

SYSTEM PHYSIOLOGY

PLANT



Dr. KAILASH CHOUDHARY

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PREFACE

Plant physiology is too often taught as a list of pathways to be memorised. It is not that. It is the study of how a rooted, silent organism solves the problems an animal solves by moving — how it captures energy from light, builds a body out of air and soil, decides when to grow and when to wait, lifts water a hundred metres without a pump, and defends itself against everything that arrives. Once a student sees the subject that way, the pathways stop being lists and start being answers to questions worth asking.

This book is arranged to follow that logic rather than the order in which the discoveries happened. It opens with the light reactions, because every joule a plant later spends is captured there, and then follows the carbon: into the Calvin–Benson cycle and the flawed, indispensable enzyme at its centre, through the C₄ and CAM pumps that evolved to hide that enzyme from oxygen, and on into sucrose and starch. Photorespiration and respiration then account for what that carbon costs. Nitrogen follows, from the triple bond of N₂ to the amino acids. The middle of the book belongs to the hormones — auxin and cytokinin, gibberellin and abscisic acid, ethylene and the later messengers — treated together in pairs and families so that the antagonisms between them are visible on the page rather than scattered across separate chapters. Light then returns, no longer as fuel but as information: the circadian clock, photoperiodism and florigen, the phytochromes and the blue-light receptors. The last three chapters deal with what the whole plant must manage — long-distance transport of water and photoassimilate, the private chemistry of secondary metabolism, and the abiotic and biotic stresses a plant cannot walk away from.

Two principles governed the writing. The first is currency. Plant physiology has changed a great deal in twenty years, and textbook accounts have not always kept pace; where the modern evidence overturns a familiar story — the source of the abscisic acid that closes stomata, the receptor that perceives each hormone, the status of the mobile florigen signal, the architecture of the resistosome — the current position is given, and the older view is named as the older view rather than quietly dropped. The second principle is that a concept should be explained once, properly, in the place where it belongs, and referred to from everywhere else. Each of the eleven chapters therefore has a defined territory, and the cross-references between them have been checked one by one.

Every chapter opens with a short orientation and a set of learning objectives, and closes each section with a KEY POINT box that states, in a few lines, what an examiner is entitled to expect. More than two hundred figures and thirty tables carry the detail that prose carries badly. The language is deliberately plain: precision has not been sacrificed, but nothing has been made to sound harder than it is.

The book is written for students preparing for CSIR-NET, GATE, ICMR and the university postgraduate examinations, and for the teachers who prepare them. It assumes an honest undergraduate background in biology and nothing more.

I am grateful to the students whose questions, over many years of teaching, showed me where the standard explanations fail, and to my colleagues at IFAS for the care they have taken over this edition. Errors that remain are mine, and I will be glad to hear of them.

Dr. Kailash Choudhary

ACKNOWLEDGEMENTS

A textbook carries one name on its cover and the work of many people on every page. This one is no exception, and it is a pleasure to record here what I owe to each of them.

My first and deepest thanks are to Er. Radheshyam Choudhary, Chief Executive Officer of IFAS Publication, whose conviction that this subject deserved a book written from first principles is the reason the project began at all, and who gave it the time and the freedom it needed to be done properly rather than quickly. I am equally indebted to Mr. Dadasaheb Sondge, Chief Operating Officer, whose steady oversight carried the manuscript through every stage from first outline to final proof, and who kept a long and demanding schedule moving without ever letting the standard slip.

Pranshu Dwivedi and Vishal Bhujbal assisted in the writing. They gathered and checked source material, drafted and redrafted, and argued with me about explanations until the clearer one won. A great deal of what reads simply in these chapters reads that way because of their patience.

Dr. Rakesh Kumawat and Lalji Kannoujiya read the entire manuscript for accuracy and consistency. Proof reading a scientific text is unglamorous and unforgiving work — a misplaced subscript or a transposed enzyme name can mislead a student for years — and their care has saved this book from more errors than I care to count.

The illustrations are the work of Vikendra Metha, who redrew and designed every diagram in the volume. In a subject where a single good figure can replace a page of prose, his contribution is not decoration but explanation, and the book is far stronger for it.

Deepak Nambani carried out the typesetting and formatting of all eleven chapters. The consistency of layout across more than two hundred pages, the placement of every figure beside the text it belongs to, and the clean final pages are entirely his doing.

Many others contributed in ways that do not appear in any list — colleagues who answered a question at short notice, teaching staff who tested explanations in the classroom, and members of the IFAS team whose work behind the scenes made everything else possible. Their names are not printed here only because I could not print them all; my gratitude to them is no smaller for that.

I thank my students most of all. Their questions, and their honest confusion at the places where the standard accounts fail, shaped this book more than any other single influence.

Finally, I thank my family for their patience through the long months this volume took, and the readers who will now put it to use. Any errors that remain are mine alone, and I shall be grateful to have them pointed out.

Dr. Kailash Choudhary

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CHAPTER

1

Photosynthesis — The Light Reactions

Every breath you take and almost every calorie you eat traces back to a single light-driven event on a green membrane. Photosynthesis is the only large-scale entry point of energy into the living world — the reaction that turns sunlight, water and carbon dioxide into the chemistry of life and fills the air with oxygen. This chapter follows that energy from the instant a photon strikes a pigment to the moment ATP and NADPH leave the thylakoid, ready to build sugar.

LEARNING OBJECTIVES

- Describe chloroplast architecture and locate the light and carbon-fixation reactions within it.
- Explain how a photon excites a pigment, and distinguish the singlet and triplet excited states and their fates.
- Compare the structures and absorption properties of chlorophylls and carotenoids and define photosynthetically active radiation.
- Trace electron flow through PSII, the cytochrome b_6f complex and PSI in the Z-scheme, naming every carrier in order.
- Account for water oxidation by the S-state (oxygen-evolving) clock and for proton accumulation in the thylakoid lumen.
- Distinguish cyclic from noncyclic photophosphorylation and relate them to ATP : NADPH supply.
- Identify the sites of action of the herbicides DCMU, DBMIB and paraquat.
- Explain photoprotection (carotenoids, the xanthophyll cycle, nonphotochemical quenching) and the molecular basis of photoinhibition and D1 repair.
- State the quantum requirement of photosynthesis and account for it from the chemistry of water oxidation.

Photosynthesis is an anabolic process in which green plants, algae and some bacteria convert light energy from sunlight into chemical energy inside chlorophyll-containing cells. It is the principal mechanism of energy input into the biosphere. The overall reaction is often written as:



Water is oxidised, releasing oxygen gas together with protons (H^+) and electrons (e^-); these electrons are ultimately transferred to carbon dioxide, which is reduced to organic products. The light reactions, treated in this chapter, harvest light to make ATP, NADPH and O_2 ; the carbon-fixation (Calvin–Benson) reactions, treated in Chapter 2, use that ATP and NADPH to reduce CO_2 to sugar.

That oxygen comes from water and not from CO_2 was first shown by Robert Hill (1937) and confirmed in 1941 by Samuel Ruben and Martin Kamen, who supplied algae with water labelled with the heavy isotope ^{18}O and recovered that label in the oxygen evolved, not in the sugar. The idea rested on C. B. Van Niel's earlier work on bacterial photosynthesis (1931), which proposed that water is the source of reducing power: when split, H_2O yields H^+ and electrons used to reduce CO_2 , so the O_2 released by plants is derived from water. This light-driven splitting of water is called photolysis of water (Van Niel's theory).

1.1 The Chloroplast — Seat of Photosynthesis

In higher plants, photosynthesis takes place entirely within the chloroplast, which is therefore called the seat of photosynthesis. A chloroplast is bounded by a double envelope membrane separated by a periplastidial space (10–20 nm). Internally it contains an elaborate system of membranes, the lamellae, embedded in a matrix called the stroma. The lamellae are sealed into flattened, closed disks called thylakoids. Some thylakoids are stacked into grana (appressed granal thylakoids); others are unstacked stromal thylakoids exposed to the stroma. The thylakoid membranes are interconnected and enclose a continuous internal space, the lumen (Figure 1.1). Grana are joined to one another by the stromal lamellae, so the lumen of every thylakoid in a chloroplast is a single connected compartment — which is why a proton gradient built at one point serves ATP synthases everywhere in the organelle (Figure 1.2).

