Plant Solute Transport

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Preface

Plants generate oxygen, consume carbon dioxide and convert the energy of the sun into food. Life on earth, as we know it, could not have developed and cannot exist without them. Human physical needs are serviced by plant products, from using their molecules as pharmaceuticals to using their bodies for timber. The extent of interest in flowers, gardens and landscapes indicates the psychological importance of plants to us. The acquisition and transport of solutes is fundamental to plant processes at all levels of organisation, and underlies their ability to colonise the land. The purpose of this book is to examine solute transport as a subject in its own right and consider it from the molecular to the ecological and agricultural contexts.

Plant cells are full of a vast array of solutes, some in very large quantities. Plants expend considerable amounts of energy and resources upon acquiring or synthesising these solutes that are necessary for the plant's existence. The need for such quantities arises from the way plants grow and the environments in which they grow.

Plants increase in size principally through cell expansion: the volume of individual cells increases over time. To achieve this, water must move into the cell. The structure of plants and plant parts, as well as their rigidity and shape, depends largely upon a hydrostatic skeleton whereby the solution within the cell is contained under pressure by a viscoelastic cell wall. Even the leaves of trees wilt without this. Accumulating and retaining water against hydrostatic pressure requires an opposing force, and this is provided by the osmotic effect of accumulated solutes. This is one reason why the concentration of solutes inside the plant must be much greater than that in the external medium.

Plants also face a continual battle to acquire water at least as fast as they lose it. In order to grow, plants must obtain carbon dioxide from the air. The stomatal pores that allow this automatically permit water vapour to pass in the opposite direction from the moist leaf to the usually much drier air. Plant cells must be able to replace this water from the soil as well as compete with the atmosphere to retain some of this water in the plant. Once again, the osmotic forces provided by the accumulation of solutes are the plants' main weapons.

Plants have also evolved in a marine environment where the concentration of inorganic ions was considerable. To avoid dehydration, early plant cells also needed to have an equivalent concentration of solutes. Vital processes such as protein synthesis developed at this time and have since been conserved rigidly. This can explain the requirement plant cells retain for elevated and specific concentrations of inorganic ions in their cytoplasm.

The marine environment provides abundant and fairly continual replacement of nutrient requirements, but this is not true for all soils. Since plants colonised the land, they have needed to forage and apply strategies to seek and mobilise nutrients from the limited quantities available in many field situations.

In addition to these inorganic ions, plant cells contain a vast array of solutes, from small molecules up to proteins and nucleic acids. These are components of cellular biochemistry; as materials, intermediates, products and co-factors in pathways and cycles. Solutes are important for the storage and mobilisation of reserves. The co-existence of all these different processes is dependent upon compartmentalisation by membranes within and between organelles (e.g. vacuoles, chloroplasts and mitochondria), and for this a multitude of transport processes of varying specificity and capacity are required.

Directly or indirectly, most of these processes are energy-driven. In plants, the primary energy currency is the proton motive force: proton gradients set up by conversion from high-energy chemical bonds and by the photosynthetic and respiratory electron transport chains.

Plant solute transport today has to meet these requirements for the uptake, synthesis and movement around the plant of sufficient quantity and quality of solutes for all of these needs. This must be achieved across the range of ecological conditions in which plants grow, ranging from the relatively sufficient conditions provided in agriculture to those severely limited by the availability of water, of nutrients, and those affected by non-optimal temperatures and by mineral toxicity.

This book sets out to provide a coherent coverage of solute transport in plants. The first section covers the physical concepts behind the solute and water movement and the roles of solutes in the plant. The second section covers the transport of solutes at the molecular, cellular, tissue and whole-plant levels of organisation; from the nanometre distances across a membrane to the 100 or more metres required to traverse a tall tree. This section includes a discussion of the membranes that provide the compartmentalisation central to living processes and that allow different cells to perform different functions and different processes to go on within the same cell. The methods of measuring solute transport at different levels of organisation are also addressed. The movement of solutes by pumps, carriers and ion channels is discussed, covering movement from within an organelle to movement around the plant. The two long-distance transport systems – the xylem and phloem – and the forces that drive movement in the two systems link the tissue and whole-plant levels of organisation. The final section of the book examines how solute transport has been adapted in plants growing in a range of conditions from carefully tended horticulture to those of environmental stress. The conflicting priorities of ecological and agricultural adaptation are highlighted.

