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The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants

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Summary

The visual deficiency symptoms developing on plants constitute the ultimate manifestation of suboptimal nutrient supply. In classical plant nutrition, these symptoms have been extensively used as a tool to characterise the nutritional status of plants and to optimise fertilisation. Here we expand this concept by bridging the typical deficiency symptoms for each of the six essential macronutrients to their molecular and physiological functionalities in higher plants. We focus on the most recent insights obtained during the last decade, which now allow us to better understand the links between symptom and function for each element. A deep understanding of the mechanisms underlying the visual deficiency symptoms enables us to thoroughly understand how plants react to nutrient limitations and how these disturbances may affect the productivity and biodiversity of terrestrial ecosystems. A proper interpretation of visual deficiency symptoms will support the potential for sustainable crop intensification through the development of new technologies that facilitate automatised management practices based on imaging technologies, remote sensing and in-field sensors, thereby providing the basis for timely application of nutrients via smart and more efficient fertilisation.

I. Introduction

The 14 inorganic elements required by plants to complete a full life cycle are coined the *essential plant nutrients,* and grouped into macronutrients and micronutrients on the basis of their concentration in plant dry matter. The macronutrients are comprised of nitrogen (N), phosphorus (P), sulphur (S), potassium (K), calcium (Ca) and magnesium (Mg). In situations in which a nutrient is not present in sufficient amounts to support its functional roles, it will lead to a state of deficiency, with specific responses characteristic for each nutrient (van Maarschalkerweerd & Husted, 2015). The severity of a nutrient deficiency can range

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from mild and transient to severe and chronic, and plants may experience multiple deficiencies during their lifespan, some even occurring simultaneously. In addition, widespread interactions with a range of different abiotic and biotic growth factors such as water, light, pests and pathogens, may lead to atypical symptoms of nutrient-related disorders.

On a global scale, various nutrient deficiencies have major negative consequences for crop production, resulting in reduced yields and a poor quality of food and feed. At the same time, inappropriate use of fertiliser may lead to pollution of terrestrial and aquatic environments, while fertiliser production itself is highly energy demanding, thus contributing to climate change (Sharpley *et al.*, 2018; Thompson *et al.*, 2019). In natural terrestrial ecosystems, nutrient availability is, next to that of water, the environmental factor that most strongly affects the responses of plants to climate change and their ability to cope with environmental stress, thus having a major effect on species biodiversity (Isbell *et al.*, 2013; Lambers & Oliveira, 2019; Terrer *et al.*, 2019).

A large body of knowledge concerning the functional properties of macronutrients in plants already exists (Hawkesford *et al.*, 2012). However, in the recent years significant new knowledge on the molecular processes underlying the direct responses of plants to limitation of a given nutrient has appeared. In addition, starvation networks, describing how individual nutrients interact and cross-talk along the pathway from uptake to integration in metabolism, have been unravelled (Kopriva *et al.*, 2015; Dong *et al.*, 2017; Maeda *et al.*, 2018; Courbet *et al.*, 2019; Hu *et al.*, 2019; Medici *et al.*, 2019).

In this review, we highlight recent insights into the physiological and molecular functions of plant macronutrients. Special emphasis is given to linking their physiological functions with the typical deficiency symptoms that may appear when nutrient availability is suboptimal for proper growth. Understanding the coupling between visual deficiency symptoms and their interactions with the functional properties of the triggering nutrient(s) is relevant for breeding of new nutrient-efficient genotypes that are able to withstand unfavourable climatic events and adverse soil conditions. Moreover, a proper understanding and interpretation of visual deficiency symptoms will support the potential for sustainable intensification of crop production systems by enabling development of new technologies that support automatised and data-driven management practices based on bioimaging, remote sensing, in-field spectrometers and/or sensors, thereby providing a basis for timely application of nutrients to crops.

II. Nitrogen

Nitrogen is an integral constituent of purines and pyrimidines that make up nucleic acids (DNA and RNA). The amine group of amino acids in all peptides, proteins and enzymes also have N as an essential component. The majority of shoot N is present in the chloroplasts as RuBisCo, photosystem proteins and chlorophyll molecules that contain four N atoms in the tetrapyrrole (chlorin) ring. The N containing ions/molecules nitrate (NO_3^-), ammonium (NH_4^+) and nitric oxide (NO) are important signalling molecules (Table 1).

Plants predominantly take up N in the form of NO_3^- and NH_4^+, but also organic N in the form of peptides and amino acids may be absorbed (Ganeteg *et al.*, 2017). The uptake of the different N forms is mediated via transport proteins with different specificity and affinity. In addition, a substantial amount of N may be provided by N-fixing microbial associations of which the best studied is that between legumes and rhizobia, the latter fixing N_2 into NH_4^+ in special root organs called nodules.

Once taken up, N is either reduced for assimilation directly in the roots or translocated to the shoot via the xylem as amino acids, amides, NO_3^-, NH_4^+ or as ureides in tropical legumes. In the shoot, N is stored, metabolised or further translocated via the phloem to N sinks, such as developing leaves, roots, fruits and seeds. N is stored as protein, primarily RuBisCo, or in vacuoles as NO_3^-, while translocation to sinks primarily occurs as amino acids (Tegeder & Masclaux-Daubresse, 2018). Recent studies have shown that N translocation in the phloem of *A. thaliana* may also occur as NO_3^- (Chen *et al.*, 2020) or ureides (Takagi *et al.*, 2018).

Primary N metabolism begins with assimilation of NO_3^-, which is first reduced to NO_2^- by nitrate reductase (NR) in the cytosol, then further reduced and converted to NH_4^+ by nitrite reductase (NiR) in plastids or chloroplasts. The formed NH_4^+ is, together with NH_4^+ generated by other processes such as photorespiration, protein catabolism and lignin biosynthesis, assimilated into glutamine by the enzyme glutamine synthetase (GS). Glutamine may subsequently react with 2-oxoglutarate to yield two glutamate molecules, a reaction catalysed by glutamate synthase (GOGAT). Alternatively, glutamine may react with aspartate to yield asparagine and glutamate as catalysed by asparagine synthetase (AS).

1. Molecular and physiological responses to N deficiency

Plants inadequately supplied with N induce a myriad of changes to their transcriptome for example affecting root architecture, shoot development and flowering. A central component in this response is NO_3^- and its function as signalling molecule.

2. The nitrate transceptor NRT1.1 is essential for adaptation to fluctuating N conditions

Plants respond within minutes to changes in external NO_3^- levels by changing the expression of hundreds to thousands of genes as part of the primary nitrate response (PNR), including NiR, NR, NO_3^- transporters and genes involved in organic acid metabolism. Nitrate is sensed by the NO_3^- transceptor NRT1.1 (NPF6.3). The CBL-interacting protein kinase (CIPK) CIPK8 is involved in dephosphorylation of NRT1.1 at high NO_3^-, whereas CIPK23 phosphorylates NRT1.1 under low NO_3^- conditions (Y. Y. Wang *et al.*, 2018). In the nonphosphorylated state, NRT1.1 dimerises to a homo-dimer with low NO_3^- affinity, while phosphorylation leads to a monomeric and high-affinity state (Fig. 1.1) (Sun *et al.*, 2014). The effects of CIPK23 on the NO_3^- transport properties of NRT1.1 are counteracted by ABI2, a phosphatase that is inhibited by abscisic acid (ABA) (Leran *et al.*, 2015). CIPK23 is also involved

Table 1 Functional roles and foliar deficiency symptoms of the macronutrients.

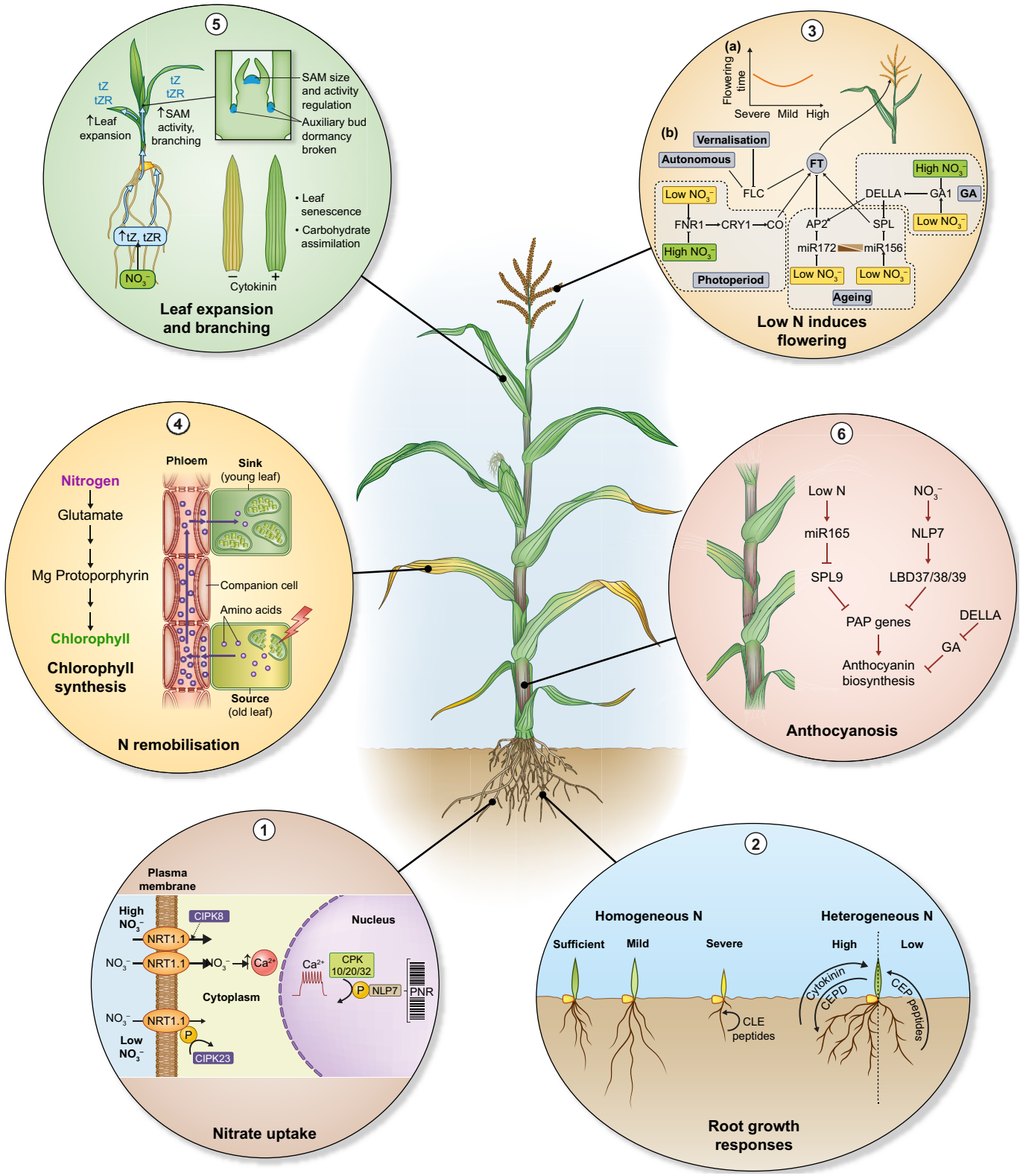
Nutrient	Dry matter conc. (%)	Functional roles	Phloem mobile	Deficiency symptoms	Stratification
Nitrogen	1–5	Major constituent of: Macromolecules (e.g. proteins, nucleic acids, coenzymes, membrane components, chlorophyll). Organic metabolites (e.g. amino acids, amines, phytohormones, secondary metabolites). Signalling ions/molecules (e.g. NO_3^-, NH_4^+, NO).	Yes	General chlorosis on oldest leaves. Stunted growth, small leaves, reduced shoot branching and early flowering. Often anthocyanosis on leaf and stem.	Acropetal
Phosphorus	0.3-0.5	Structural element of nucleic acids and phospholipids. Energy metabolism (ATP and NADPH). Signalling and enzyme activation via phosphorylation and dephosphorylation.	Yes	Anthocyanosis. Dark-green and/or purple leaves.	Acropetal
Sulphur	0.1–0.5	Constituent of organic metabolites and cellular components (e.g. cysteine, methionine, glutathione, ferredoxin and secondary metabolites like glucosinolates and alliins). Electron transport.	Conditional	Chlorosis of young leaves. Stunted growth. Anthocyanosis.	Basipetal
Potassium	1–6	Osmotic regulation and provision of turgor for cell growth. Regulation of stomatal opening and plant movement. Cation-anion balance, electro-neutrality, biochemical pH stat. Stabilisation of binding between biomolecules.	Yes	Chlorosis on tip of oldest leaves that develop into marginal necrosis. Bronzing. Slack appearance due to poor turgor and stomatal control.	Acropetal
Calcium	0.2–2	Structural element. Stabilisation of membranes, cell walls and cytoskeleton. Signalling (Ca^{2+}).	No	Disintegration of root tissue. Necrotic lesions on leaf edges and tips. Meristem death. Necrotic spots on fruits and vegetables. Leaf deformity.	Basipetal
Magnesium	0.1–1	Central element in chlorophyll. Electrostatic interactions and complex formation with enzymes and substrates, for example ATP activation through Mg-ATP complexation essential for energy-requiring transport processes.	Yes	Intervenous chlorosis on oldest leaves that eventually develop into necrosis. Accumulation of sucrose and starch in chloroplast.	Acropetal