Figure 1.1 Structure of the chloroplast. A double envelope encloses the stroma, in which an elaborate internal membrane system is folded into flattened sacs. Stacks of these sacs form the grana, joined to one another by stromal lamellae. The stroma also holds 70S ribosomes, starch grains and the circular chloroplast genome.

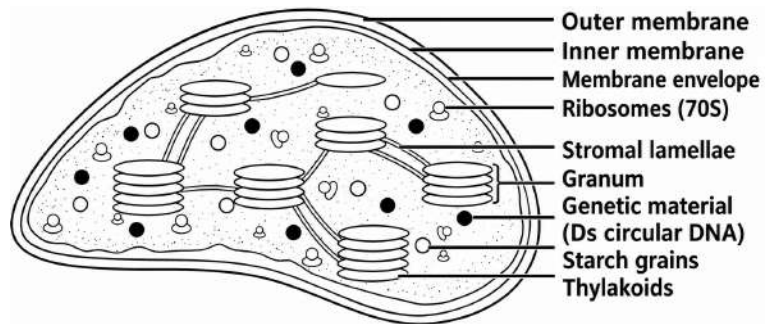
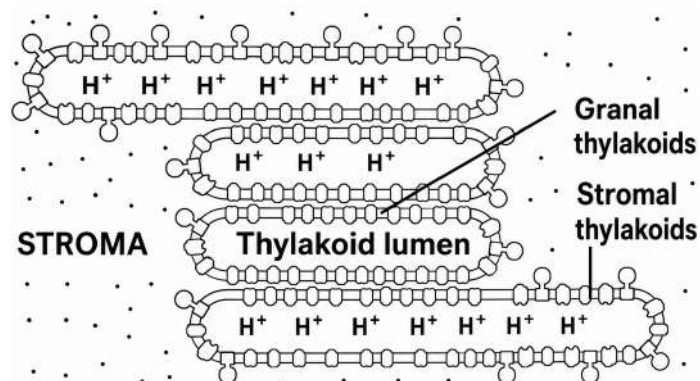
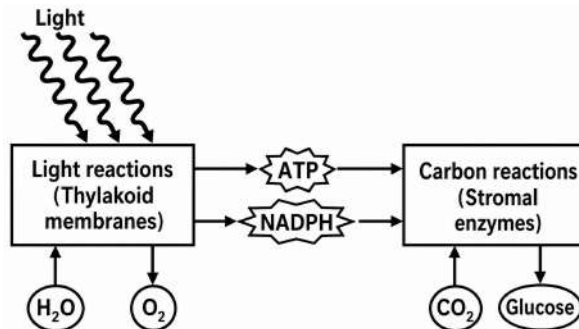


Figure 1.2 Granal (stacked) and stromal (unstacked) thylakoids. Although the membranes are folded into separate stacks, their interiors are continuous, so the whole thylakoid system encloses one lumen. Protons pumped into that lumen during electron transport are therefore available to every ATP synthase in the chloroplast.



The pigments embedded in the thylakoid membrane make it the exclusive site of the light reactions, whereas the stroma houses the enzymes of carbon fixation. Photosynthesis therefore proceeds in two stages: the light reaction, a photochemical reaction at the thylakoid membrane that generates NADPH, ATP and O_2 ; and the carbon-fixation reaction, in which the energy-rich NADPH and ATP drive CO_2 fixation by stromal enzymes (Figure 1.3).

Figure 1.3 The light and carbon-fixation reactions occupy different compartments. On the thylakoid membrane, light drives the oxidation of water to O_2 and makes ATP and NADPH; in the stroma, those two products pay for the reduction of CO_2 to sugar. The two sets of reactions are linked only by ATP and NADPH — which is why O_2 evolution can be observed with CO_2 entirely absent



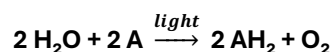
KEY POINT

The light reactions occur on the thylakoid membrane and produce ATP and NADPH (and release O₂); the carbon (Calvin) reactions occur in the stroma. The thylakoid lumen is the enclosed, interconnected internal space of the thylakoid system — the compartment that becomes acidified during electron transport.

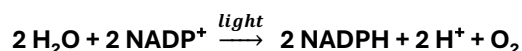
1.2 Light, Pigments and the Physics of Excitation

1.2.1 The Hill reaction

In 1937 Robert Hill found that illuminated leaf extracts containing chloroplasts evolved O₂ while reducing a non-biological electron acceptor (a 'Hill reagent', A):



A classic Hill reagent is the dye DCPIP (2,6-dichlorophenol-indophenol), blue when oxidised and colourless when reduced. On illumination the dye was decolourised and O₂ was evolved; in the dark, neither occurred. This proved that absorbed light drives electrons from water to an acceptor, and that O₂ evolution can be uncoupled from CO₂ reduction (CO₂ was neither required nor reduced). Severo Ochoa later identified NADP⁺ as the natural chloroplast acceptor, so that the physiological reaction is:



The light reaction can be divided into two stages — light absorption and energy transduction.

1.2.2 Energy in a quantum of light

Each quantum of light, or photon, carries a discrete quantity of energy given by $E = h\nu$, where h is Planck's constant (6.626×10^{-34} J s) and ν is the frequency. Because frequency and wavelength are related by $c = \nu\lambda$, the energy of a photon is inversely proportional to its wavelength: shorter (blue) wavelengths are more energetic than longer (red) ones.

For example, one mole of 490-nm blue photons carries about 240 kJ, whereas one mole of 700-nm red photons carries only about 170 kJ. Of all the light falling on a leaf, most is reflected, transmitted or lost as heat. The theoretical ceiling for converting total incident solar energy into biomass is about 4.6% for a C₃ plant and 6% for a C₄ plant; field crops in practice achieve only 1–2%, and it is this gap that makes photosynthetic efficiency such an active target for crop improvement.

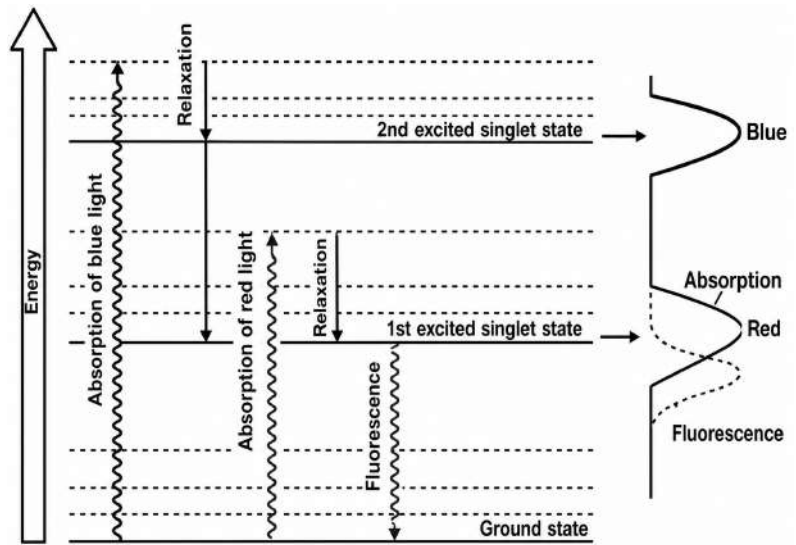
1.2.3 Excited states, de-excitation and quantum yield

When a pigment absorbs a photon it is promoted from its lowest-energy ground state to an excited state (pigment*), as one electron jumps to a higher-energy orbital. By the rules of quantum mechanics this transition occurs only when the photon energy matches the gap between the ground and excited states — not all transitions are allowed. Unlike isolated atoms, pigment molecules have many closely spaced vibrational and rotational sub-states, so they show broad absorption bands rather than sharp lines.

Absorption of blue light lifts chlorophyll to a higher excited state than red light; the molecule then relaxes internally to the lowest excited state, losing the surplus as heat (Figure 1.4). Whatever colour of light is absorbed, therefore, photochemistry always begins from the same lowest excited singlet state.

A pigment can exist in two kinds of excited state. The singlet state is short-lived and contains electrons with opposite (antiparallel) spins; the triplet state is comparatively long-lived, lies at lower energy, and contains electrons with parallel spins.