Plant Solute Transport aims to provide an in-depth coverage of this substantial topic, from the molecular to the ecological scale. There is a gap, which we seek to fill, between the large general textbook covering all of plant physiology (perhaps including growth and development and/or biochemistry and molecular biology) and the highly detailed multi-author volume addressing one specific area (such as membrane transport). This volume is directed particularly at research workers and graduate students, but has a wide enough coverage to be of use to third-year students in plant sciences. The up-to-date research is grounded in the underlying physics and chemistry and placed in the context of what solute transport must achieve for plants in both ecological and agricultural contexts.

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1 General introduction

Anthony Yeo

1.1 Introduction

Plant cells are full of solutes, both dissolved inorganic ions and low-molecular-mass organic molecules. The concentration of solutes inside plant cells is higher than that in the growing medium, and it is much higher for the large majority of terrestrial plants. Plants expend considerable amounts of energy and resources upon acquiring or synthesising these solutes, so perhaps the first question to ask is, 'why do they do it?'

In part the reasons are historical. The salinity of the early oceans was substantially greater than it is today (Knauth, 1998). The conditions in which life evolved are still debated. It is believed that life might have been evolved in situations where freshwater diluted this salinity; however, the great majority of early life arose in the oceans. For simple physical reasons (water flows across their semipermeable membranes influenced by osmotic forces; see Chapter 3), it was necessary for cells to match the water potential of the seas to remain hydrated; so an equivalent concentration of solutes was needed. Some fundamental living processes of cells were laid down during this arcane period – long before life colonised the land. Observation has shown that these processes have been rigidly conserved; for instance the ionic requirements for protein synthesis (see Chapters 3 and 14). The ghost of the past commands the conditions that plants have to maintain in the cytoplasm of their cells today (a hundred or hundreds of mM of solutes). This is even though the concentration of salts in the growing medium may now be of the order of μM and mM, a tiny fraction of that in the present-day or ancient oceans.

In part the reasons are physical. The first challenge of life on land was to remain hydrated. As plants evolved from wetlands to dry land, the availability of water became less. Retaining water against the non-osmotic components of water potential became a priority for the first time. The soil was periodically dry, and the cells of plant roots had to retain water against the water potential of the drying soil. In addition to this, the leaves of plants were in a medium that was hardly ever saturated with water, that is the air. The moist surfaces of cells lost water to the demands of the unsaturated air – because of the vapour pressure difference. This has been the no-win situation of plant life on land. The need to acquire atmospheric carbon dioxide for photosynthetic carbon fixation meant that the cells could not be permanently waterproofed – letting in carbon dioxide meant letting out water. Cells not only had to obtain their water from drying soil, but also had to compete with the voracious demands of transpiration – some 98% of water used (see Chapter 3) – and for this they had to depend upon their own osmotic pressure.

In part the reasons are structural. Without enough water, plants and even the leaves of trees wilt. Plants still rely largely on a hydrostatic skeleton maintained by turgor pressure; that is the positive hydrostatic pressure that the cell contents exert upon the surrounding structural cell walls (see Chapter 3). Cells use the osmotic component of water potential (hence the dissolved solutes) to build the turgor pressure. Without this, leaves (or large parts of the plant in the absence of the structural thickening found in woody stems) become flaccid. Such leaves are then unable to fulfil the needs of photosynthesis and may be irreversibly damaged. The large majority of plant growth is by cell expansion. In contrast with animals, mature cells of plants contain a large central vacuole (which may be 90% or more of the volume). This is the principal way in which plants generate size, be it to get up into the light or down into the wet soil, or to expand leaves and ramify roots to capture carbon dioxide, water and nutrients. A continual increase on the quantity of solutes is needed to sustain the concentration within the growing cells, without this the turgor pressure would decrease and there would be no growth.