Stratification: Basipetal, symptoms first appear on youngest leaves; acropetal, symptoms first appear on oldest leaves.

in NH_4^+ uptake as it phosphorylates and inhibits the ammonium transporter AMT1 at high NH_4^+ concentrations (Straub *et al.*, 2017). Nitrate sensing by NRT1.1 is concentration dependent and triggers Ca^{2+} signalling by the three calcium-sensor protein kinases (CPKs) CPK10, CPK30 and CPK32, that translocate to the nucleus in response to NO_3^- (K-h. Liu *et al.*, 2017). The CPKs then phosphorylate Nin-like protein 7 (NLP7), which appears to be a master regulator of the primary N response (Marchive *et al.*, 2013).

3. Low N induces morphological changes to root systems architecture

Mild N deficiency leads to elongation of lateral roots and the primary root, while severe or prolonged N deficiency inhibits primary root growth and total root length (Fig. 1.2) (Gruber *et al.*, 2013). Stimulation of lateral root growth during mild N deficiency has been shown to be auxin-dependent (Ma *et al.*, 2014). During prolonged N deficiency, inhibition of lateral root growth is controlled by NRT1.1 which removes auxin from lateral root primordia inhibiting their growth. By contrast, high NO_3^- inhibits auxin transport away from lateral root primordia, thereby stimulating root growth (Krouk *et al.*, 2010; Bouguyon *et al.*, 2015). The local inhibition of lateral root growth during N starvation is, in addition to auxin, also regulated by CLAVATA3 (CLV3)/EMBRYO SURROUNDING REGION-related (CLE) peptides, which are induced to specifically inhibit emergence of lateral roots through a CLAVATA1-dependent signalling pathway (Fig. 1.2) (Araya *et al.*, 2014). Two systemic signalling pathways known as the N-demand and N-supply pathways promote root growth and N uptake in root parts exposed to high N (Poitout *et al.*, 2018). In the N-demand pathway, C-TERMINALLY ENCODED PEPTIDES (CEP) peptides are produced in low N root parts that, via shoot-acting receptors and phloem-mobile Class III glutaredoxin polypeptides (CEPD1/CEPD2), promote root growth and N uptake in high N root parts (Tabata *et al.*, 2014; Ohkubo *et al.*, 2017). The N-supply pathway is initiated by synthesis and translocation of cytokinin from the root to the shoot, which both induces N-dependent leaf expansion and increased expression of NO_3^- transporters in high N roots (Poitout *et al.*, 2018). NH_4^+ toxicity inhibits primary root growth, while local NH_4^+ may stimulate lateral root branching (Liu & von Wirén, 2017). N-deficient plants exposed to a local supply of NH_4^+ respond by developing more second and third order lateral roots through a mechanism dependent on the NH_4^+ transporter AMT1;3 (Lima *et al.*, 2010).



4. Transition from vegetative to reproductive stage

In general, low N leads to earlier flowering compared with high N conditions, however prolonged N starvation may result in a flowering time comparable with that of high N in *A. thaliana* (Lin & Tsay, 2017). Several mechanisms are responsible for linking N status and flowering, of which a central one revolves round the balance between the microRNAs miR156 and miR172 (Fig. 1.3).

Fig. 1 Schematic model showing how nitrogen (N) affects plant growth and physiology. (1) NRT1.1 (NPF6.3) is a dual-affinity nitrate transporter that senses external nitrate concentrations. At high NO_3^-, the calcineurin B-like interacting protein kinase 8 (CIPK8) regulatesdephosphorylation of NRT1.1, causing dimerisation in a low-affinity state. Cytosolic Ca^{2+} concentrations increase and the Ca^{2+} signal is further transmitted by three calcium-sensor protein kinases (CPK): CPK10, CPK30 and CPK32 that phosphorylate Nin-like protein 7 (NLP7) in the nucleus. NLP7 induces expression of hundreds of genes as part of the primary nitrate response (PNR). At low NO_3^-, CIPK23 phosphorylates NRT1.1 resulting in a high-affinity monomeric state. (2) Spatial distribution and concentration of N affects root development. Mild N deficiency results in a root foraging response, that is existing lateral roots grow longer and deeper, a response caused by high auxin in root tips. At severe deficiency, root growth is inhibited through auxin removal from root tips. In addition, CLAVATA3/Embryo Surrounding Region-Related (CLE) peptides inhibit lateral root growth locally. Under conditions with heterogeneous N distribution, systemic signalling via C-TERMINALLY ENCODED PEPTIDES (CEP-CEPD) from low N roots induces lateral root growth and N uptake in high N roots. Cytokinin promotes root proliferation in high N roots via root–shoot–root signalling. (3) N levels affect flowering time. (a) Mild N deficiency promotes flowering compared with severe starvation and high N levels. (b) Nitrogen availability and flowering are correlated via different pathways related to ageing, photoperiod, gibberellic acid (GA) and vernalisation, as well as the autonomous pathway. Central to this regulation is the flowering gene FLOWERING LOCUS T (FT). In the photoperiod pathway, low and high NO_3^- oppositely affect expression of Ferredoxin-$NADP^+$-oxidoreductase 1 (FNR1) that positively affects FT via Constans (CO) and Cryptochrome 1 (CRY1). In the ageing pathway, the balance between miR156 and miR172 oppositely affects FT via expression of APATELA2 (AP2) and SPL. In the GA pathway, NO_3^- modulates GA1 levels and DELLA protein activity that in turn affects flowering via AP2 and SPL in the ageing pathway. The vernalisation pathway and the autonomous pathway both inhibit FLOWERING LOCUS C (FLC) that in turn inhibits FT. (4) (left) Glutamate is the precursor for chlorophyll synthesis and is stimulated by nitrogen; (right) Nitrogen deficiency results in proteolysis and released amino acids are translocated through the phloem from old source leaves to young sink leaves. (5) Leaf growth and shoot branching are regulated by cytokinin in a NO_3^--dependent manner. Biosynthesis of the root cytokinin trans-zeatin (tZ) and the cytokinin precursor trans-zeatin-riboside (tZR) is correlated with nitrate levels. Root-derived cytokinin positively regulates shoot apical meristem (SAM) size and activity, and breaks auxiliary bud dormancy. Collectively this leads to more shoot branches and more leaves. In addition, cytokinin delays senescence and activates photosynthesis resulting in improved carbohydrate assimilation. (6) In some species, N deficiency induces anthocyanin biosynthesis in stems and leaves. Low N induces the expression of PRODUCTION OF ANTHOCYANIN PIGMENT (PAP) transcription factors via miR165-mediated downregulation of SQUAMOSA PROMOTOR BINDING PROTEIN-LIKE 9 (SPL9). High N represses the expression of PAP genes via NLP7-mediated expression of LATERAL ORGAN BOUNDARY DOMAIN (LBD) genes. Furthermore, DELLA proteins positively affect anthocyanin accumulation during N deficiency through repression of negative effects of gibberellic acid (GA) on anthocyanin biosynthesis.

During plant ageing, miR156 levels decrease relative to miR172, promoting transition from the vegetative stage to the reproductive stage. miR156 represses expression of several SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors, which in turn affects expression of both miR172 and several flowering genes (Spanudakis & Jackson, 2014). Low N conditions induce expression of miR156 and repress miR172, hence affecting flowering time and stage transition depending on developmental stage (Liang *et al.*, 2012; Lin & Tsay, 2017). Another recently discovered mechanism linking N status and flowering is based on the intensification of circadian rhythms controlled by the blue light photoreceptor cryptochrome 1 (CRY1). Here, low N induces ferredoxin-$NADP^+$-oxidoreductase (FNR1), which in turn controls activity of CRY1, leading to earlier flowering (Yuan *et al.*, 2016). Three other pathways are known to link N status and flowering, namely the autonomous, gibberellic acid (GA) and the vernalisation pathways (Fig. 1.3).

5. Coupling of visual leaf symptoms with functional roles

Low N leads to developmental responses such as stunted growth, early flowering, reduced tillering in cereals, shoot branching and smaller leaves due to decreased cell division and expansion (Table 1). The most prominent visual symptom of N deficiency is chlorotic leaves, where the chlorosis spreads uniformly (general chlorosis) across the entire leaf due to either reduced chlorophyll synthesis or breakdown of existing chlorophyll binding proteins in the photosystems (Table 1; Fig. 2). Nitrogen assimilation in the chloroplast is tightly coupled with chlorophyll biosynthesis via the GS/GOGAT pathway as glutamate is the precursor for all chlorophylls produced in the tetrapyrrole biosynthetic pathway (Fig. 1.4). Hence, there is a strong correlation between the N status of plants and the chlorophyll content, which can be used to optimise N fertiliser application (Hudson *et al.*, 2011). Although the mutual regulation of chlorophyll biosynthesis and breakdown largely remains unknown, it is well established that N deficiency induces chlorophyll breakdown via proteolysis leading to the release of amino acids, amides and NH_4^+ (Fig. 1.4) (Havé *et al.*, 2016). All released N compounds are highly mobile in the phloem. Older leaves consequently act as source tissue during low N situations supplying young and developing tissue like leaves, flowers and seeds with N. As a consequence, visual symptoms are first visible on the oldest leaves (acropetal stratification).

Reduced shoot branching, stunted growth and inhibition of leaf expansion constitute central physiological responses to N deficiency. These responses are closely linked to cytokinin produced in roots in response to NO_3^- (Rahayu *et al.*, 2005) (Fig. 1.5). Subsequent root-to-shoot translocation of trans-zeatin (a cytokinin) and trans-zeatin-riboside (a cytokinin precursor) affects leaf growth through stimulation of leaf expansion and meristem activity (Osugi *et al.*, 2017). In addition, root-derived trans-zeatin-riboside directly stimulates shoot apical meristem (SAM) growth and thereby overall shoot biomass (Landrein *et al.*, 2018). Shoot branching is controlled by formation and activation of axillary buds. Axillary buds are activated by NO_3^- due to a stimulatory effect on cytokinin production, whereas low NO_3^- maintains bud dormancy and thereby reduce branching (Fig. 1.5) (Müller *et al.*, 2015). This effect further relies on interaction with auxin and strigolactones (de Jong *et al.*, 2014).