Figure 1.4 Energy levels in the chlorophyll molecule. Blue light lifts the pigment to the second excited singlet state and red light to the first; relaxation from the higher state to the lower is very fast and the surplus is lost as heat. Emission therefore always occurs from the first excited singlet, so fluorescence is of longer wavelength than the light absorbed, whatever colour was absorbed.



Transitions from singlet to triplet are low-probability events. From the excited state the energy can be dissipated by one of several competing routes: (i) nonradiative decay as heat; (ii) fluorescence — re-emission of a photon of longer wavelength than that absorbed, always from the lowest excited singlet state; (iii) resonance (Förster) energy transfer to a neighbouring pigment; and (iv) photochemistry — the excited pigment donates an electron to an acceptor in a charge-separation event. Photochemistry is the route that converts light into chemical products and underlies photosynthesis (Figure 1.5).

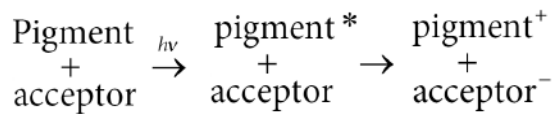


Figure 1.5 Photochemical charge separation, the event that converts light into chemistry.

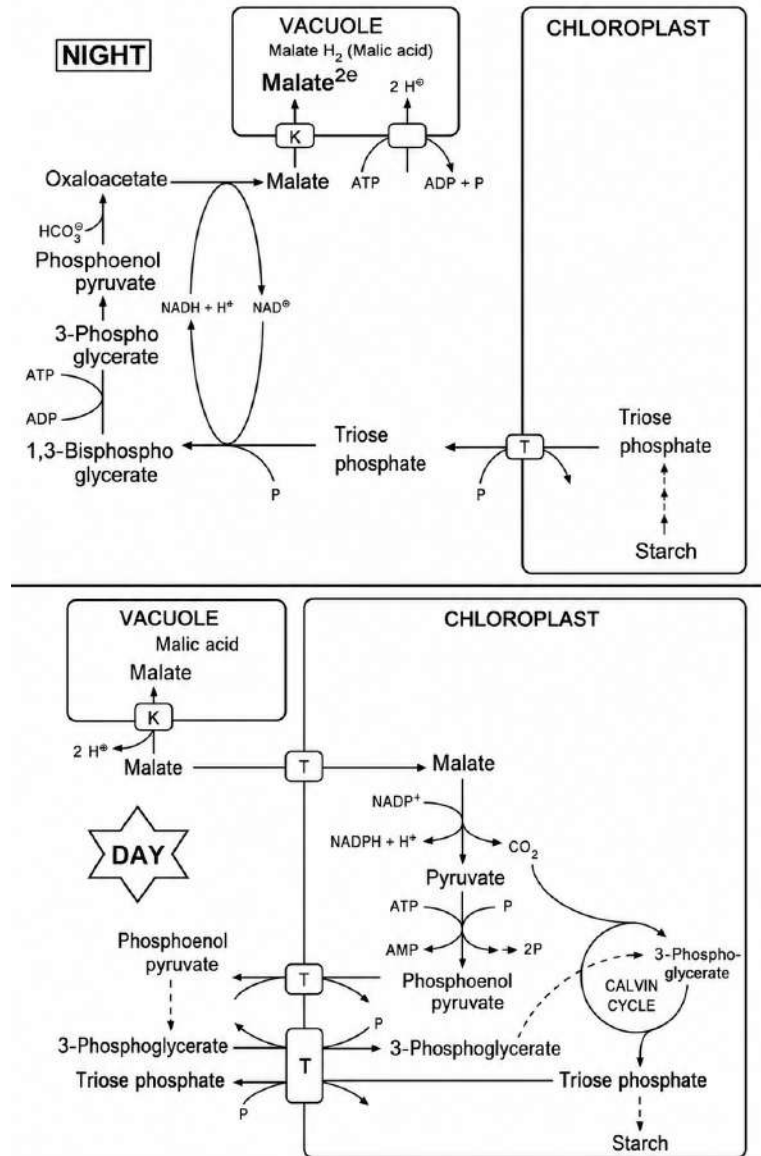
The fastest available pathway predominates. Fluorescence occurs in nanoseconds (10^{-9} s), but photochemical charge separation occurs in picoseconds (10^{-12} s) — about 1,000-fold faster — so when photochemistry is available little fluorescence is seen and photosynthesis proceeds with high efficiency. The singlet state of chlorophyll participates in energy transfer and photochemistry; the long-lived triplet state does not act as an intermediate in charge separation. The efficiency of a photochemical event is its quantum yield (Φ), the fraction of absorbed photons converted to product, ranging from 0 to 1.

Under optimal conditions the measured quantum yield of photosynthetic charge separation approaches 1, indicating that almost every absorbed photon is used. Because the four routes compete for the same excited state, the fluorescence a leaf emits reports indirectly on the other three, and chlorophyll fluorescence has become the standard non-invasive probe of photosynthetic performance. The ratio F_v/F_m , measured after dark adaptation, estimates the maximum quantum efficiency of PSII; it sits close to 0.83 in an unstressed leaf and falls whenever PSII is damaged or down-regulated.

1.2.4 Photosynthetic pigments

Plants, algae and cyanobacteria make chlorophyll, while anaerobic photosynthetic bacteria make a variant, bacteriochlorophyll. In the thylakoid membrane, lipids and proteins occur in roughly equal amounts; about one-quarter of the lipid fraction consists of pigments and associated co-factors. The chlorophyll molecule has a cyclic tetrapyrrole (porphyrin) ring with a magnesium (Mg) atom coordinated at its centre, and a long C_{20} hydrophobic phytol tail that anchors it in the membrane and makes it strongly nonpolar. Chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) carries a methyl ($-CH_3$) group on the ring; in chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) this is converted to a formyl ($-CHO$) group by an oxygenase (chlorophyll a oxygenase).

Figure 2.9 *Crassulacean acid metabolism.* At night the stomata open, starch is broken down to provide PEP, and PEP carboxylase fixes CO_2 into malate, which is pumped into the vacuole and stored as malic acid. By day the stomata close, malate returns to the cytosol and is decarboxylated, and the CO_2 released is fixed by Rubisco behind closed stomata — the same two carboxylations as in a C_4 plant, separated in time instead of in space.



2.6.3 Evolutionary significance of C_4 and CAM

C_4 and CAM occur in many unrelated monocot and dicot families, showing that both have evolved independently and repeatedly from C_3 ancestors — at least 61 independent origins of the C_4 pathway are now recognised, spread across 19 families — three of monocots (the grasses, the sedges and the Hydrocharitaceae) and sixteen of eudicots.

CAM has arisen independently at least as often, and probably more. Because the structural elements and enzymes of C_4 and CAM are already present in C_3 plants (for example in stomatal guard cells), the evolutionary conversion of C_3 to C_4 or CAM appears to be relatively easy — a classic case of convergent evolution. The route is now reasonably well understood, and it runs through an intermediate stage.

In C_3 – C_4 intermediate species such as those of *Flaveria* and *Moricandia*, glycine decarboxylase is confined to the bundle-sheath mitochondria, so the glycine produced by photorespiration in the mesophyll must travel there to be broken down and its CO_2 is released where Rubisco is concentrated. This 'C2 photosynthesis', or photorespiratory CO_2 pump, is a modest CO_2 -concentrating mechanism in its own right, and it seems to be the stepping stone by which almost every C_4 lineage arose.

Table 4.1 Rates of natural and anthropogenic nitrogen fixation

Source	N fixed (Tg yr ⁻¹)*
Lightning	< 10
Biological fixation, terrestrial (natural + agro-ecosystems)	90–140
Biological fixation, marine systems	30–300
Chemical synthesis of N fertiliser	100
Fossil-fuel combustion	> 20

*One teragram (Tg) = 10¹² g. Once fixed into ammonium or nitrate, nitrogen enters the biogeochemical cycle, passing through several organic and inorganic forms before returning to molecular N₂ (Table 4.2).