For all these reasons, it is a fundamental requirement for survival that plants fill their cells with solutes, whether this is in the form of inorganic ions concentrated from the growing medium or with organic solutes synthesised from sources (of principally: carbon, nitrogen, phosphorus, oxygen and hydrogen) in the atmosphere and soil.

Plants need both the major inorganic ions (for instance, potassium, magnesium and nitrates) and the numerous ions that serve the role of specific ‘micronutrients’. On land these resources had to be found from an environment in which they could become rapidly depleted – in contrast to the sea, where, even at low concentrations, there was normally continual replacement. Nowadays, ‘fertigation’ and nutrient film techniques are common in commercial horticulture to prevent such depletion. In the soil, plants must often forage for the materials they need. Overall the flows of water and solutes are locked together in a dance of physical laws. Evapotranspiration causes a mass flow of water through the soil-plant-atmosphere system and the accumulation of salts drives localised flow of water which brings with it dissolved salts. It takes two to tango.

The solutes of plant cells and their roles are diverse. Quantitatively, the largest components are dissolved inorganic ions and low-molecular-mass organic molecules. But the term solute also includes compounds of greater molecular mass as components and products of biosynthetic and catabolic pathways and cycles, up to and including soluble proteins and nucleic acids. Not all soluble species always exist in, or are always transported in, solution. Soluble inorganic ions must often be transported anhydrously across the membrane bilayer by protein carriers. Also, there are species that are not soluble in water but are nonetheless transported throughout the plant; for instance insoluble proteins and viral particles.

The transport of solutes occurs over a large range of scale, some 10 orders of magnitude, from the order of 10 nm to cross a cell membrane to the order of 100 metres to ascend the tallest tree. The nature of the events and driving forces that underlie transport over such differences in scale are extremely different for the same solute. A potassium ion carried to the top of a tree in the xylem is in solution in water, but a potassium ion being transported across a membrane by a carrier is not in solution but is bound reversibly to a transport protein. Movement up the xylem of a tall tree is by a mass flow of solution driven largely by the evaporation of water at the leaf surface, while accumulation across a membrane is driven either directly or indirectly by energy derived from a biochemical process.

Membranes provide the compartmentalisation that is central to living processes; allowing different cells to perform different functions and allowing different processes to go on within the same cell. The concentrations of soluble metabolic intermediates of the citric acid cycle within the mitochondrion can be made relatively independent of the concentrations of the same solutes in the cell as a whole. This allows the same solute to be used for different purposes in different parts of the same cell. Extreme examples are the vacuolar compartmentalisation of malate in CAM plants (see Chapter 13) and of salts in halophytes (see Chapter 14); in both cases permitting the retention of concentrations that would destroy the cytoplasm. More generally, compartmentalisation within membrane-bound compartments provides efficiency, allowing high concentrations to exist in one place without the need for the enormous quantities that would be needed to provide the same concentration throughout the cell. The compartmentalisation of protons is universal in plant cells, with pumping out of the cytoplasm both across the plasma membrane to the outside and across the tonoplast into the vacuole. This not only provides the neutral-to-alkaline pH needed in the cytoplasm, but the electrochemical potential gradient of protons. In plant cells, it is this proton motive force (PMF) that is used both to store and couple the energy derived from biochemical processes (ATPases and pyrophosphatases, the photosynthetic and respiratory electron transport chains) with the active transport of other solutes.

1.2 Synopsis

There is a wide range of inorganic and organic solutes in plants. Chapter 2 is an introduction to methods for their extraction and analysis. Inorganic elements can be measured by optical properties (by flame emission and atomic absorption spectroscopy), mass spectroscopy, X-ray fluorescence, with ion-specific electrodes, and by ion chromatography. Analysis of organic solutes is usually achieved by chromatographic separation, often in conjunction with mass spectroscopy and nuclear magnetic resonance. Intracellular localisation can be achieved either via transmission or scanning electron microscopy preceded by precipitation, freezing or freeze-substitution. Ion-specific intracellular electrodes can also be used, as can direct sampling using a modified pressure probe. Individual ions can be monitored in cells loaded with fluorescent probes, and tracer fluxes can be interpreted using analysis of compartmental models. Chapter 2 also introduces the roles of solutes in the vacuole, cytoplasm, organelles and cell walls.