Another prominent visual symptom of N deficiency is reddish coloration due to anthocyanin production (Fig. 1.6). This is only seen in some species and may also be influenced by other abiotic factors such as high light intensity, low temperatures and P deficiency. In *A. thaliana*, low N induces expression of key

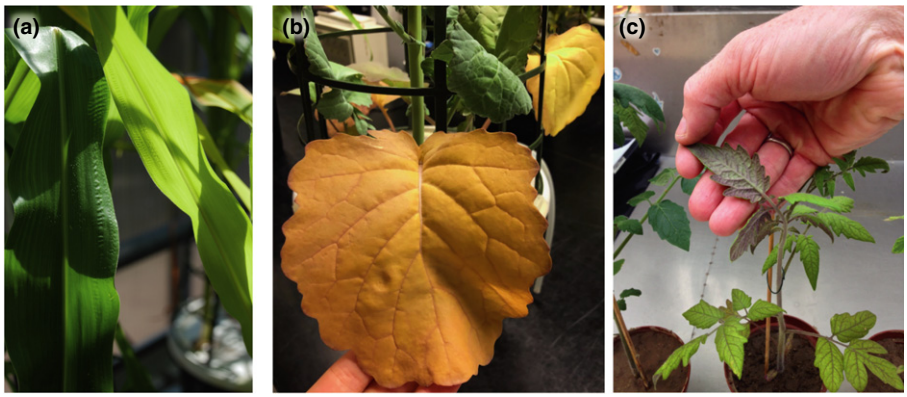


Fig. 2 Nitrogen (N) deficiency always appears on the oldest leaves first and is characterised by a general chlorosis. (a) Leaf of healthy maize plant to the left and N-deficient leaf with general chlorosis to the right. (b) Anthocyanin accumulation in a N-deficient oilseed rape leaf with general chlorosis. (c) General chlorosis in tomato, with anthocyanosis on veins and on the abaxial side of leaves.

transcription factors positively regulating anthocyanin synthesis, including PRODUCTION OF ANTHOCYNANIN PIGMENT1 (PAP1), PAP2 and GL3 (Xu *et al.*, 2015). Repression of GA signalling by DELLA proteins is required for the induction of several biosynthesis genes, including genes related to anthocyanin accumulation during N deficiency (Y. Zhang *et al.*, 2017). By contrast, three members of the LATERAL ORGAN BOUNDARY DOMAIN (LBD) protein family LBD37/38/39, which are NO_3^- induced, act as negative regulators of anthocyanin synthesis by repressing PAP1 and PAP2 expression (Rubin *et al.*, 2009). In parallel, the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factor, SPL9, is a negative regulator of anthocyanin synthesis, as it represses PAP1, PAP2 and other transcription factors that positively regulate anthocyanin production (Fig. 1.6). SPL9 is repressed by miR156, which itself is induced by N deficiency, thus linking low N conditions to anthocyanin production by increasing expression of PAP1 and the biosynthetic gene DFR (Cui *et al.*, 2014).

III. Phosphorus

Phosphorus is a structural element of essential biomolecules involved in energy metabolism (ATP, NADPH), of nucleic acids (DNA, RNA) and of phospholipids in cell membranes (Table 1). The hydrolysis of the P–O–P bond in adenosine triphosphate (ATP) to yield ADP and inorganic phosphate (Pi) is the principal source of biochemical energy within the cell (Kamerlin *et al.*, 2013). Plants predominantly take up P as the inorganic orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-}, depending on soil pH.

A wide range of enzymes has evolved to catalyse reactions involving phosphates. These enzymes catalyse phosphorylation and dephosphorylation processes affecting the activity of proteins, stabilising them or labelling them for breakdown, facilitating or inhibiting their movement between subcellular compartments or regulating protein–protein interactions. The importance of protein phosphorylation/dephosphorylation in cellular signalling is emphasised by the fact that one third of all proteins in the cell are phosphorylated at any given time (Manning *et al.*, 2002).

1. Functional properties of P in photosynthesis

Phosphorus is an essential element in the light reaction of photosynthesis, where absorbed light drives the electron transport chain in the thylakoid membrane to generate ATP and NADPH. Phosphorus also plays an essential role in the dark reactions (Calvin–Benson cycle), in which ATP and NADPH are used to convert CO_2 to carbohydrates in the chloroplast stroma (Fig. 3.1). When plants are exposed to P deficiency, lowered chloroplast Pi levels lead to a reduction in ATP production, because Pi together with CO_2 and H_2O, are the primary substrates for photosynthesis. Moreover, during P deficiency a larger fraction of $NADP^+$ remains in the reduced form (NADPH), because it cannot be utilised in the Calvin–Benson cycle due to ATP limitation (Fig. 3.2) (Carstensen *et al.*, 2018). A prominent feature of P deficiency is therefore a marked decrease in CO_2 assimilation and reduced biomass production.

2. Molecular and physiological responses to P deficiency

In response to P limitation, plants trigger a series of biochemical adjustments, including activation of organic acid (OA) exudation to mobilise inorganic P trapped in precipitated Al, Fe and Ca salts in the rhizosphere. OA are mainly produced in the mitochondria through the tricarboxylic acid (TCA) cycle (Krebs cycle) and to a lesser extent in the glyoxysomes as part of the glyoxylate cycle. OA have a high affinity for Al, Fe and Ca and can displace Pi from insoluble complexes through ligand exchange, increasing the P solubility and make soil Pi available for plant uptake.

The activity of key enzymes such as citrate synthase (CS) and PEP carboxylase involved in OA biosynthesis increases in response to P starvation and OA are subsequently released to the rhizosphere as a response. The proteins responsible for OA secretion belong predominately to the ALMT (aluminium-activated malate transporters) and the MATE/AACT (multidrug and toxic compound extrusion/aluminium-activated citrate transporter) families (Delhaize *et al.*, 2012).

There is a strong correlation between the P status of plants and cellular respiration (Wang *et al.*, 2015). Plant mitochondria possess

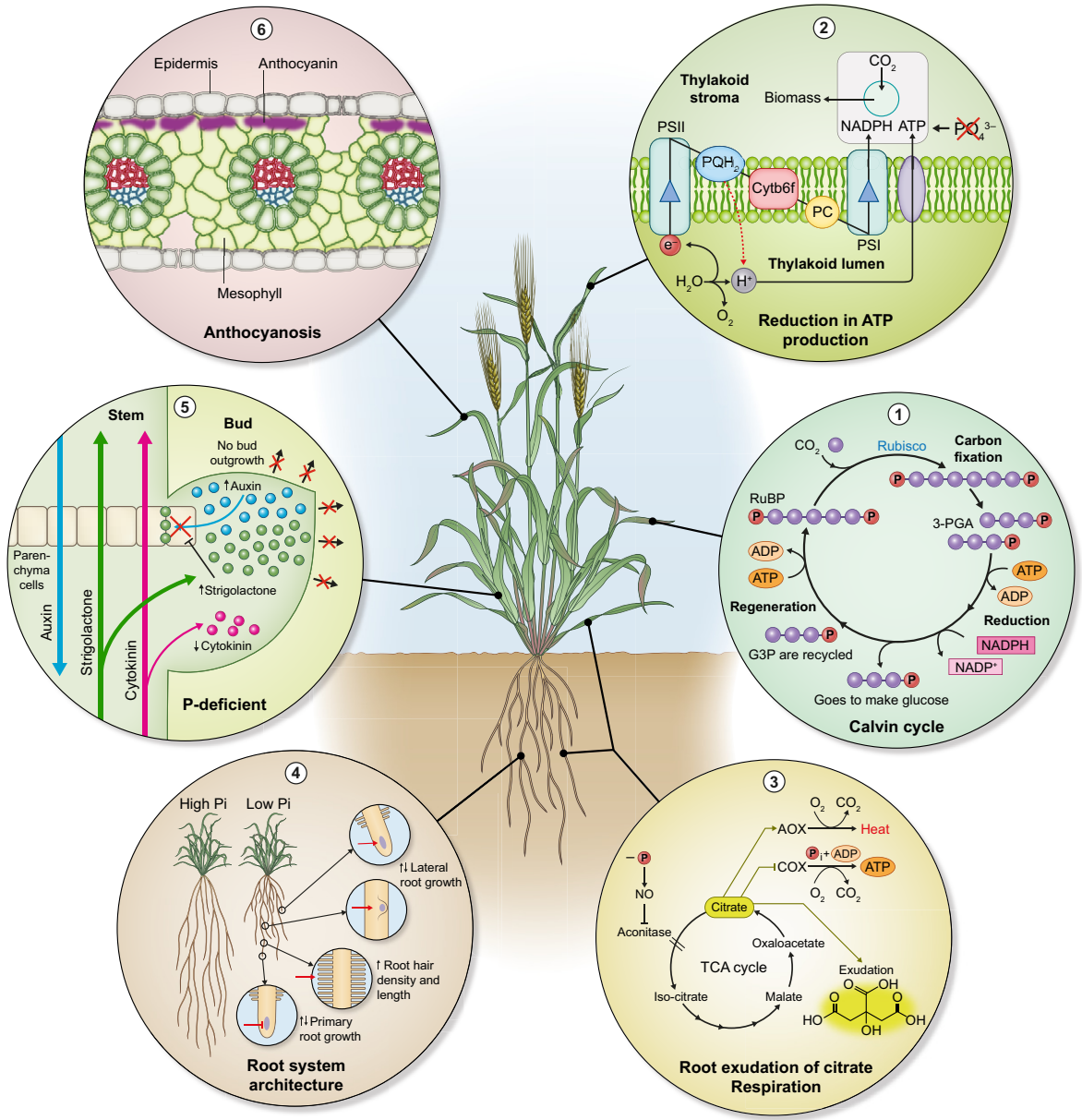


Fig. 3 Schematic model showing how phosphorus (P) affects plant growth and physiology. (1) P the chloroplast stroma affects CO_2 assimilation through thecarboxylation rate of RuBisCo and the regeneration of ribulose-1,5-bisphosphate (RuBP). 3-PGA, 3-phosphoglycerate; G3P, glyceraldehyde-3-phosphate. (2) Lowered chloroplast PO_4^{3-} (Pi) levels lead to a reduction in ATP production. The reduced ATP synthesis impacts the flow of protons from the thylakoid lumen to the chloroplast stroma, causing lumen acidification, which triggers energy dissipation via nonphotochemical quenching. (3) When the Pi concentration of the cell decreases, less substrate is available for oxidative phosphorylation (ATP synthesis) via the conventional cytochrome oxidase pathway (COX). This triggers the alternative oxidase (AOX) pathway (no ATP synthesis), ensuring that electron transport in the respiration process continues under P starvation. Moreover, P deficiency leads to NO accumulation, which impacts the TCA cycle and leads to aconitase inhibition. As a consequence, citrate accumulates, leading to stimulation of AOX relative to COX and promoting citrate exudation from the root to the rhizosphere. (4) Root system architecture is affected during P deficiency. In cereals, the response is complex and much more variable than observed in Arabidopsis, in which the architecture is shaped to allow a more thorough exploration of the top soil where most P is present. P deficiency may enhance/repress primary root and lateral root growth, whereas the root hair density and length generally are enhanced. (5) During P deficiency, strigolactone biosynthesis increases in the root leading to increased strigolactone transport to the shoot and higher levels of strigolactone in the bud, which initiate degradation of the auxin efflux protein PIN1, inhibiting auxin transport away from the bud. When the auxin level in the bud is high, the cytokinin level is low and bud outgrowth is inhibited. (6) Anthocyanosis: photoabatement by anthocyanin shields photosynthetic systems from light stress.

two distinct pathways for the transfer of electrons to molecular oxygen during respiration: the cytochrome *c* oxidase (COX) and the alternative oxidase (AOX) pathways (Fig. 3.3). Generally, the AOX expression is very low until a stress condition emerges. When the Pi concentration of the cytosol goes down, less substrate is available for oxidative phosphorylation (ATP synthesis) via the

conventional COX pathway, triggering the AOX pathway, which ensures that electron transport in the respiration process continues under P starvation. Simultaneously, the root exudation of citrate increases. The interactions between Pi, AOX and citrate exudation are not yet fully resolved at the mechanistic level (Del-Saz *et al.*, 2018). However, P deficiency leads to accumulation of the messenger molecule NO in roots (Wang *et al.*, 2010), which inhibits aconitase and thereby prevents the conversion of citrate to iso-citrate (Gupta *et al.*, 2012). The resulting accumulation of citrate subsequently triggers the AOX pathway and allows continuation of respiration when the COX pathway is hampered. By linking these processes, P sensing is related to the TCA cycle intermediate citrate, modulation of mitochondrial respiration, OA exudation and eventually mobilisation of P in the rhizosphere, allowing for an increased Pi uptake.