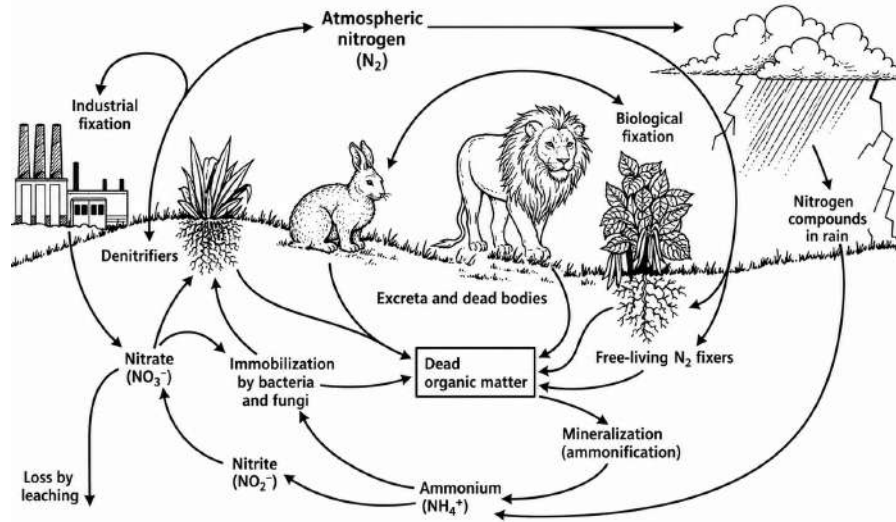


Figure 4.1 The biogeochemical nitrogen cycle. Molecular N₂ enters the biological world only through fixation — industrial, atmospheric or biological — and is then passed between ammonium, nitrite, nitrate and organic nitrogen by microbial oxidation, assimilation and mineralisation. Denitrification returns it to the atmosphere as N₂O and N₂, closing the cycle, while leaching and volatilisation remove it from the soil altogether.

Table 4.2 Major processes of the biogeochemical nitrogen cycle

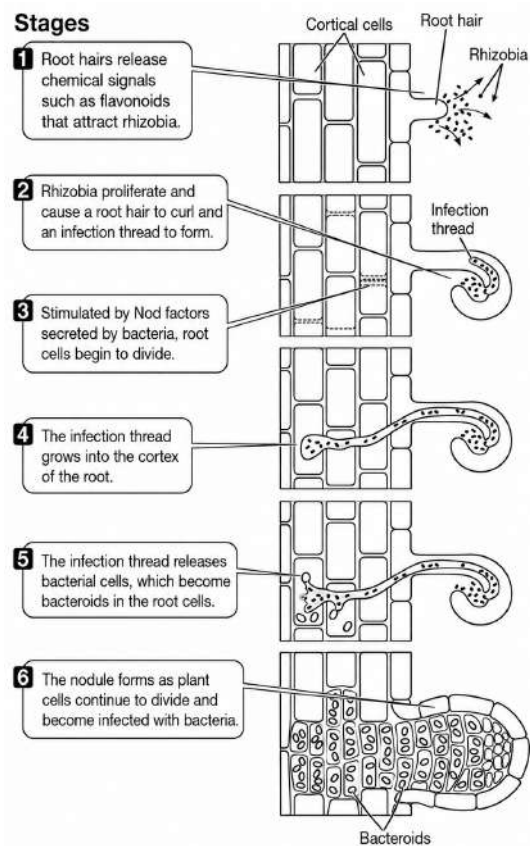
Process	Definition
Industrial fixation	Industrial conversion of N ₂ to ammonia
Atmospheric fixation	Lightning/photochemical conversion of N ₂ to nitrate
Biological fixation	Prokaryotic conversion of N ₂ to ammonia
Plant acquisition	Plant uptake and assimilation of ammonium or nitrate
Immobilisation	Microbial uptake and assimilation of ammonium or nitrate
Ammonification	Microbial catabolism of soil organic matter to ammonium
Nitrification	Oxidation of ammonium → nitrite (<i>Nitrosomonas</i>) → nitrate (<i>Nitrobacter</i>)
Volatilisation	Physical loss of gaseous ammonia to the atmosphere
Denitrification	Bacterial conversion of nitrate to N ₂ O and N ₂ (e.g. <i>Pseudomonas</i>)
Nitrate leaching	Flow of dissolved nitrate out of topsoil into groundwater

KEY POINT

The atmosphere is by far the largest nitrogen reservoir (78% N₂), but plants cannot use N₂ directly. Remember the cycle's signature microbes: nitrification is a two-step oxidation by *Nitrosomonas* (NH₄⁺ → NO₂⁻) then *Nitrobacter* (NO₂⁻ → NO₃⁻); denitrification (NO₃⁻ → N₂) is carried out by bacteria such as *Pseudomonas*.

Temperate legumes such as pea, clover and alfalfa keep a persistent apical meristem and form elongated, cylindrical indeterminate nodules, in which every stage of infection and bacteroid development can be seen at once along the length of a single nodule. Tropical legumes such as soybean, bean and peanut lack a persistent meristem and form spherical determinate nodules, in which the whole population of cells develops in step (Figure 4.4). The distinction matters beyond morphology, because the two kinds of nodule export their fixed nitrogen in chemically different forms — a point taken up in Section 4.8.

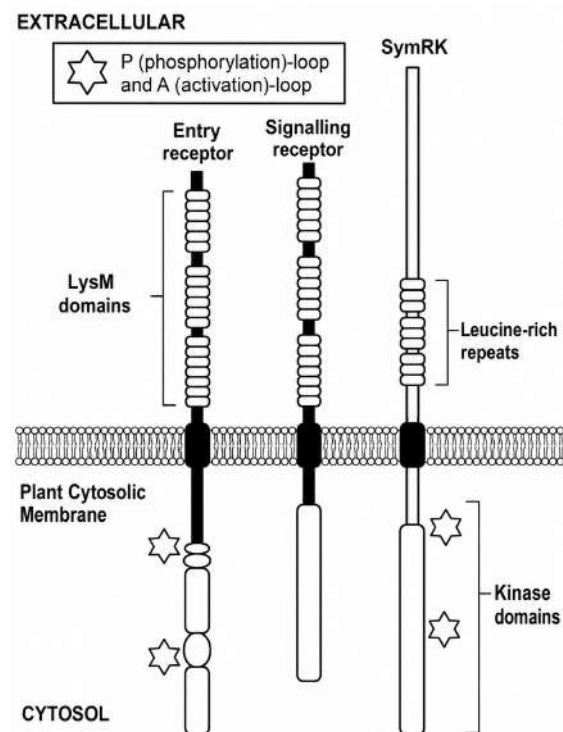
Figure 4.4 *Infection and nodule formation. Nod factor causes the root hair to curl sharply and trap the attached bacteria; the wall is locally digested and an infection thread grows inward as a tubular invagination of the plasma membrane. While the thread advances, cortical cells divide to form the nodule primordium, and when the two meet the bacteria are released into host cells inside symbiosome membranes and differentiate into nitrogen-fixing bacteroids*



4.3.5 Plant genes and proteins required for nodulation

Nod-factor perception needs several plant receptors: distinct genes encode an inferred 'entry' receptor and a 'signalling' receptor, and a mutation in either abolishes nodulation and the response to bacteria or Nod factor. Each is a membrane-spanning receptor-like protein whose extracellular region carries several LysM domains — chitin-binding motifs of 44–65 residues. A partner in that perception is the symbiosis receptor kinase SymRK, which has three extracellular leucine-rich-repeat (LRR) domains and is required both for rhizobial symbiosis and for symbiosis with arbuscular mycorrhizal fungi (Figure 4.5).

Figure 4.5 *The plant receptors of nodulation. The Nod-factor receptors are receptor-like kinases whose extracellular regions carry LysM domains — the chitin-binding motifs that recognise the Nod-factor backbone. The symbiosis receptor kinase SymRK instead carries leucine-rich repeats, and is required not only for rhizobial symbiosis but for the far older arbuscular mycorrhizal one.*



CHAPTER

8

Light, the Clock & Flowering

A plant cannot see, yet it knows the season, the time of day and whether a neighbour is shading it. This chapter is about light as information rather than as fuel. It begins with the internal clock that keeps time in constant darkness and the photoperiodic machinery that measures the night and sends florigen from leaf to shoot tip; it then turns to the pigments themselves — phytochrome, which reads the ratio of red to far-red and doubles as a thermometer, and the cryptochromes, phototropins, ZEITLUPE proteins and UVR8 that read the blue and ultraviolet ends of the spectrum. Together they let a rooted organism navigate a world it cannot move through.