Chapter 3 begins by describing the properties of water that are important to its behaviour in biological systems: the hydrogen-bonding that confers structure and order, latent heat, thermal capacity, tensile strength, surface free energy (tension) and incompressibility. The large dielectric constant gives water its solvent properties, its ability to perform charge shielding and provide hydration shells, which link to its roles in maintaining the higher order structure of macromolecules.

It is difficult to understand how plants acquire and transport solutes without understanding the physical bases of ion and water movement. What are the driving forces? Which way do ions and water 'want' to go? How do plants move and accumulate solutes against physical and chemical gradients? Chapter 3 continues with a consideration of Gibbs free energy and chemical potential, water potential and water potential gradients, osmosis and other colligative properties. It includes the derivation of equations for water movement in cells and in the soil–plant–atmosphere system (resistances and the Ohm's law analogy), and of how surface tension develops negative hydrostatic pressures in drying soils and cell walls. The chapter then moves on to solute movement; diffusion and Fick's law, and to permeabilities and fluxes. The contribution of electrical charge is explored in the derivation of the Nernst equation, Donnan systems and the Goldman equation. Finally, irreversible thermodynamics is introduced as it applies to the analysis of coupled flows of solutes and solvents. With this background, the subsequent section of chapters (4 to 10) looks at how solutes are moved at individual membranes and, on an increasingly integrative scale, within and between cells and around the plant, both up in the xylem and down in the phloem.

Chapter 4 considers the structure and composition of plant membranes – of which there are about 20 types, all comprised of lipids, proteins and carbohydrates in the approximate ratio of 40:40:20. The amphiphatic nature (both hydrophobic and hydrophilic) of lipids underlies the formation of bilayer membranes. These have little intrinsic solute permeability. This is conferred in biological membranes by embedded transporter proteins mediating either active or passive movement and providing varying degrees of regulation. The overall structure of membranes is currently considered to consist of lipid-ordered microdomains, with rather less freedom of movement in the plane of the membrane that was inherent in the first fluid mosaic model. The transport proteins are often multimeric and distributed in membranes in clusters.

Techniques for studying solute transport in membranes are discussed next beginning with those applicable to intact (or semi-intact) tissues and moving on to adaptation of these techniques for use with isolated membranes. There is an emphasis on design and composition of experimental solutions, particularly their osmolarity, and on consideration of unstirred layers, and the difference between the study of net transport and unidirectional fluxes. Methods available include inhibitor studies, radioactive tracers, fluorescent probes and electrophysiology – the last including multi-barrelled electrodes. Individual membrane types can be isolated via protoplasts, and sometimes by direct mechanical means, separated by differential centrifugation and identified by marker analysis. Aqueous polymer two-phase isolation provides information regarding sidedness. Analysis can be performed on vesicles or tiny pieces of a membrane attached to a micro-electrode. Techniques such as fluorescence microscopy and patch-clamping can yield considerable spatial and temporal resolution, enabling the detection of the activities of single ion channels.

Molecular techniques now allow the *in silico* characterisation of the possible function of membrane proteins where there is sufficient information on available databases. Forward and reverse genetic screens can be used to endeavour to relate gene to function, as can the use of over-expression and expression in heterologous systems (generally in yeasts or in *Xenopus laevis* oocytes). The location of proteins within the plant and cell can sometimes be determined by expression using reporter gene constructs. For all techniques of investigations there is a compromise between resolution and invasiveness (or distance from physiological reality). The importance of confirming a result obtained with one technique using a different approach, cannot be overstated.