Plants respond to P deficiency by increasing the root-to-shoot ratio and by changing the root architecture to facilitate a more thorough exploration of soil P resources, particularly in the top soil where most P tends to accumulate. The root response to P limitation differs across the plant kingdom. Some plants, including *A. thaliana*, respond to P starvation by a process called root apical meristem (RAM) exhaustion, where cells within the root tip loose meristematic activity and cells in the elongation zone are arrested, eventually leading to a reduction in growth of the primary root (Fig. 3.4) (Gutiérrez-Alanís *et al.*, 2018). The RAM process is triggered by direct exposure of the root tip to low P levels in the soil. The local response appears to be independent of the general root P status and is not caused by metabolic disturbances triggered by the P deficiency. It has been shown that accumulation of Fe^{3+} within the root tip is essential for the inhibition of primary root growth under low P conditions (Ward *et al.*, 2008). In P-starved *A. thaliana*, Fe accumulates in specific zones of the primary root, which triggers the STOP1-ALMT1 module, resulting in exudation of malate within the apoplast of the root tip. As malate forms strong Fe complexes, this leads to Fe accumulation and Fe toxicity in RAM tissue, causing generation of reactive oxygen species (ROS) though Fenton reactions. The accumulation of ROS appears to stimulate callose formation and a stiffening of the cell walls, preventing cell elongation in RAM. Mutations in STOP1-ALMT1 restore the growth of the primary root under P limitation (Balzergue *et al.*, 2017).

Compared with *A. thaliana*, the root system of, for example, cereals have a more complex response to P deficiency and marked species and genotype differences have been observed (Péret *et al.*, 2014). Maize plants tend to allocate relatively more carbon resources to their roots during P deficiency and shows no reduction or even a slight enhancement in root elongation. Also *japonica* rice roots respond with elongation of the primary root under P deficiency (Dai *et al.*, 2012).

Apart from changes to primary root growth under P deficiency, plants respond to P limitation by increasing the root hair density and/or the frequency and length of lateral roots. The length and density of root hairs and fine roots are important hot spots for P acquisition as they provide the largest surface area for P uptake with the lowest carbon investment in root biomass. Transcriptomic analyses of root hair and tips have shown that accumulation of Pi transporters belonging to the PHT1 family are markedly more frequent at the interface between the newly developed root tissue and the soil to be explored. In *A. thaliana* seedlings, only the cap seems to play an important role in P acquisition, whereas the elongation zone above the cap seems to play a minor role (Kanno *et al.*, 2016).

Auxin is a key regulator of root system architecture, and plays an important role in shaping the architecture of roots during P starvation. Especially RAM appears to be important in that respect as auxin accumulates here when P becomes limiting, which inhibits further growth of the root tip, irrespective of the shoot P status. Strikingly, this inhibitory response of root tip growth to auxin is opposite to the elongation of lateral roots, which are promoted by auxin accumulation. During P deficiency, the expression of the auxin receptor TIR1 is upregulated, thereby increasing the sensitivity of the pericycle cells towards auxin. This leads to activation of the auxin response factor (ARF), a transcription factor that promote lateral root emergence. The auxin accumulation in RAM tissue during P deficiency is also involved in triggering the formation of trichoblast cells to form root hairs when the auxin levels are upregulated at the root tip. At low P levels, auxin is mobilised and enters the root hair differentiation zone via the auxin influx carrier (AUX1). Elevated auxin levels in trichoblasts subsequently trigger a gene expression cascade, mediated by a series of transcription factors, to promote root hair formation and elongation (Bhosale *et al.*, 2018).

Also, shoot branching is modified during P limitation (Fig. 3.5). Even moderate- and short-term P deficiency in barley is known to affect grain yields significantly via a reduction in the number of fertile tillers (Carstensen *et al.*, 2019). This effect is mediated via strong interactions between tissue P levels and the hormones strigolactones, auxin and cytokinin. During P deficiency, strigolactone biosynthesis is increased in the roots, promoting strigolactone transport towards the shoot (Kohlen *et al.*, 2011). When the level of strigolactone increases in the bud, degradation of the auxin efflux carrier (PIN1) is initiated, which inhibits export of auxin from the bud to the stem (Kebrom *et al.*, 2013). When the auxin levels in the bud are high, the cytokinin levels are low and bud outgrowth is prevented. During P sufficiency, strigolactone levels are not increased in the bud and auxin is transported away from the bud by the PIN1 proteins. At low auxin levels, the cytokinin levels increase, which in turn triggers bud outgrowth.

3. Coupling of functional roles and visual deficiency symptoms for P

A reddish-purple cast to the leaves due to anthocyanin accumulation (anthocyanosis) is a marked symptom of P deficiency across a wide range of plant species (Table 1; Fig. 4). Anthocyanins are flavonoid pigments synthesised on the cytoplasmic face of the endoplasmic reticulum and stored in vacuoles where they are protected from oxidation by the low pH. The anthocyanins typically accumulate on a dark-green coloured background (Fig. 3.6), due to a high chlorophyll concentration caused by a

Fig. 4 Phosphorus (P) deficiency always appears on the oldest leaves first and is characterised by anthocyanosis on a nonchlorotic background. (a) Leaf of P-deficient maize plant showing anthocyanosis on a dark nonchlorotic background. (b) Anthocyanosis on the abaxial side of P-deficient tomato leaf.

reduced cell division and extension in P-deficient plants (Hughes and Lev-Yadun, 2015).

However, not all plant species develop anthocyanosis when exposed to P deficiency, important exceptions are for example potato, sugar beet and rice (Hughes and Lev-Yadun, 2015). It is also striking that symptoms develop very differently across species (Fig. 4). Tomato develops symptoms on the abaxial side, whereas maize seems to express symptoms on the adaxial side and other species tend to accumulate anthocyanins on the stems or along the leaf margins. The majority of species, however, seem to accumulate anthocyanins across the whole leaf or in major parts of the leaf. As the chlorophyll concentration on a leaf area basis is maintained or even increased during P deficiency this typically leads to the development of a red-purple-bluish hue across the leaf surface. The severity of P symptoms appears to be heavily influenced by abiotic factors, such as leaf temperature and light intensity.

Phosphorus deficiency triggers an increased expression of genes in the anthocyanin biosynthetic pathway. Some of these genes appear to be GA dependent. When plants are exposed to P deficiency, transcript levels of genes encoding enzymes activated by GA are reduced, whereas those that deactivate GAs are increased. This leads to a decrease in bioactive GA levels, which in turn triggers the synthesis of specific growth-regulating proteins (DELLAs). Increased levels of DELLA in P-deficient plants upregulate transcripts encoding for enzymes involved in anthocyanin biosynthesis, which include *F3H* and *LDOX*, but not *UF3GT* and *PAP1* (Jiang *et al.*, 2007).

Currently, it is believed that the primary role of anthocyanins is photoprotection of the light-harvesting complexes (LHCs) of PSII, which theoretically should provide a functional advantage to red leaves when the capacity for thermal energy dissipation is exceeded by excessive absorption of photons (Gould *et al.*, 2018). As a consequence, anthocyanosis can be seen as a feedback response to P deficiency in order to prevent over-excitation and damage to PSII. However, using closely related genotypes of maize and coleus (*Solenostemon scutellarioides*) with different regulation of anthocyanin production, Henry *et al.* (2012) showed over a range of light intensities that leaves with anthocyanosis did not better tolerate the impacts of P deficiency than green leaves. Thus, the putative adaptive significance of anthocyanin accumulation under P deficiency stress still remains enigmatic.

IV. Sulphur

Atmospheric S (e.g. sulphur dioxide, SO_2) can be taken up and utilised by aerial plant parts, but the main source of S is inorganic sulphate (SO_4^{2-}), which is taken up by roots and assimilated into a variety of key metabolites, including the amino acids cysteine and methionine and various prosthetic groups.

Plant species differ considerably in their demand for S, which generally decreases in the order: Cruciferae > Leguminosae > Graminae. The occurrence of S deficiency has increased since the 1990s, mainly because of decreased deposition of atmospheric SO_2. Consequently, research on S sensing, uptake, assimilation and functional properties have increased substantially during the past decades (Weissert & Kehr, 2017).

1. Sulphur sensing, uptake and assimilation

Sulphur-deficient plants sense the S status of the soil–root environment via the tissue level of cysteine precursors (Dong *et al.*, 2017). Intermediates in SO_4^{2-} and NO_3^- reduction (SO_3^{2-} and NO_2^-) are toxic and S fluxes must be balanced relative to the fluxes of N and C. Sulphate assimilation is therefore downregulated when C or N is limiting (Long *et al.*, 2015; Kopriva *et al.*, 2019).

When plants are exposed to S deficiency, high-affinity SO_4^{2-} transporters in the SULTR family, which function by co-transporting SO_4^{2-} along with protons, are upregulated to facilitate uptake from the rhizosphere (Fig. 5.1). An ethylene insensitive-like (EIL) transcription factor, SLIM1, has been isolated and shown to be involved in this response (Koprivova & Kopriva, 2016). In *A. thaliana,* SULTR1;1 and SULTR1;2 are responsible for the initial SO_4^{2-} uptake from the soil. *SULTR1;1* is mainly expressed in the epidermis, including root hairs, while *SULTR1;2* is mainly expressed in the cortex (Kimura *et al.*, 2019).

Plants incorporate SO_4^{2-} into organic molecules after reduction to sulphide (S^{2-}). Among the macronutrients, S is remarkable due to its ability to change between the oxidation states +6 and −2. The change in oxidation state from +6 to −2 is highly energy dependent and consumes 732 kJ mol^{-1}. In comparison, NO_3^- and C assimilation requires only 347 and 478 kJ mol^{-1}, respectively. SO_4^{2-} is first activated to adenosine 5′-phosphosulphate (APS) by catalytic reaction with ATP sulphurylase. APS can then either be channelled into cysteine synthesis or used for formation of secondary metabolites. Cysteine may be further converted to methionine, glutathione and all other metabolites containing reduced S (Long *et al.*, 2015; Maruyama-Nakashita, 2017).