LEARNING OBJECTIVES

- Define the properties of circadian rhythms
- Classify plants by photoperiodic response
- Describe florigen — the FT protein — its movement from leaf to apex and the florigen activation complex
- Explain vernalization and the epigenetic silencing of FLC
- Describe the meristem-identity genes and the ABC / floral-quartet model of organ identity.
- Explain phytochrome photoreversibility, dark reversion and the photostationary state,
- Classify phytochrome responses as VLFR, LFR and HIR, and distinguish the roles of phyA and phyB.
- Describe phytochrome signalling
- Describe cryptochrome, phototropin, ZEITLUPE and UVR8 structure and signalling,
- Explain blue-light stomatal opening from the photoreceptor

8.1 Light as a Developmental Signal

Light is not only the energy source for photosynthesis but also a master signal for development. Seedlings grown in darkness are pale, tall and spindly — etiolated growth, or skotomorphogenesis — adapted to pushing through the soil on stored reserves.

On reaching light, a single dim flash triggers de-etiolation within hours: stem elongation slows, the apical hook straightens and pigment synthesis begins. This light-triggered switch to the stockier, green, above-ground form is photomorphogenesis (Greek, 'light-form-beginning'); photosynthesis cannot drive it, since chlorophyll is still absent.

Phytochrome — a pigment most sensitive to red and far-red light — directs much light-controlled vegetative and reproductive development, while phototropins and cryptochromes (and the ZEITLUPE family) sense blue light. The hormonal side of that switch — gibberellin falling in the light, DELLA proteins accumulating and binding the PIF transcription factors — was described in Section 6.4.1.

This chapter concerns the rhythmic and photoperiodic responses these receptors govern; the receptors themselves are treated in the second half of this chapter. What unites it is a single idea: light here is not fuel but information, and the plant reads it for time of day, time of year, and the presence of neighbours.

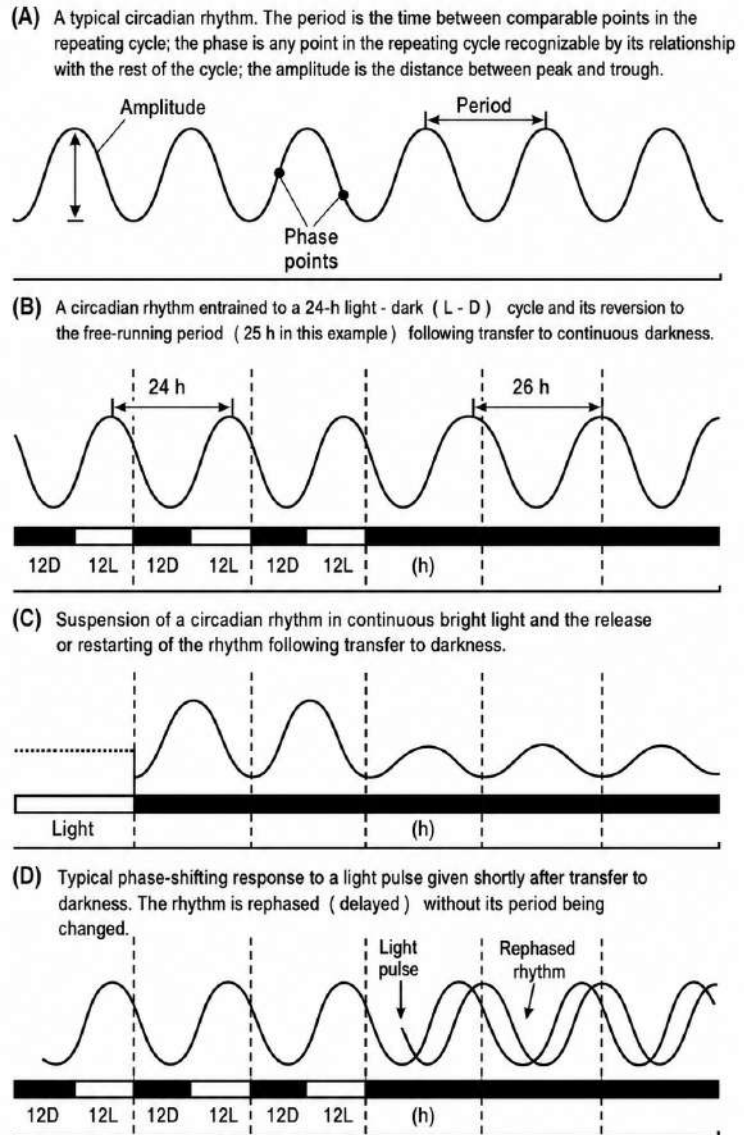
8.2 Circadian Rhythms and the Biological Clock

Circadian rhythms are endogenous ~24-hour cycles defined by three parameters: period (the time between equivalent points, e.g. successive peaks), phase (a recognisable point such as a peak or trough) and amplitude (the peak-to-trough distance, which can change while the period stays fixed).

In constant light or darkness the rhythm departs slightly from 24 hours and drifts relative to solar time — it is then free-running, reverting to the organism's characteristic period. In nature the clock is reset (entrained) each day by environmental signals called zeitgebers ('time-givers'), chiefly the light transitions at dawn and dusk.

Many rhythms damp out (lose amplitude) in a constant environment and need a zeitgeber to restart. Crucially, the clock keeps accurate time despite changing temperature — temperature compensation — because the temperature-sensitivities of its biochemical steps cancel out (Figure 8.1). A clock that ran faster in the afternoon would be useless for measuring a season, so temperature compensation is not a curiosity but a design requirement.

Figure 8.1 The vocabulary of circadian rhythms. Period is the time between equivalent points, phase a recognisable point such as a peak, and amplitude the peak-to-trough distance. In constant conditions the rhythm free-runs at its own period and slowly damps; a light pulse shifts its phase, advancing or delaying it according to when it is given.



Because a single oscillator can drive several rhythms, the clock must adjust to seasonally changing day lengths by phase shifting — moving the whole cycle forward or back without changing its period. This is probed by giving a light pulse at different points of a free-running rhythm: a pulse early in the subjective night delays the rhythm (read as 'end of the previous day'), while a pulse late in the subjective night advances it (read as 'start of the next day').

Such phase shifts allow entrainment to ~24-hour cycles of different day length. The light that resets the clock is sensed by specific photoreceptors (not by photosynthesis): in *Arabidopsis* four of the five phytochromes plus CRY1/CRY2 entrain the clock (CRY proteins are even needed for red-light entrainment, probably as phytochrome-signalling intermediates), and the ZEITLUPE family controls the stability of clock components.

The sleep (nyctinastic) movements of legume leaves are a classic plant circadian rhythm: leaflets open horizontally by day and fold by night, driven by turgor changes in the pulvinus at the petiole base. The rhythm persists in constant darkness but its phase can be reset by red or blue light — blue light opens closed leaflets, and red light (reversible by far-red, hence phytochrome) closes open ones within ~30 minutes.

The turgor changes come from K^+ and Cl^- fluxes between the dorsal and ventral motor cells of the pulvinus, one group swelling as the other shrinks (Figures 8.2 and 8.3) — the same osmotic machinery, and much the same channels and proton pump, that open and close a stoma (Section 6.9.3), used here to move an organ rather than a pore.

Figure 8.2 The circadian rhythm of leaf movement in *Albizia*. Leaflets open by day and fold by night, and the rhythm continues in constant darkness at reduced amplitude — the definition of an endogenous rhythm rather than a direct response to light.