The details of transport across membranes is considered for simple inorganic solutes, anions and cations (see Chapter 5). Any membrane protein involved in cross-membrane movement of substrates is defined as a transporter. Transporters can be classified as to whether the event they mediate is active or passive, and if it is active, whether it is a primary process or a secondary one utilising energy already stored in proton gradients. Transporters may also be classified as pumps, ion channels, or carriers – the last includes the provision of passive transport at higher selectivity but lower capacity than ion channels. A further form of classification is that of uniport, symport and antiport. All these terms will be met in different combinations in the literature. Broadly speaking, primary, ATP-driven, pumps set up proton gradients to drive secondary transport. Primary pumps are also directly involved in the transport of calcium and heavy and transition metals. Secondary transport in plants is generally coupled to proton gradients, and participates in the uptake and movement of hundreds, if not thousands, of different substrates. Finally, it is the ion channels that are almost exclusively responsible for passive transport. They mediate only passive transport and are either open or closed, known as gating, which may be regulated by voltage, ligands, or may be mechano-sensitive (e.g. stretch-activated). In addition, there is a selectivity filter that operates on the basis of physical size and charge properties. Channels may be inward- or outward-rectifying according to whether they are permitting passage into or out of the cell. The transport rate of channels may be millions per second. Water movement across membranes is always passive and directed by the gradient in water potential. Water may cross membranes via their intrinsic permeability to water and also through proteinaceous pores: aquaporins. The selectivity of aquaporins is related to size and they have very high capacity (over 10^9 per second).

Primary pumps use chemical, redox or light energy to move solutes against their electrochemical gradient. ATPases have low capacity (around 100 per second), and consequently large numbers of these proteins are required. There are also primary pumps for calcium and some other metals, such as for copper in chloroplasts. Transport rates of primary pumps are hundreds per second. Secondary active transport pumps solutes against their free energy gradient, but the energy derives from coupling to the proton gradient set up by the primary pump(s) and can be either symport (in the same direction as protons) or antiport (in opposite directions). These are often termed carriers, as they are neither *primary* pumps nor channels. Such carriers have higher selectivity than channels but lower capacity (hundreds or thousands per second). Major nutrients, such as potassium, are taken up through channels at high external concentrations, and by active processes that are induced upon potassium starvation, at low external concentrations.

Plants have many transport processes that need to operate in different ways to address different environmental conditions and developmental stages, as well as differences between different cells and tissues. The processes require regulation (Chapter 6), which occurs at several levels, e.g. gene expression, mRNA degradation, protein turn-over, protein activity and membrane trafficking. Regulation involves both positive and negative feedback, and the transporters themselves are both components and targets of signalling pathways (e.g. calcium, auxin and ABA). Chapter 6 considers examples of the regulation of transporters in adaptive processes, the molecular mechanisms underlying transcriptional and post-transcriptional regulation, and the regulation of transporters by membrane trafficking.

Regulation of solute transport is required to effect changes in cell volume, both for sustained growth and for the cyclical changes in volume needed in stomatal guard cells for control of stomatal aperture. The pathways leading to co-ordinated regulation of potassium and chloride channels during stomatal closure are examined. High-affinity uptake of nutrients is often induced by deficiency situations, since there may be less costly pathways of uptake when the same nutrient is in abundant supply. Some transporters are induced by a change from high supply to low supply, and some transporters are induced by a change from nil to low supply. Fine-tuning may be via differential regulation of apparently functionally redundant isoforms. Nutrient transport is regulated not only by availability but by the nutrient status of the plant. Transport is also linked to carbon status, and thus is controlled indirectly by environmental factors that affect photosynthesis.