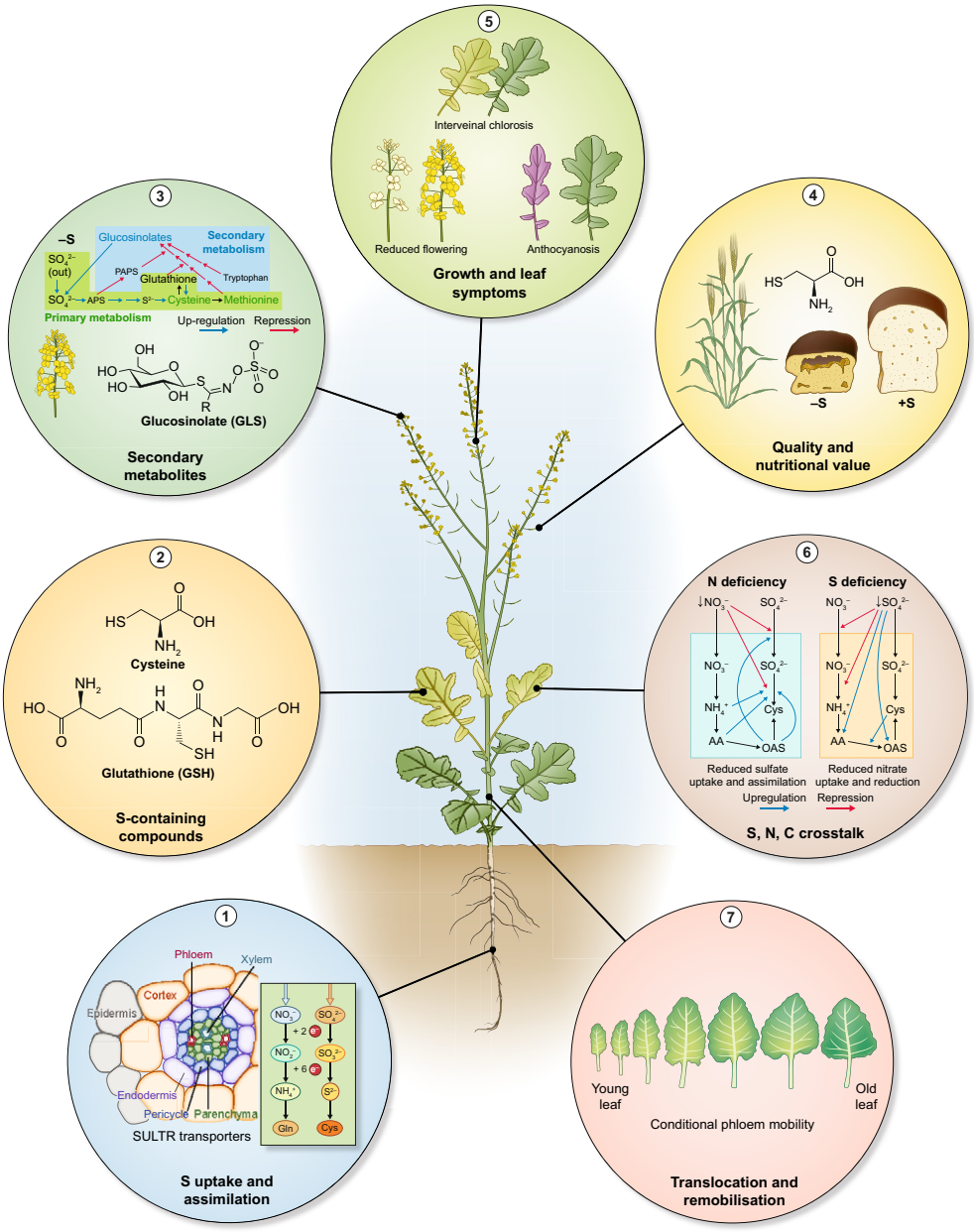


Fig. 5 Schematic model showing how sulphur (S) affects plant growth and physiology. (1) The main plant available S source is inorganic sulphate (SO_4^{2-}), which is taken up by membrane localised transporters (SULTRs). Plants incorporate SO_4^{2-} into bioorganic molecules after reduction to sulphide, much like NO_3^- reduction and assimilation. (2) S is a key component of essential metabolites and cellular constituents in plants, including the amino acid cysteine and the low molecular soluble compound glutathione.(3) Plants contain numerous S-containing secondary metabolites,for example glucosinolates (GSLs), of which many serve as defences against microbes and pests. During S deficiency, GSH and GSLs are degraded in order to up-regulate primary S metabolism (APS, adenosine 5′-phosphosulphate; PAPS, 3′-phosphoadenosine-5′-phosphosulfate). (4) S deficiency reduces the protein content and the content of S-containing amino acids. This has consequences for the quality and nutritional value of food and feed. (5) S deficiency leads to reduced protein synthesis, functionality and quality, anthocyanin production, stunted growth, chlorotic young leaves and premature/reduced flowering. (6) SO_4^{2-} uptake is coordinated alongside the uptake and assimilation of N. S deficiency leads to reduced nitrate uptake and NR activity. N deficiency leads to reduced sulphate uptake and APS reductase activity. (7) S deficiency symptoms appearon youngest leaves first (basipetal stratification). S remobilisation is increased by low N availability. Adapted from Long *et al*. (2015) and Maruyama-Nakashita (2017).

2. Functional roles of S

Sulphur deficiency interacts massively with the transcriptome and ionome of plants (Courbet *et al*., 2019) resulting in a decrease in photosynthetic carbon assimilation and a reprogramming of metabolism to ensure seed production (Jobe *et al*., 2019). In reduced form, S is a key component of various prosthetic groups in Fe–S proteins, such as ferredoxin, which mediate electron transfer in the photosynthetic electron transport chain (Forieri *et al*., 2013; Zheng & Leustek, 2017). Thus, a key feature of S deficiency is a

marked suppression of the photosynthetic efficiency and rapid development of chlorotic leaves. In S-deficient rice, the chlorophyll content was 50% lower due to a general decrease in the chlorophyll binding proteins of the light-harvesting complex of both PSII and PSI (Lunde *et al.*, 2008).

Glutathione (GSH) is a low molecular soluble S compound with complex and multifaceted functions associated with its reactive thiol group (Noctor *et al.*, 2012) (Fig. 5.2). This thiol group allows glutathione to donate electrons for enzymatic catalysis, to control redox homeostasis and to function as an antioxidant and a signalling molecule in stress responses and development. Glutathione is also involved in storage and long-distance transport of reduced S from shoots to roots (Zheng & Leustek, 2017) and acts as substrate for phytochelatin synthesis, thereby playing an important role in metal hyperaccumulation (Ahmed *et al.*, 2019).

The primary S compounds are complemented by numerous secondary S-containing metabolites of which many are involved in the defence against pathogens (Fig. 5.3). Examples of important S-containing metabolites are glucosinolates, alliins, camalexin and S-rich peptides of the thionin and defensin groups. Glucosinolates are a diverse group of compounds consisting of a thioglucose core with two S atoms ligated to different amino acids resulting in > 100 different compounds. They constitute a large pool of S, up to 30% of the total S, in *Brassica* tissues and the glucosinolate content of plants directly reflects their S status, being increased at high-S nutrition and decreased under S-limited growth (Aghajanzadeh *et al.*, 2014; Long *et al.*, 2015).

3. Sulphur and plant quality

Sulphur deficiency is known to have significant effects on the quality of plant-based food and feed (Fig. 5.4). Methionine is an essential amino acid in human nutrition and often represents a limiting nutritional factor in seed-rich diets (Dung Tien *et al.*, 2016). Sulphur has a pronounced effect on the protein composition of cereal grain as opposed to N, which more generally affects the protein content (Yu ZT *et al.*, 2018). Reduced S availability favours the synthesis and accumulation of S-poor or low-S storage proteins such as ω-gliadin and high-molecular-weight subunits of glutenin. This affects the baking quality of wheat, because disulphide bridging during dough preparation is essential for polymerisation of the glutelin fraction to create optimum loaf volume (Liu *et al.*, 2011; Järvan *et al.*, 2017).

S-containing secondary metabolites such as glucosinolates and alliins are not only important defence compounds, but are also the basis of smell and taste of cruciferous vegetables, onion and garlic. The positive influence of S nutritional status on the content of secondary S-containing metabolites has been documented for a range of crop species, in some cases showing a positive interaction with N status, in others not (Bloem *et al.*, 2005; Poisson *et al.*, 2019) (Fig. 5.3).

4. Sulphur deficiency symptoms linked to functional properties

Plants growing at suboptimal S supply display stunted growth, chlorosis, anthocyanosis, as well as premature and reduced flowering, and reduced seed setting (Table 1; Figs 5.5, 6). These symptoms are most pronounced in S-demanding Cruciferae species (Schnug & Haneklaus, 2005). Sulphur-deficient plants become chlorotic and stay chlorotic for an extended period until developing necrosis. Symptoms are typically very similar to the general chlorosis found in N-deficient plants, but in some species intervenous chlorosis is prevalent, similar to symptoms occurring in Fe-deficient plants. The differences in leaf appearance are not fully understood, but are probably related to extensive cross-talk with N and Fe during S deficiency (Fig. 5.6) (Forieri *et al.*, 2017). During S limitation, plants are unable to fully induce their Fe-uptake machinery, which includes relevant transport proteins and the release of phytosiderophores in strategy II plants such as barley, maize, and wheat (Courbet *et al.*, 2019). A characteristic feature across different plant species is the marked accumulation of NO_3^- in root tissue combined with a reduction in NR activity, showing that S-deficient plants are unable to utilise N in their metabolism and consequently becomes N deficient (Kaur *et al.*, 2011) and display N deficiency symptoms.

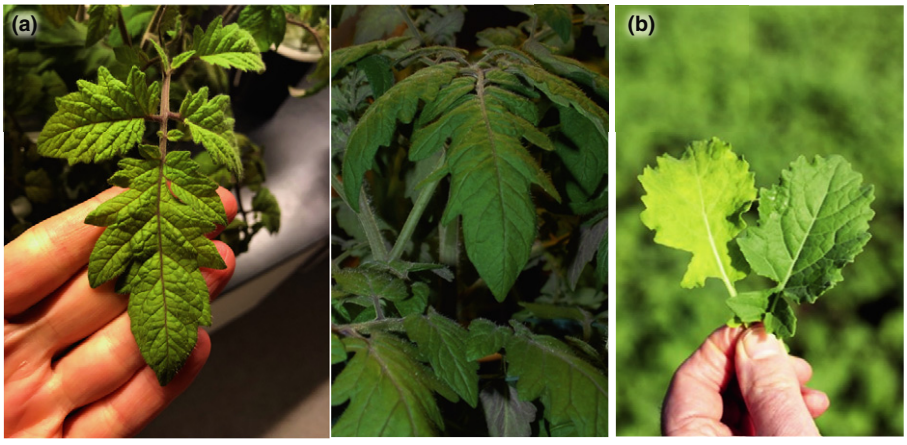


Fig. 6 Sulphur (S) deficiency always appears on the youngest leaves first and spreads rapidly in basipetal direction. (a) Left, leaf of S-deficient tomato plant showing interveinal chlorosis with anthocyanin accumulation on veins and petiole. Right, a healthy control plant without any symptoms. (b) Left, S-deficient leaf of oilseed rape plant. Right, healthy control.

The chlorosis will in most cases be interveinal due to a relatively higher S supply around leaf veins (Fig. 5.5). However, high anthocyanin content may, in some cases, mask this symptom. Chlorosis induced by S deficiency rarely develops into necrosis, as is for example the case for Mg, K and N, but the stratification of symptoms is dependent on N status. Mature (source) leaves becomes chlorotic under N deficiency due to protein degradation and amide export to young sink leaves (acropetal stratification) (Dubousset *et al.*, 2009). By contrast, S starvation first and strongest affects the young leaves (basipetal stratification). The reason for this difference is a much higher mobility of NO_3^- between vacuole and cytosol upon demand than is the case for SO_4^{2-} (Miller *et al.*, 2009). In addition, organically bound S only becomes exported to the phloem under N deficiency, because the latter is required to trigger protease activity leading to remobilisation of S-containing amino acids (conditional mobility) (Fig. 5.7). In oilseed rape, S deficiency induces 'white blooming', where white flower colour develops from an overload of petal cells with carbohydrates caused by disorders in the protein metabolism. This finally ends up in the formation of leuco-anthocyanins that often occurs during periods of high photosynthetic activity (Schnug & Haneklaus, 2005).

V. Potassium

Potassium forms no covalent bonds and the K^+ ion is pivotal for maintaining healthy cellular conditions by affecting for example electro-neutrality, osmotic regulation, anion–cation balancing and the biochemical pH stat (Table 1). The majority of K^+ is found in vacuoles, where it acts as a turgor provider. K^+ also facilitates protein and enzyme stability through optimal hydration, hence a large number of enzymes (> 70) are either stabilised, activated and/or inhibited by K^+ as a co-factor, for example pyruvate kinase, phosphofructokinase and starch synthase (Anschutz *et al.*, 2014).