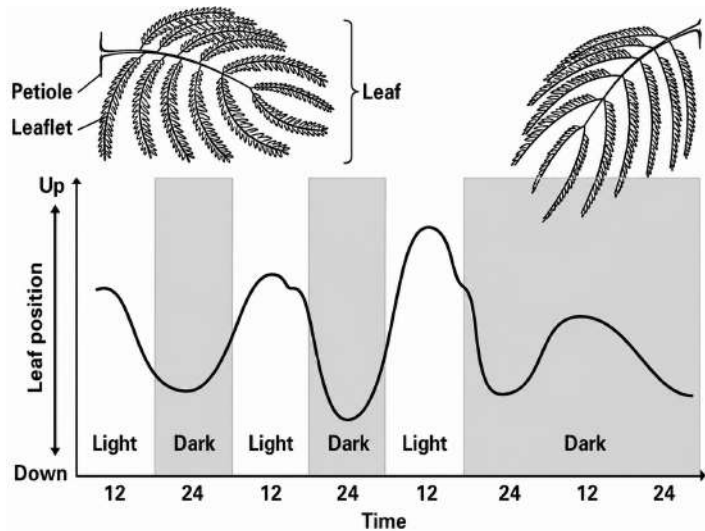
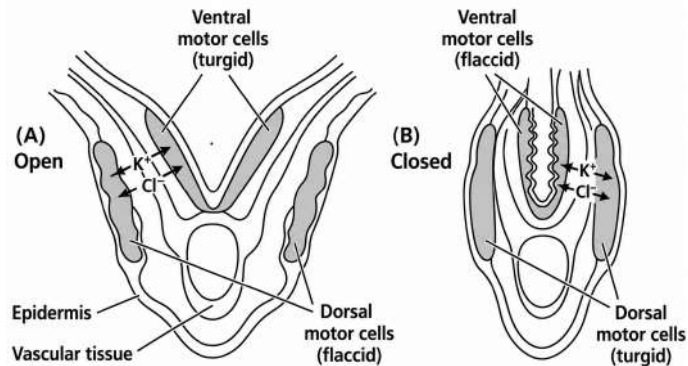


Figure 8.3 How the pulvinus moves a leaflet. K^+ and Cl^- move between the dorsal and ventral motor cells at the base of the petiole so that one group becomes turgid as the other becomes flaccid; water follows osmotically and the resulting difference in turgor bends the joint — the same ion machinery a guard cell uses to open a stoma.



Phytochrome also controls rhythms at the level of gene expression — the LHCB (light-harvesting chlorophyll a/b protein) genes oscillate, rising in the morning. Clock genes were found by fusing the LHCB promoter to luciferase and screening transformed *Arabidopsis* for altered bioluminescence rhythms, yielding the *toc* (timing of CAB expression) mutants. *TOC1* is part of the core oscillator, and the oscillator itself is now understood in outline.

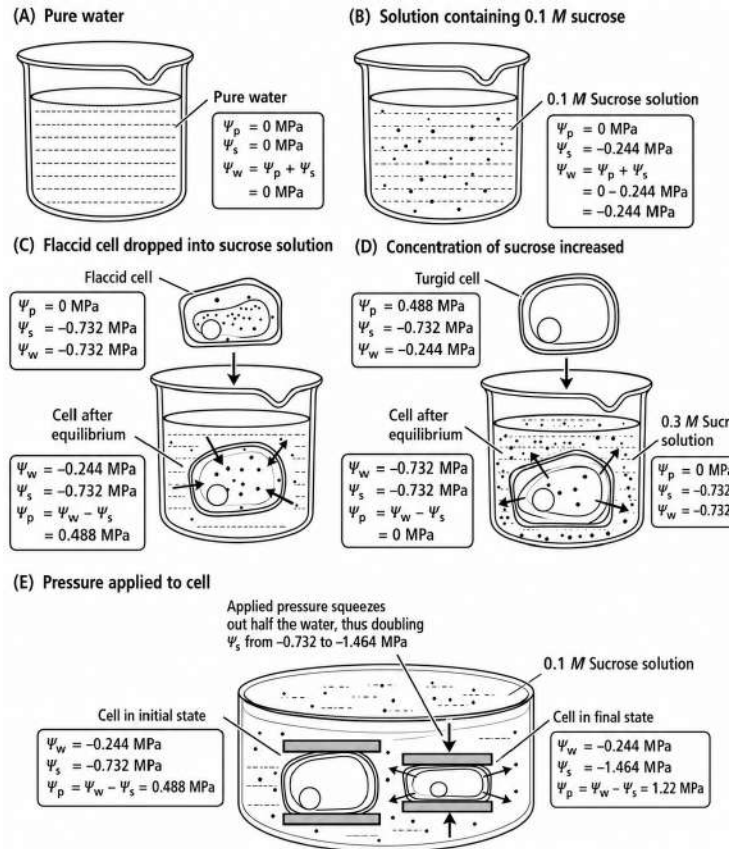
Two Myb-like transcription factors, CCA1 and LHY, peak at dawn and repress *TOC1*; *TOC1* peaks at dusk and — this is a correction to the model taught for a decade — represses CCA1 and LHY rather than activating them, so the core is a loop of mutual repression rather than the positive–negative pair originally proposed.

Around it run two further layers: the PRR9, PRR7 and PRR5 proteins, switched on in sequence through the day, which progressively repress CCA1 and LHY; and the evening complex of ELF3, ELF4 and LUX, which assembles at dusk and represses growth and the PRR genes. The ZEITLUPE proteins, being both blue-light receptors and F-box proteins, sit across the two ideas of this chapter — they degrade *TOC1* and PRR5 in the dark, and are protected by GIGANTEA in the light, so end-of-day light is written directly into the stability of clock proteins. A non-transcriptional oscillator also exists: the redox state of peroxiredoxin proteins cycles with a 24-hour period in organisms from cyanobacteria to humans, and persists even when transcription is blocked.

Water potential has two uses: it governs transport across membranes, and it indexes a plant's water status. Cell growth is the process most sensitive to water deficit, followed by cell division, wall and protein synthesis, solute accumulation, stomatal closure and photosynthesis. Well-watered leaves have ψ_w of -0.2 to -1.0 MPa (down to -2 to -5 MPa in arid plants); since uptake is passive, a plant takes up water only when its ψ_w is below the soil's.

A second status measure is relative water content, $RWC = [(fresh - dry)/(turgid - dry)] \times 100$, typically 85–95% in transpiring leaves; below a critical value, often under 50%, the tissue dies (Figures 9.1 and 9.2).

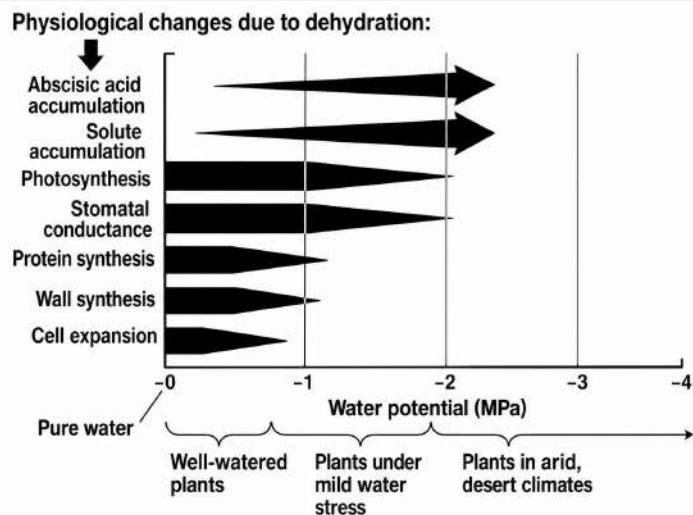
Figure 9.1 Water potential in practice. The panels show a cell taking up water and gaining turgor in a solution of higher water potential, and losing water and plasmolysing in one of lower water potential, with the solute and pressure components accounted for in each case.



KEY POINT

$\psi_w = \psi_s + \psi_p + \psi_g$. Solutes LOWER water potential (ψ_s is negative); positive (turgor) pressure raises it while negative (tension) pressure lowers it; gravity adds pgh . Water flows passively toward lower water potential — there is no water 'pump'. A flaccid cell in a higher- ψ_w solution takes up water and gains turgor; in a lower- ψ_w solution it loses water and may plasmolyse.

Figure 9.2 The range of plant water potentials and the sensitivity of physiological processes to water deficit. Growth is the first process to fail as ψ_w falls, long before photosynthesis is affected — which is why a mildly droughted crop stops growing while still looking green.



9.2 Water in the Soil

Soil water content and movement depend on soil type. Sandy soils have low surface area and large channels from which water drains; clay soils (particles $<2\ \mu\text{m}$) have huge surface area and tiny channels that hold water tightly. The water held after saturation and drainage is the field capacity — about 40% by volume for clay or humus-rich soils and about 3% for sandy ones (Table 9.1). The difference between field capacity and the permanent wilting point is the available water, and it is the number that matters agronomically: a sandy soil holds little and dries quickly, a clay holds a great deal but grips much of it too tightly for a root to recover.