Response to many environmental stresses is dependent upon regulation. For example, 'unwanted' entry of sodium into the root cells in saline conditions will lead to membrane depolarisation, which will open depolarisation-activated calcium channels leading to a rise in cytoplasmic calcium activity, which is in turn a signal to enhance the activity of the sodium-proton antiport carrier at the plasma membrane, which pumps sodium out again. The limited information regarding the molecular components of the transcriptional regulation of nutrient transporters are summarised. Post-transcriptional regulation involves auto-inhibitory domains, protein–protein interactions (e.g. with protein kinases, calmodulins and 14-3-3 proteins), and ligand binding (e.g. ion channel gating by cyclic nucleotides). The 14-3-3 proteins are highly conserved and regulate a wide range of targets including a number of ion channels. Calmodulins are small calcium-binding proteins that are able to translate intracellular calcium signals into a variety of cellular responses. Cyclic nucleotides are widely used in signal transduction, and evidence is building that higher plants use cGMP as a secondary messenger. Finally, the role of membrane trafficking is reviewed. SNARE (soluble NSF attachment receptor) proteins have been identified in higher plants; they are a group of membrane proteins that are highly conserved in eukaryotes and are at the centre of the molecular machinery involved in vesicle trafficking and membrane fusion.

Plant processes involve a complex traffic between organelles, and between organelles and the cytoplasm. Organelles have their own transport systems and these are integrated with cellular metabolism (Chapter 7).

Chloroplasts are part of the plastid family that includes storage plastids and amyloplasts. They contain the light-harvesting centre and the photosynthetic electron transport chain. Chloroplasts have distinct outer and inner membranes, plus the thylakoid system. The outer envelope (OE) has a range of proteins (OEPs) which are selective channels for solutes essential to plastid function. The inner membrane contains the phosphate translocator family and members of the major facilitator superfamily. There are transporters for di- and tri-carboxylates and carbohydrates, for ATP/ADP exchange, and for a range of specific ions (including nitrate and sulphate which are reduced in the plastid) and there are also symporters for transition metals.

Mitochondria are semi-autonomous organelles with a smooth outer membrane and a much-folded inner membrane, which is the energy-transducing membrane. The compartments are the intermembrane space and the protein-rich core, or matrix. One key role of mitochondria is the synthesis of ATP formed by oxidative phosphorylation – the PMF generated by the respiratory chain drives the ATP synthase complex. The outer membrane contains the VDAC porin which is freely permeable to solutes of up to 4–5 kDa: specific permeability barriers reside with the inner membrane. Carriers on the inner membrane include the phosphate carrier, the ATP/ADP carrier, and carriers for intermediates of the tricarboxylic acid cycle, amino acids, and a carrier for succinate/fumarate (which links β-oxidation in the peroxisomes with the TCA cycle). There are also ion channels for potassium and calcium.

Peroxisomes are bounded by a single membrane. They are involved in β-oxidation and are part of the photorespiratory cycle; they also generate reactive oxygen species and contain appropriate protective mechanisms. Glyoxisomes convert lipid reserves to sucrose. The peroxisome family has a ‘specific porin’ as well as transporter proteins including the peroxisomal ATP/ADP carrier. The photorespiratory pathway is split between the chloroplast, mitochondrion and peroxisome.

Vacuoles are multifunctional and are involved in the storage of different metabolites, quantitatively extreme examples being malate (in CAM) and sucrose (in storage tissue). The vacuole is the largest organelle and usually comprises the major volume fraction: it is bounded by a single membrane, the tonoplast. The tonoplast contains proton ATPases and pyrophosphatases which together generate a PMF. A major facilitator imports malate. Tonoplast intrinsic proteins (TIPs, aquaporins) mediate water flow. There are ABC transporters for the accumulation of secondary metabolites and xenobiotics. There are a range of ion channels and carriers mediating the movement of solutes needed for cell expansion, guard cell movement, and compartmentalisation (such as of sodium).

Chapter 8 addresses the main factors affecting and controlling the uptake of charged solutes by plants, from the soil solution to the transpiration stream. It describes root anatomical and physiological responses to the availability of nutrients in the soil and the general processes involved in the transport of solutes into and out of root cells. The Casparian strip blocks apoplastic radial movement of water and solutes when it develops, and in many species this barrier is backed up with an hypodermis. Some leakage may occur, particularly when lateral roots are initiated. There is also a symplastic continuity from cell to cell via plasmodesmata. Root hairs are modified epidermal cells that increase surface area and root radius, and appear to be most important in the acquisition of immobile nutrients. In some instances, epidermal cells are modified as transfer cells. The cells of the cortex may be involved in nutrient uptake depending upon whether the epidermal cells can satisfy the needs of the plant and upon whether they have already depleted the concentration to which the cortex is exposed. The tissue and cell expression pattern of high-affinity transporters varies between different nutrients. Cortical cells may also be involved in re-uptake of nutrients that have been effluxed by cells in outer layers of the root.