All plant movements are conveyed by K^+ fluxes. Guard cells increase their osmotic potential through an influx of K^+, which results in an accompanying water uptake from adjacent cells, leading to stomatal opening. This process is light induced and starts with H^+-ATPase driven acidification of the stomatal apoplast, stimulating K^+ influx via polarisation of the plasmamembrane potential. Electro-neutrality during K^+ influx is maintained by Cl^-, NO_3^- and malate ions. Conversely, during darkness, K^+ efflux is correlated with water efflux and stomatal closure. Abscisic acid (ABA) induces stomatal closure through H_2O_2 and NO along with subsequent ion channel-mediated loss of K^+ and anions (Chen *et al.*, 2016). In barley, SLAC1 (Slowly Activating Anion Channel) transports NO_3^- and mediates stomatal closure via interaction with ABA (Schäfer *et al.*, 2018), hence linking N and K dependent metabolic processes.

K^+ is highly mobile in the phloem and vital for phloem loading of photoassimilates, which at the site of source cells is ATP driven. The phloem is loaded with sucrose via H^+-coupled sucrose transporters (SUT), requiring channel-mediated K^+ transport from the mesophyll apoplast into the phloem in order to maintain the activity of H^+-ATPases transporting H^+ in the opposite direction. As much as 50–80% of K in the xylem may be recycled to the roots and sink tissues via the phloem (Noa *et al.*, 2014). This recycling is increasingly being understood as a sophisticated system for whole-plant communication of nutritional demands, while at the same time providing a distribution system for sink tissues with variable demands for sucrose and nutrients along the phloem pathway (Gajdanowicz *et al.*, 2011; Dreyer *et al.*, 2017).

In exchange for H^+, K^+ is vital for establishing the transmembrane pH gradient required for ATP production during photosynthesis. K^+ is also necessary for photosynthetic CO_2 fixation through proper stomatal functioning and maintenance of chloroplast conditions with respect to turgor, pH and enzyme activities. In chloroplasts of *A. thaliana*, the K^+ antiporter KEA3 is important for the ability to maintain high photosynthetic efficiency under fluctuating light conditions (Kunz *et al.*, 2014; Armbruster *et al.*, 2017).

1. Molecular–physiological responses to K deficiency

Root epidermal cells, particularly root hairs, represent the main sites for K^+ uptake (Fig. 7.1). High-affinity K^+ uptake at low external K^+ concentrations is mediated by K^+/H^+ symporters in the HAK/KUP/KT family, whereas voltage-gated K^+ channels, for example AtAKT1 in *A. thaliana*, mediate low-affinity K^+ uptake at higher external concentrations. The flexibility of K^+ transport is also reflected in a diversity of functionally different channels, for example outward rectifiers (K^+ release channels) and weak-rectifiers allowing both K^+ efflux and uptake (Hedrich *et al.*, 2011). In xylem parenchyma cells in the stele, K^+ loading is controlled by SKOR-like (Stelar K^+ Outward Rectifier) channels, which release K^+ into the xylem stream (Gaymard *et al.*, 1998). Stomatal closure is conveyed by K^+ efflux via GORK (Guard Cell Outwards Rectifying K^+) channels (Ivashikina *et al.*, 2005).

Plants have evolved sophisticated sensing and signalling systems that respond to K deficiency. These systems involve specific signal molecules, ROS, Ca^{2+}, phytohormones and microRNAs. Hyperpolarisation of the plasma membrane is the first detected response, occurring within a few minutes after a significant decrease in external K^+ concentration. After prolonged deficiency (6–30 h), ROS generation and ethylene signalling are typical K deficiency responses (Behera *et al.*, 2017). K deficiency also triggers two successive and distinct Ca^{2+} signals involving a calcineurin B-like (CBL1)-CIPK23 Ca sensor–kinase complex activating AKT1 K^+ channels in postmeristematic tissues in the stele. This activation happens rapidly (minutes) after onset of low external K^+ concentration. After several hours of deficiency, activation of AKT1 in the elongation and root hair differentiation zones takes place mediated via another Ca^{2+} signal (Behera *et al.*, 2017). MicroRNAs are likely to play a role in the K^+ starvation responses linked to Ca^{2+}. By high-throughput sequencing and degradome analysis, ata-miR1432-5p was identified as a regulator participating in Ca^{2+} signalling associated with the low K response in barley (Zeng *et al.*, 2019). Transcriptional regulation of HAK1 and HAK5 in rice and *A. thaliana* involves the transcription factor ARF2, which after phosphorylation initiates HAK5 transcription at low K^+ but suppresses it during K sufficiency (Wang & Wu, 2017). A number of additional hormonal interactions are also activated during K

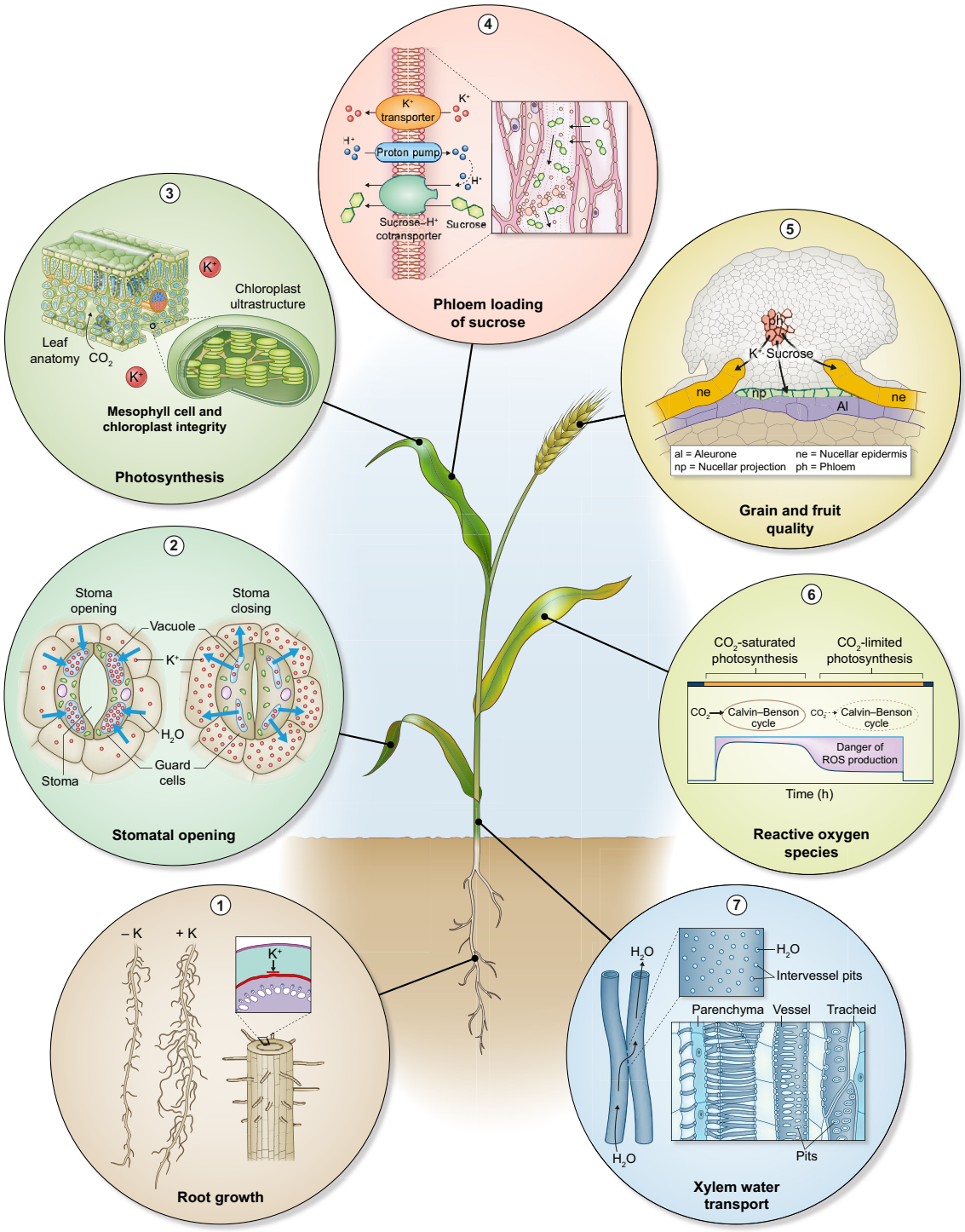


Fig. 7 Schematic model showing key functions of potassium (K) in plant growth and physiology. (1) Root growth is dependent on sucrose loading into the phloem, which is a process that require K. Hence, during K deficiency, lateral root growth is reduced. In addition, the endodermis of K-deficient roots become more suberised than that of nondeficient roots. This increased suberisation improves translocation of K to the shoots, as it improves water conductance and reduces leakage of K out of the stele. (2) Influx of K is necessary for guard cells to become fully turgid so that the stomatal pore opening can maintain CO_2 conductance. (3) K sustains photosynthesis by maintaining mesophyll cell and chloroplast integrity. (4) K is necessary for sucrose loading into the phloem, hence K deficiency leads to sucrose accumulation in source leaves. (5) Due to poor sucrose loading into the phloem, K deficiency leads to reduced sucrose supply to sink tissues like seeds, grains and fruits. (6) By maintaining proper CO_2 conductance, K supresses the number of electrons available for side reactions with O_2, which would otherwise create reactive oxygen species (ROS). (7) K facilitates proper xylem vessel maturation and functioning. K-deficient plants hence have reduced water conductance due to poor interaction between K and the pectin matrix of intervessel pits. These effects tend to give K-deficient plants a slack appearance, as well as a lowered capability to handle water-related stress, that is drought and salinity.

stress, including ethylene, auxin, cytokinin and jasmonic acid (Schachtman, 2015; Wang & Wu, 2017; G. Li *et al.*, 2017). In *A. thaliana*, K starvation induces ethylene production, which also increases ROS levels, ultimately leading to upregulation of low-affinity K transporters. Some proteins and enzymes related to the jasmonic pathway are upregulated in wheat plants during K stress (G. Li *et al.*, 2017).

2. Coupling of visual symptoms and K functionalities

Inadequate K supply has a range of negative effects on plant growth and vigour as well as on plant resistance towards environmental stresses like drought and salinity. During K stress, organic acids are exuded (Zöorb *et al.*, 2014) and lateral root growth is inhibited. The root endodermis tends to become more suberised under K deficiency (Fig. 7.1), a process triggered by ABA (Barberon *et al.*, 2016) and affecting the translocation of K^+ (Chen *et al.*, 2019).

In the shoot, the earliest visual symptoms of K deficiency are chloroses at the tip of the oldest leaves, which rapidly turn into marginal necrosis spreading from the tip along the leaf margins (Table 1; Fig. 8). Leaves with these symptoms contain lower K levels in the leaf tips, compared with the basal parts of the leaf, and nonnecrotic symptoms can be reverted by re-supply of K. Some jasmonate-related genes are expressed in K-deficient leaf tips and may promote leaf senescence (Ueno *et al.*, 2018). These symptoms often appear together with bronzing, which is due to accumulation of polyamines (PAs), for example putrescine (Gupta *et al.*, 2013). The polyvalency of PAs, along with the stimulation of their synthesis during low pH, suggests that PAs may aid the cellular charge and pH buffering during K deficiency. The PAs, however, seem to play multiple additional roles in plants, including scavenging of ROS, activation of the antioxidant machinery, alleviation of stress-induced membrane injury and electrolyte leakage (Pottosin *et al.*, 2014).