Table 9.1 Physical characteristics of soils

Soil	Particle diameter (μm)	Surface area per gram (m^2)
Coarse sand	2000–200	<1 –10
Fine sand	200–20	<1 –10
Silt	20–2	10–100
Clay	<2	100–1000

Soil water potential has two components: an osmotic potential (usually trivial, $\sim -0.02\ \text{MPa}$, but significant in saline soils) and a hydrostatic pressure. In wet soil $\psi_p \approx 0$, but as the soil dries, water recedes into the wedges between particles, forming curved air–water interfaces whose surface tension creates a negative pressure (ψ_p can reach -1 to $-2\ \text{MPa}$ as the radius of curvature shrinks).

Water moves through soil by bulk flow down a pressure gradient: as roots absorb water they lower ψ_p at the root surface, drawing water through the interconnected pores. The rate depends on the pressure gradient and the soil's hydraulic conductivity (high in sand, low in clay; falling sharply as the soil dries and air replaces water). When ψ_w falls so low that the plant cannot regain turgor even if transpiration stops, the soil is at the permanent wilting point — which depends on the plant's ψ_s , not solely on the soil.

9.3 Water Absorption by Roots

Effective absorption needs close root–soil contact, maximised by root hairs (microscopic epidermal extensions that can be $>60\%$ of root surface area). Water enters most easily through the root-hair zone near the apex; older regions develop a hydrophobic exodermis.

From epidermis to endodermis, water takes three routes: the apoplast (through cell walls only, crossing no membrane), the transmembrane/transcellular pathway (entering and exiting cells across the plasma membrane, sometimes via the tonoplast/vacuole), and the symplast (cell to cell through plasmodesmata) — Figures 9.3 and 9.4.

Figure 9.3 *Root hairs. These epidermal extensions press into the water films around soil particles and can account for well over half the absorptive surface of a root*

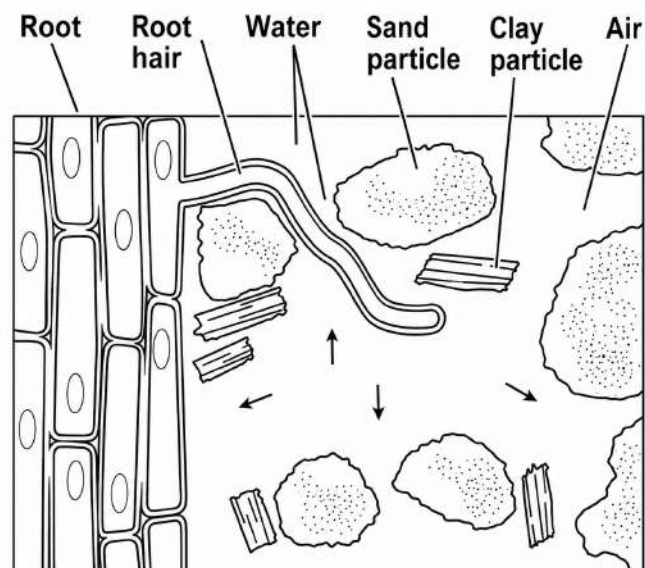
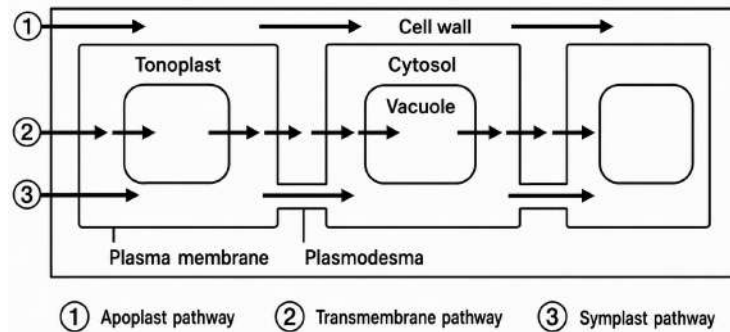


Figure 9.4 The three radial routes across the root: the apoplast, through cell walls and crossing no membrane; the transcellular route, in and out of cells across plasma membranes; and the symplast, from cytoplasm to cytoplasm through plasmodesmata.

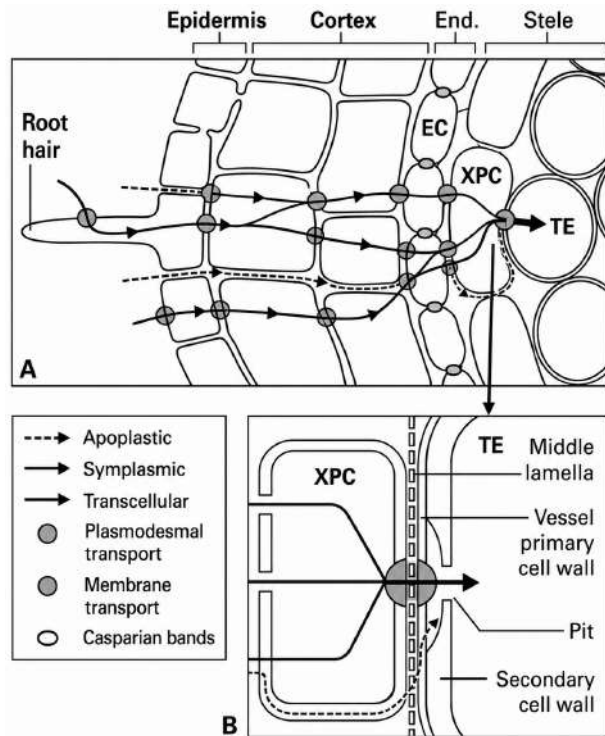


At the endodermis the apoplast route is blocked by the Casparian strip — a band in the radial endodermal walls impregnated with hydrophobic lignin (in young roots) and suberin (in the maturation zone). It breaks the apoplast's continuity and forces water and solutes to cross the plasma membrane, so movement across the endodermis is symplastic.

The Casparian strip forms several millimetres behind the root tip, as the first protoxylem matures (Figure 9.5). How the plant builds so precise a seal has been worked out recently and is worth knowing. A family of small membrane proteins, the CASPs, assemble into a sharply bounded band in the plasma membrane exactly where the strip will form; they recruit the peroxidase and the dirigent protein ESB1 that polymerise lignin in the wall at that spot, and nowhere else.

The plant then checks its own work: a receptor kinase, SGN3, sits in the endodermal membrane and detects CIF peptides that leak through from the stele whenever the barrier is incomplete, and the resulting signal reinforces lignification until the leak stops.

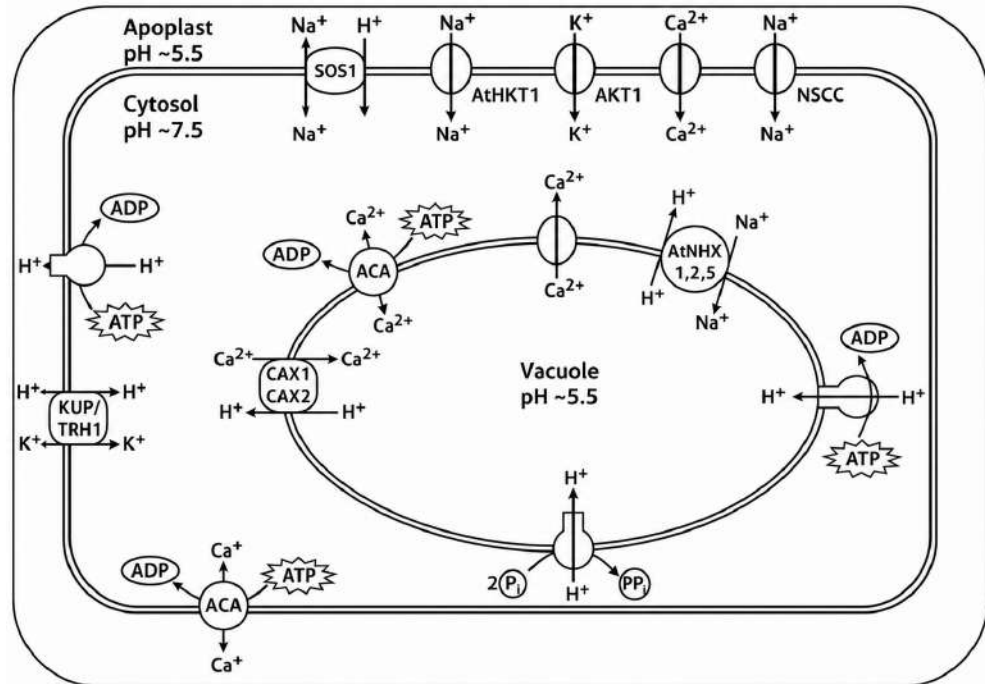
Figure 9.5 Radial movement of water and nutrients, and where it is controlled. The Casparian strip in the radial walls of the endodermis interrupts the apoplast, so every ion must cross a plasma membrane before it reaches the stele; xylem parenchyma cells then load the tracheary elements.