Uptake varies along the length of the root, being minimal at the apex (which is phloem-supplied). Root hairs are usually concentrated behind the apex. Uptake of mobile nutrients may occur along the root but uptake of immobile nutrients is mainly near the tip. Uptake of calcium occurs in young roots only where an apoplastic radial pathway remains available. Xylem loading varies longitudinally, clearly affected by the stage of xylem development. Xylem loading is independent of initial uptake, at least for some solutes. There is evidence that shoot requirements can dictate root uptake and translocation rates. Net uptake is the sum of influx and efflux, and the latter can be a very high percentage of the former. Analysis of tracer uptake is usually related to a three-compartment model: (1) the cytosol of cells of the outer root, (2) vacuoles and (3) transport to the shoot. After the initial uptake, filling of vacuoles and transport to the shoot are in parallel. The kinetics of tracer uptake have been interpreted as dual isotherms since the 1960s. This is now considered to represent the co-existence of low-capacity-high-affinity systems at low external concentration, and high-capacity-low-affinity systems at higher external concentration. These may be either channels or carriers.

The xylem has evolved for long-distance upward transport of water and solutes (Chapter 9). The xylem has a large capacity to carry the replacement of transpirational losses and is a leak-proofed conduit with the mechanical strength to avoid collapse under negative hydrostatic pressure. Xylem comprises vessels and tracheids (collectively, tracheary elements, the conducting pathway), fibres and parenchyma (the only living cells in the xylem). The xylem parenchyma cells are densely cytoplasmic with ER, ribososmes and mitochondria. Vessel elements are 5–500 μm (typically 40–80 μm) in diameter and joined end-to-end via perforation plates into vessels that may be several metres long. Tracheids are 10–25 μm and are interconnected by pit fields at their overlapping, tapered ends. The classic interpretation of water movement in the xylem is the cohesion-tension theory. There have also been additional mechanisms suggested which include: mucopolysaccharides to help maintain water flow, osmotic water lifting (root pressure), ionic control of xylem conductance and an electrical driving force.

The concentration of major solutes in the xylem is mostly in the mM range though these concentrations are variable between species and may depend upon shoot demand. The osmotic pressure of the xylem is usually not considerable. Sampling of the xylem is difficult because most methods are very invasive, though there has recently been use of xylem-feeding insects. Loading of potassium into the xylem is probably via depolarisation-activated outward-rectifying potassium channels. There are three types of anion channels involved in xylem loading, and this is mostly a passive process. Sodium loading could be via a non-selective channel but probably via sodium-proton antiport. Unloading of solutes from the xylem into the leaf is plausibly under hormonal control, and a complex network of veins exists to reduce damage due to excessive concentration of xylem contents when water is withdrawn. Proton ATPases are probably the driving force behind both active and passive unloading, with co-transport processes important, for example, for sugars.

The other long-distance transport system in plants is the phloem (Chapter 10). The transport pathway consists of sieve tubes which are an end-to-end arrangement of sieve elements (each 40–500 μm by 5–50 μm) joined at a sieve plate. The plate is perforated by pores and is the major resistance to flow. The sieve tube contains mitochondria but is anucleate. Sieve tubes may live many years and have protection against oxidative damage. The other main component of the phloem is the companion cells which are connected to the sieve tubes via plasmodesmata, through which all proteins destined for the anucleate sieve element must pass from the companion cell.