Tip and marginal chlorosis/necrosis is closely linked to ROS production, or more precisely the handling of ROS in the chloroplasts. Light-exposed chloroplasts are the most potent ROS generating organelles, producing up to 20-fold more ROS than for example mitochondria (Pottosin & Shabala, 2016). During K deficiency, photosynthesis is strongly decreased as a result of reduced stomatal conductance (Fig. 7.2), increased mesophyll resistance (Fig. 7.3) and lowered ribulose bisphosphate carboxylase activity. Furthermore, efficient photosynthesis is dependent on the export and utilisation of photoassimilates, and a typical feature of K deficiency is sucrose accumulation in the leaves (Fig. 7.4), typically accompanied by sucrose deficiency in the roots, which offer an explanation for the decrease in lateral root growth (Cakmak, 2005). K deficiency also leads to reduced sucrose supply to sink tissues like seeds, grains and fruits (Fig. 7.5). The severe reduction in CO_2 fixation caused by K deficiency results in an excess of electrons, ultimately leading to an increase in ROS via an intensified electron transfer to O_2 and ROS production (Fig. 7.6). As a result, K-deficient plants are sensitive to light intensity, which drives the development of K deficiency symptoms (Cakmak, 2005).

K-deficient plants are sensitive to water limitation, and the leaves of affected plants typically appear slack. Low K status negatively effects stomatal closure, as complete closure of stomata requires backpressure from fully turgid epidermal cells in order to close properly (Fig. 7.2). Another reason for the poor control of stomatal closure is increased ethylene production, which interferes with ABA signalling during K deficiency. Potassium deficiency leads to swelling of the pectin matrix in xylem cell walls and a reduction in the porosity of xylem pit membranes, restricting transpiration via a decreased hydraulic conductance (Fig. 7.7) (Anschutz *et al.*, 2014).

VI. Calcium

Calcium is essential for higher plants because it is required as a structural element and plays an essential roles in cellular signalling. The role of Ca in signalling is achieved by transducing, integrating and multiplying incoming signals, thus linking environmental stimuli with physiological responses (Thor, 2019). By far the major part of Ca is present in the cell walls, on the outside of cell membranes and in cell organelles. The concentration of free Ca^{2+}

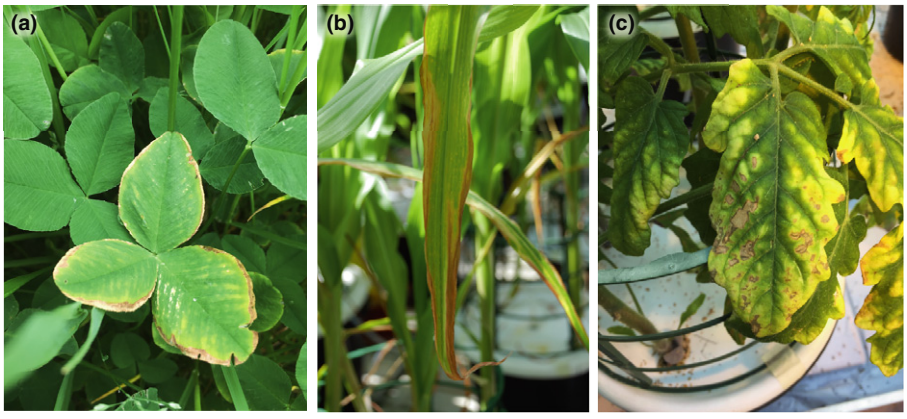
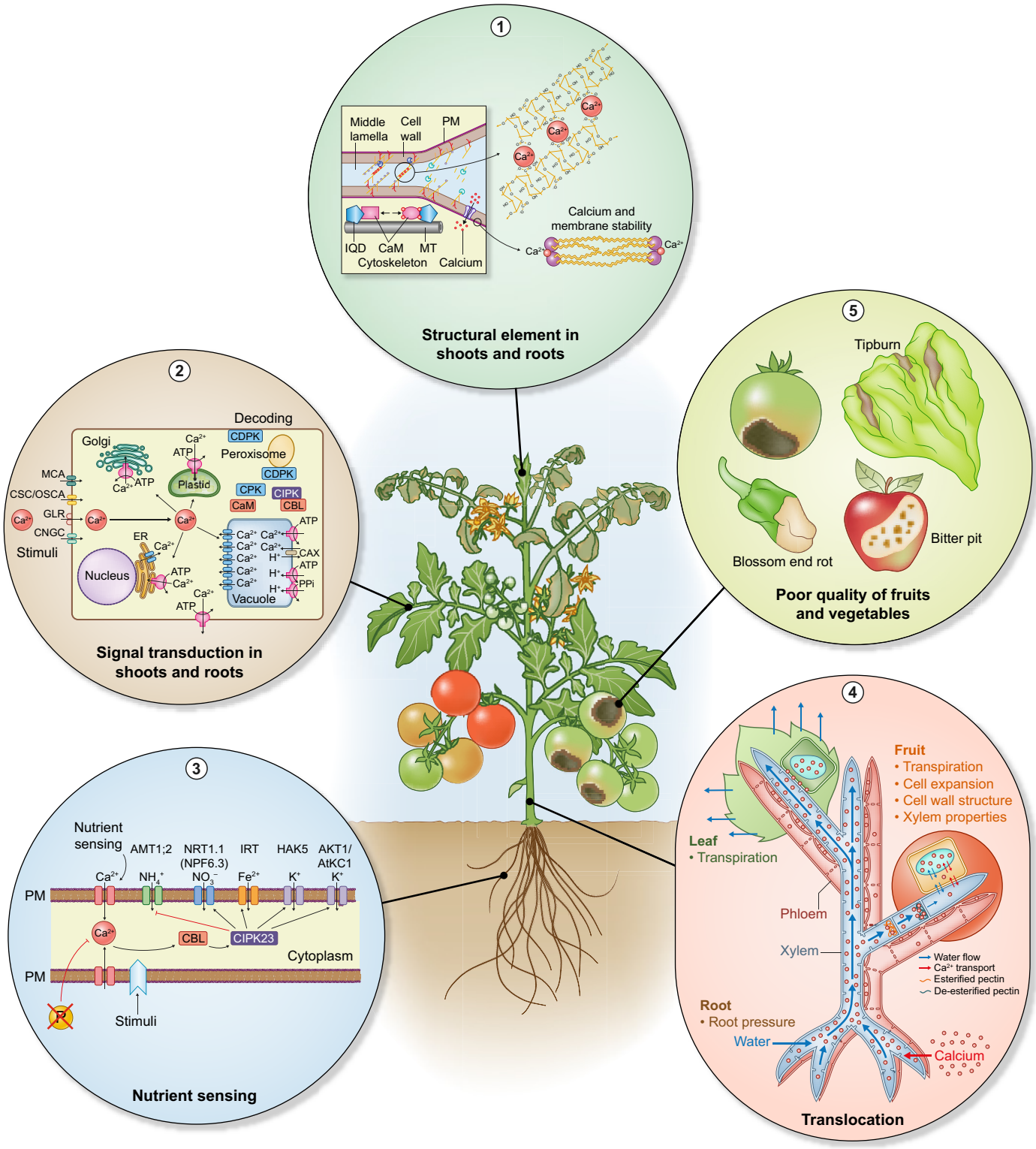


Fig. 8 Potassium (K) deficiency always appears on the oldest leaves first and spreads as a necrosis from the tip along the margins (marginal necrosis) and subsequently appears as intervenous necrotic spots on the entire leaf. (a) Marginal necrosis in white clover. (b) Marginal necrosis in maize. (c) Intervenous necrosis in tomato.



ions in the cytosol has an extremely low (< 0.1 μM) resting value, but with transient spikes. Ca is virtually immobile in the phloem, implying that there is very limited translocation of Ca from old plant parts (source organs) to young leaves, shoots and fruits (sink organs). These organs therefore depend on the xylem for delivery of Ca.

1. The role of Ca as a structural element

The functions of Ca as structural element are primarily related to the fact that Ca confers rigidity to the cell wall system and stabilises it (Fig. 9.1). During the biosynthesis of cell walls, acidic pectin residues (e.g. galacturonic acid) are secreted from the cells as methyl

Fig. 9 Schematic model showing how calcium (Ca) affects plant growth and physiology. (1) Structural element in shoots and roots. Ca is bound to pectic carboxyl groups in the cell wall and to negative charges on cell membranes, thereby stabilising them. Ca also plays an essential role in the regulation of the microtubule (MT) cytoskeleton mediated via the plant-specific IQ67 DOMAIN (IQD) family in interaction with calmodulin (CaM)-dependent Ca signalling. (2) Signal transduction in shoots and roots. Stimuli are perceived via different membrane receptors leading to transient perturbations of the cytosolic free Ca^{2+} concentration via influx mediated by cyclic nucleotide-gated channels (CNGCs), ionotropic glutamate receptor homologues (GLRs), mid1-complementing activity proteins (MCAs), and calcium permeable stress-gated cation channels (CSCs)/reduced hyperosmolarity-induced $[Ca^{2+}]$ increase channels (OSCAs). P-type Ca^{2+}-ATPases mediate Ca^{2+} removal from the cytosol via efflux or via sequestration in different organelles (endoplasmic reticulum, Golgi apparatus and plastids). Ca^{2+} can also be sequestered in the vacuole by Ca^{2+}-ATPases or Ca^{2+}/H^{+} exchangers (CAXs), the latter driven by ATP or pyrophosphate (PPi). Ca^{2+} is released from the vacuole via Ca^{2+} permeable channels. Changes in cytosolic Ca^{2+} trigger a cascade of downstream processes mediated via sensor relays, viz. calmodulins (CaM) and CaM-like proteins, and responders, namely Ca^{2+}-dependent protein kinases (CPKs/CDPKs) and calcineurin B-like (CBL) proteins, which control the activity of CBL-interacting protein kinases (CIPKs). (3) Nutrient sensing. Changes in external nutrient availability affect the cytosolic Ca^{2+} concentration and activate or repress nutrient transporters via the Ca^{2+}-dependent CBL–CIPK pathway with CIPK23 activating transporters/channels for K^{+} (HAK5, AKT1/AtKC1), NO_3^{-} (NRT1.1/NPF6.3) and Fe^{2+} (IRT) upon starvation, while inhibiting NH_4^{+} transport via AMT1;2 in response to high NH_4^{+}. Plant phosphorus starvation may negatively affect the oscillations in cytosolic Ca^{2+} concentration caused by mechanical, salt, osmotic, and oxidative stress (adapted from Kudla *et al*., 2018). (4) Translocation. The Ca concentration in the phloem is very low. Root-to-shoot Ca translocation is therefore determined by the xylem Ca concentration and xylem sap flow related to transpiration and root pressure. With respect to fruits, Ca import is controlled by xylem sap inflow as determined by fruit water uptake and loss driven by cell expansion and transpiration. Xylem sap flow into fruits is affected by xylem vessel development, vessel diameter and connectivity. Ca is bound in the cell walls of xylem vessels and also in intervessel pits, where de-esterification of pectin enables formation of Ca cross-linked gels affecting pit porosity, xylem flow and Ca distribution. There may likely also be some phloem Ca transport in fruits where xylem dysfunction occurs early during fruit development. Adapted from Hocking *et al*. (2016). (5) Examples of Ca deficiency symptoms: blossom end rot in tomato and pepper, tipburn in lettuce and bitter bit in apple.

esters, which are subsequently de-esterified by pectin methylesterase, thereby providing carboxyl groups which bind Ca via covalent and ionic bonds (Hocking *et al*., 2016). When associated with Ca, the cell wall system is protected from degradation by polygalacturonases (pectin depolymerase). Dicot plant species have a much higher Ca requirement than monocots due to their higher content of pectate with a higher density of binding sites for Ca^{2+} (White *et al*., 2018). In addition to the cell wall system, Ca^{2+} stabilises cell membranes via the formation of bridges with phosphate and carboxylate groups in lipids and proteins (Fig. 9.1). Calcium deficiency therefore leads to increased plasma membrane permeability, resulting in cell death in the apical meristem and growth cessation.