A second, later barrier is laid down outside the plasma membrane in the maturation zone — a continuous lamella of suberin that seals the whole endodermal surface and blocks the transcellular route as well, which is why older parts of a root absorb far less than the young apical zone.

Water uptake falls when roots are chilled, made anaerobic or treated with respiratory inhibitors (cyanide) — explaining why plants wilt in waterlogged soil: submerged roots become O_2 -starved (O_2 diffuses $10^4\times$ faster through air than water) and transport less water. Roots can also generate root pressure: by actively pumping ions into the xylem, they lower xylem ψ_s and ψ_w , drawing in water and building a positive hydrostatic pressure (0.05–0.5 MPa), so the whole root acts as an osmotic cell. Root pressure is greatest when soil is wet and transpiration low; it drives guttation — exudation of xylem sap through hydathodes at leaf margins, the 'dewdrops' seen on grass tips at night, which are not dew at all but the plant's own sap (Figure 9.6).

Figure 11.2 Sodium and potassium at the root plasma membrane. The high-affinity transporter HKT1 normally carries K^+ , but at high external Na^+ it admits sodium instead; calcium in the medium restores selectivity. Class-1 HKT proteins in the cells around the xylem also retrieve sodium from the transpiration stream and so keep it out of the leaves.

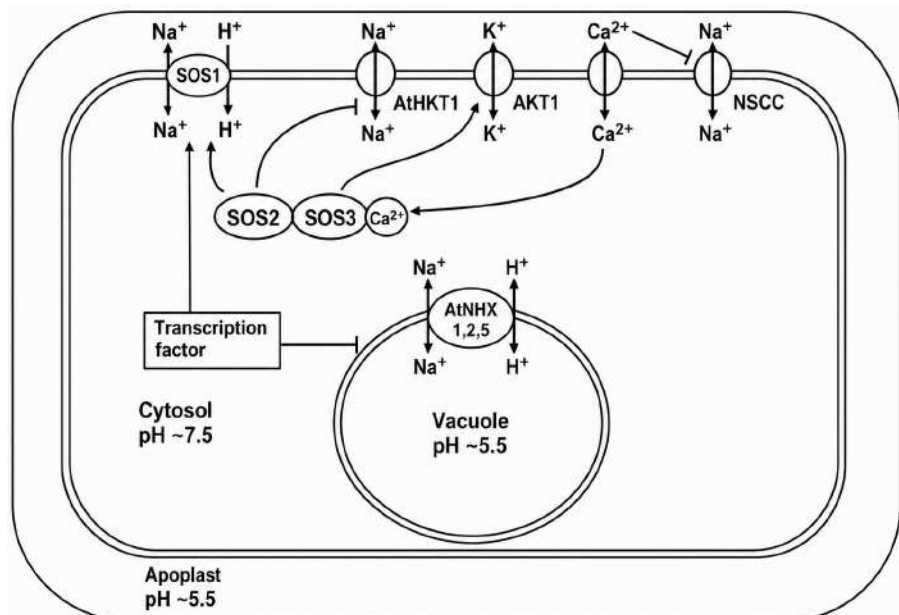


The names deserve translating, because the SOS proteins were christened from mutant phenotypes before their identities were known: SOS3 is the calcineurin-B-like calcium sensor CBL4, SOS2 is the CBL-interacting protein kinase CIPK24, and together they form a calcium-activated kinase module that phosphorylates and switches on the SOS1 antiporter (Figure 11.3).

Vacuolar Na^+ sequestration is carried out by tonoplast Na^+/H^+ antiporters such as *Arabidopsis* AtNHX1, whose overexpression makes transgenic plants more salt-tolerant. These are not laboratory curiosities.

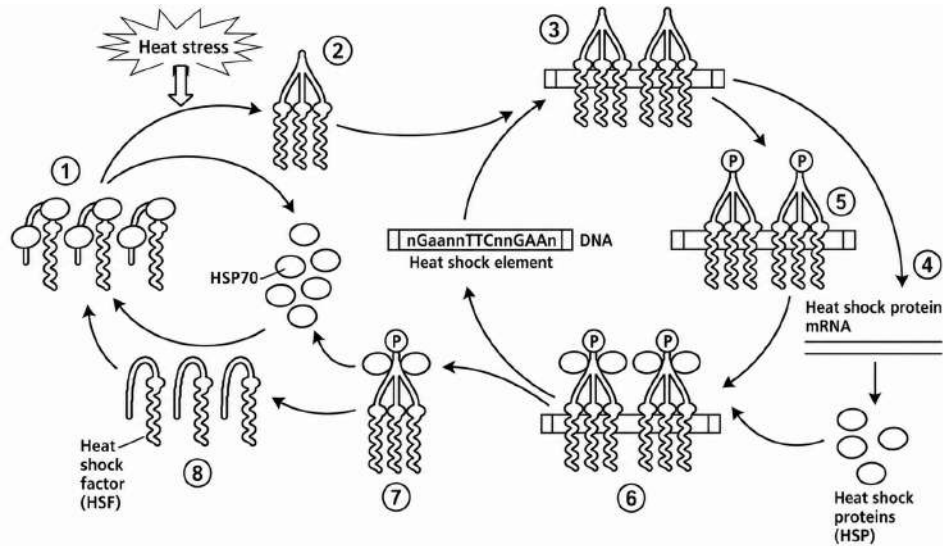
The rice quantitative-trait locus Saltol, which underlies the salt tolerance of the landrace Pokkali, is HKT1;5, and it has been bred by marker-assisted backcrossing into high-yielding varieties for the coastal saline soils of South and South-East Asia; the Nax1 and Nax2 loci of durum wheat, HKT1;4 and HKT1;5, were introgressed from a wild relative and raise grain yield by around a quarter on saline soils.

Figure 11.3 The SOS pathway. Salt raises cytosolic calcium; the calcium sensor SOS3 (CBL4) binds and activates the kinase SOS2 (CIPK24); and SOS2 phosphorylates the plasma-membrane antiporter SOS1, which extrudes Na^+ from the cytosol. In parallel the tonoplast antiporter NHX1 sequesters sodium in the vacuole, where it can serve as a cheap osmoticum instead of a poison.



Plants have far more heat-shock factors than animals — more than twenty in *Arabidopsis* — and they are organised hierarchically: HSFA1 proteins are the master regulators, held inactive by HSP70 and HSP90 until heat releases them, and they then induce a second tier including HSFA2 and the DREB2A factor already met under drought. HSFA2 is responsible for thermomemory: a plant given a mild heat episode responds faster and survives better for several days afterwards, and the memory is written as a histone modification at the HSFA2 target genes.

Figure 11.4 Regulation of the heat-shock response. Heat converts inactive heat-shock-factor monomers into trimers that bind the heat-shock element in the promoters of the HSP genes and drive their transcription; as chaperones accumulate, HSP70 binds the factor, releases it from the DNA and returns it to the monomeric state — a self-limiting circuit.



Heat tolerance is also mediated by cytosolic calcium. High temperature raises cytosolic Ca^{2+} , which through calmodulin activates glutamate decarboxylase (GAD) to make the nonprotein amino acid GABA (γ -aminobutyric acid) from glutamate; GABA accumulates six- to ten-fold and is a metabolic acclimation to heat (Figure 11.5). Its protective role is still being worked out, but GABA is now known to do more than accumulate: it binds the aluminium-activated malate transporter ALMT1 of the guard cell and so modulates stomatal aperture, which makes it the closest thing plants have to a neurotransmitter-like signalling molecule.

Figure 11.5 Heat, calcium and GABA. A rise in temperature raises cytosolic calcium, which through calmodulin activates glutamate decarboxylase; glutamate is decarboxylated to γ -aminobutyric acid, which accumulates several-fold as part of the metabolic acclimation to heat.