Analysis of sieve tube contents has been made mostly using phloem-feeding insects or by bark incision. The major carbohydrate in most species is sucrose, at hundreds of mM, though in some species the major transported carbohydrate differs; for example, sorbitol, raffinose or mannitol. Potassium and sucrose are the major osmotica and there is a reciprocity between them, maintaining turgor pressure with varying carbon supply. Phloem transports many other nutrients and, recently, the implications of the transport of mRNA and proteins is complementing and revising the understanding of the phloem. Over 200 proteins have been identified, although sieve elements are unable to synthesise proteins themselves.

Osmotic pressure in sieve tubes at ground level is generally 1–2 MPa. Phloem is loaded at sources (sites where there is synthesis, as in photosynthesis, or else breakdown of storage compounds) and solutes are removed at sinks (where contents are diluted, metabolised, or stored elsewhere). Turgor pressure differences between sources and sinks underlie the pressure flow hypothesis of bulk movement in the phloem. Solutes move into the sieve element from the companion cell. Entry of solutes into the companion cell can take one of the two routes, apoplastic or symplastic. Much evidence favours the former. Sucrose is loaded by a proton co-transport carrier, powered by the PMF set up by the proton ATPase. Loading of potassium into the sieve element-companion cell complex is important both in the transport of potassium in the phloem and in the regulation of the loading process itself. Aquaporins are also present, as are transporters for the loading of many other substances. Unloading may be by either symplastic or apoplastic routes; this differs with species, organ, and stage of development.

In the third section of the book we set out to put this information in an ecological and agricultural context (Chapters 11–15). We describe the factors, other than the transport processes themselves, which limit the supply of nutrients to plants in field conditions and even when growing in carefully tended artificial environments. Next, we look at deficiency and toxicity; some of the ways in which plants have evolved to cope with the 'not enough' and 'too much' of elements and minerals in their growth environment. We then go on to look at how the use of solutes, both in quantity and quality, has been adapted to more extreme environments: the demands of hot, dry deserts, freezing mountains and saline marshes. All of these entail dealing (by avoidance or tolerance) with some form of externally imposed dehydration. There is also a crucial stage in the life cycle of most plants, the internally controlled dehydration concomitant with seed formation. This is true desiccation tolerance and, while this is common place during reproduction, it is very rare in the vegetative tissues of vascular plants.

Many factors, in addition to the properties of the transport processes themselves, affect the rate of uptake of nutrients by plants (Chapter 11). Plants are able to take up nutrients from concentrations that are very low in comparison with those in the soil solution, certainly in fertile soils; except in the case of phosphorus which is commonly at limiting concentrations. Although present, nutrients are not always available: many processes affect the supply of nutrients from the bulk (soil) solution to uptake sites on the roots of plants. These include bioavailability and mobility (the rate of diffusion is impeded by absorption on, and chemical interaction with, the soil). Mass flow of soil solution provides a large-capacity route of nutrient supply, but the contribution of bulk flow to nutrient supply decreases with the decrease in soil water content. There will always be boundary (unstirred) layers around the root in which movement is principally by diffusion. Whenever the flux density of uptake exceeds the flux density of supply, there will be depletion zones around the root, greater than the unstirred layer, across which nutrients must also diffuse. Since diffusion becomes less effective as the distance increases, such supply is commonly limiting, and in many situations the rate of transfer across boundary and depletion zones limits the rate of uptake by the plant.

Distribution of nutrients in the soil is also heterogeneous in both space and time, and interception of nutrients also involves roots exploring and exploiting new volumes of soil. Uptake of nutrients depends on both affinity and capacity (flux density) of transport processes. High affinity transporters may provide enough capacity to avoid deficiency of major nutrients and sufficiency of trace nutrients, but are not able to supply the quantitative needs of the plant to support rapid growth. A spectrum of transport processes exists with lower affinity, higher capacity alternatives providing the uptake at higher external concentrations. Concentrations of major nutrients in the xylem are generally in the mM range, and external concentrations in the same range are generally needed to support maximal growth, even in well-mixed solutions, even though K_{m} values for high-affinity transporters are often in the μM range. Maintaining optimal growth in horticulture increasingly relies upon the controlled supply of nutrient solution to the plant in hydroponics, which has many advantages as well as