2. Functions of Ca in signalling and nutrient sensing

The function of Ca^{2+} as secondary messenger in signalling processes is mediated via transient perturbations of the cytosolic free Ca^{2+} concentration. Various external factors such as light, temperature, gravity, touch and pathogen infection in combination with internal hormonal signals, are perceived via different membrane receptors, leading to specific spatio-temporal patterns of increasing cytosolic Ca^{2+} concentrations that trigger a cascade of downstream processes (Fig. 9.2). The generation of these Ca^{2+} signals relies on influx of Ca^{2+} via coordinated actions of distinct Ca^{2+} channels (Tang & Luan, 2017; Demidchik *et al*., 2018) (Fig. 9.2). In parallel with the actions of Ca^{2+} influx channels, Ca^{2+} efflux across the plasma membrane and Ca^{2+} sequestration into intracellular organelles are important to maintain the transient nature of the Ca^{2+} signals. P-type Ca^{2+}-ATPases either pump Ca^{2+} out of the cell across the plasma membrane or sequester it into organelles so that Ca^{2+} is removed from the cytosol.

The specific Ca^{2+} signatures formed in response to various environmental stimuli lead in turn to some downstream processes, which amplify and integrate the Ca^{2+} signal via interaction with a number of Ca^{2+}-binding proteins in the cytosol acting as Ca^{2+} sensors (Fig. 9.2) (Bender *et al*., 2018). These sensors can be classified into sensor relays and sensor responders (Kudla *et al*., 2018). Sensor relays include calmodulins (CaMs) and CaM-like proteins (CMLs), which upon Ca^{2+} binding undergo a conformational change that is subsequently relayed to an interacting protein (La Verde *et al*., 2018). Two classes of Ca^{2+} sensor responders have been characterised, namely the Ca^{2+}-dependent protein kinases (CPKs/CDPKs) and the calcineurin B-like (CBL) proteins, which control the activity of CIPKs. Members of the CDPK and CIPK protein families may target receptors responsive to abscisic acid, thereby linking to signal transduction mediated by hormones (Waadt *et al*., 2017; Kudla *et al*., 2018; Vighi *et al*., 2019).

Ca^{2+} plays a central role in nutrient sensing and in adaptation to changes in nutrient status. The link between external nutrient availability and transport activities is constituted by the Ca^{2+}-dependent CBL–CIPK pathway with CIPK23 functioning as a key master regulator of K^{+} and NO_3^{-}, as well as Fe, Mg and NH_4^{+} homeostasis (Fig. 9.3) (Riveras *et al*., 2015; K-h. Liu *et al*., 2017; Kudla *et al*., 2018; X. Wang *et al*., 2018). As also mentioned in Section V, CIPK23 phosphorylates and activates the low-affinity K^{+} transporter AKT1 as well as the high-affinity HAK5 K^{+} transporter. With respect to NO_3^{-} uptake, CIPK-mediated changes in phosphorylation status affect both high- and low-affinity uptake via the membrane-located NO_3^{-} transceptor NRT1.1/NPF6.3. Downstream of NRT1.1/NPF6.3, CPK Ca-dependent kinases may induce NO_3^{-} assimilatory and regulatory genes, thus constituting the molecular link between major players in the NO_3^{-} signalling pathway. CIPK23 also activates Fe transport in *A. thaliana* roots, while inhibiting AMT1;2 (Ammonium transporter 1;2)-mediated NH_4^{+} uptake (Tian *et al*., 2016; Straub *et al*., 2017).

Calcium signalling is not only involved in intracellular regulation, but may also contribute to long-range signal propagation and regulation of physiological responses (Kudla *et al*., 2018). Besides mobile small molecules (e.g. hormones) and macromolecules (e.g. proteins, small RNAs and mRNAs), electrical signals linked to

waves of Ca^{2+} and ROS may traverse the plant and trigger systemic responses (Choi *et al.*, 2016). Over the past decade there has been limited progress in revealing the molecular mechanisms involved in regulating the transport and loading/unloading of Ca^{2+} in the phloem (Fig. 9.4). It needs to be further revealed why Ca^{2+} concentrations generally are low in the phloem and what the physiological implications of high Ca^{2+} would be. Ca^{2+} loading of sieve tubes might occur through plasmodesmata. However, increasing cytoplasmic Ca^{2+} tends to close plasmodesmata and the transport of chelated Ca^{2+} from the symplast of the phloem companion cells through plasmodesmata also seems restricted (Sager & Lee, 2014).

3. Functional properties of Ca in relation to plant growth and development

Proper responses of plants to changes in environmental conditions will, in most cases, require reprogramming of the growth rate as determined by cell division and subsequent cell expansion. The adjustment in growth rate involves dynamic reorganisation of the cytoskeleton, that is the complex network of interlinked protein filaments that extends from the cell nucleus to the cell membrane of all cells. Part of the cytoskeleton consists of microtubules (polymers of tubulin) that provide structure and shape to the cytoplasm, thus coordinating and controlling plant cell shape and cell growth. Ca plays an essential role in the regulation of the microtubule cytoskeleton and Ca^{2+}-mediated regulation of microtubule-associated proteins provides hubs for cross-talk with other signalling pathways (Fig. 9.1) (Kölling *et al.*, 2019). The regulation of cell function, shape and growth has been suggested to occur via the plant-specific IQ67 DOMAIN (IQD) family, which may act as platform proteins for integration of CaM-dependent Ca^{2+}-signalling (Bürstenbinder *et al.*, 2017; Kölling *et al.*, 2019).

Via its role in signalling and stabilisation of cell walls and membranes, Ca plays a crucial role for root growth and adaptation. Starvation of P, but not N, strongly dampens oscillations in cytosolic Ca^{2+} concentration caused by mechanical, salt, osmotic and oxidative stress (Matthus *et al.*, 2019). Gradients and oscillations in Ca^{2+} concentration also play a key role in the growth of root hairs (S. S. Zhang *et al.*, 2017; Kwon *et al.*, 2018) as well as for establishment of symbiotic associations (Miller *et al.*, 2013).

4. Calcium deficiency symptoms linked to functional properties

As Ca in the phloem is maintained at a very low concentrations, different parts of the shoot depend on transpiration or root pressure to provide Ca via the xylem (Fig. 9.4). For organs with low transpiration, such as fruits and internal heart leaves in vegetables, root pressure may be insufficient leading to a range of unique Ca deficiency symptoms (Song *et al.*, 2018). These symptoms reflect the essential role of Ca for proper formation of cell walls and for protecting them from degradation by cell wall-degrading enzymes. Typical foliar symptoms of Ca deficiency are necrotic lesions on leaf margins and tips, brownish leaf veins and leaf deformities (Table 1). In dicots, this leads to cup-shaped leaves while, in grasses, leaf spiralisation is a typical deformity. Upon continued Ca deficiency, apical meristems die.

Special problems with Ca deficiency occur in fruits. Fruit Ca uptake is believed to be determined by the Ca^{2+} concentration in the xylem sap, as well as xylem sap influx, which is related to fruit stomatal density, transpiration, root pressure and growth (Fig. 9.4) (Hocking *et al.*, 2016). However, in many fruits, permanent loss of xylem functionality occurs. Under these circumstances, the phloem may constitute a transport pathway for Ca mobilised from the pedicle (Song *et al.*, 2018).

The deficiency symptoms for Ca are so characteristic that they carry specific names in specific crops (Figs 9.5, 10). Examples are blossom end rot in tomato, pepper and zucchini, in which sunken, necrotic pits appear at the blossom end of the fruits (Djangsou *et al.*, 2019). The leaf edges of cabbage, lettuce and brussels sprouts experiencing Ca deficiency are likely to develop necrotic edges, called tipburn. Lee *et al.* (2016) demonstrated that the relationship between intracellular Ca concentration and tipburn in *Brassica oleracea* differed between genotypes and were correlated with differential expression of a number of transporter and stress response genes. In apple, Ca deficiency is called bitter bit, reflecting that the fruit skin develop pits and that brown spots appear on the skin and/or in flesh, resulting in a bitter taste (Buti *et al.*, 2018; Yu XM *et al.*, 2018). Calcium deficiency in carrot leads to cavity spot, characterised by oval necrotic spots developing into craters. The proportion of Ca pectate in the cell wall has great importance for resistance against fungal and bacterial pathogens and thereby for the storage and postharvest quality of fruits (Fig. 9.5) (Winkler & Knoche, 2019).

VII. Magnesium

Depending on the nutritional status, about 5–25% of Mg in plants is present in the chloroplasts, where Mg constitutes the central element of the tetrapyrrole ring in chlorophyll (Fig. 11.1), fine tuning its electric properties. Mg thus participates in photon capturing and transfer of excitation energy from the LHCs to the PSII reaction core.

Besides the mainly covalent binding in chlorophyll, Mg forms ion bonds with negative charges in the cell walls. Differences in cell wall chemistry and cation exchange capacity of the cell walls of different species results in a distinct stoichiometric relationship between shoot concentrations of Mg and Ca in major groups of angiosperm species (White *et al.*, 2018).

The key functionalities of Mg are mainly related to its strong electropositivity, which provides capacity to interact electrostatically with nucleophilic ligands. The Mg^{2+} ion forms ternary complexes with enzymes, thereby establishing a specific geometry between the enzyme and the substrate and increasing the efficiency of the catalytic reaction (Fig. 11.2). Magnesium ions exhibit fast ligand exchange kinetics, which means they can easily be replaced by other divalent metal ions, including Mn^{2+}, Ca^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+}. Substitution of Mg^{2+} with other ions typically changes the catalytic rate of the enzyme and in many cases it also

Fig. 10 During the vegetative stage, calcium(Ca) deficiency always affects the roots before the shoot. Roots turn brown and the tissue disintegrates. Fruits are also a main target for Ca deficiency. (a) Brown and disintegrating roots in barley. (b) Blossom end rot necrotic plaques in tomato.

changes the functional role of the enzyme. RuBisCo (Ribulose-1,5-bisphosphate carboxylase/oxygenase) represents an illustrative example of this, as differential binding of Mg^{2+} and Mn^{2+} changes both the catalytic rate and the substrate preference of the protein (Schmidt & Husted, 2019). When RuBisCo binds to Mg^{2+}, carboxylation is favoured and proceeds 4–11 times faster than oxygenation, but when RuBisCo binds to Mn^{2+}, carboxylation and oxygenation occur at similar rates. The ability of Mg^{2+} to perform ligand exchange is essential for the coupling of ATP with substrates and enzymes in phosphorylation processes, energy-demanding transport processes and ATP synthesis. An important example is Mg·ATP, which is the substrate for the H^+-ATPase that drives the export of sucrose from the leaf cells into the phloem. This makes Mg^{2+} critical for the phloem loading of sucrose and thus for long-distance transport of photosynthates and carbohydrate partitioning between source and sink tissues (Fig. 11.3) (Tränkner *et al.*, 2018).

1. Molecular and physiological responses to Mg deficiency

Reflecting its importance in many central reactions, cytosolic Mg^{2+} levels are tightly regulated. The concentration of free Mg^{2+} in the cytosol has been estimated to be around 0.4 mM compared with around 4 mM total Mg, the latter including Mg^{2+} complexed with ATP and other ligands. The major family of Mg^{2+} transport proteins in higher plants belongs to homologues of the bacterial CorA Mg^{2+} transporters, the so-called MGTs (Fig. 11.4) (Tang & Luan, 2017). In *A. thaliana*, rice and maize, 10-12 members of the MGT family have been identified (Li *et al.*, 2016; Sun *et al.*, 2017). A higher number of MGT genes, viz. 36, were identified in *B. napus*, clustering in five groups and showing clear homology with MGT genes in *A. thaliana* and rice (Zhang *et al.*, 2019). AtMGT6, and its homologue ZmMGT10 in maize, appear to mediate high-affinity Mg^{2+} uptake, hence is upregulated under Mg-limited conditions. Knockout of AtMGT6 impaired *A. thaliana* growth under Mg-limited conditions (Yan *et al.*, 2018). *AtMGT7* is preferentially expressed in the roots and also important for plant adaptation to low Mg conditions. The double mutant *mgt6 mgt7* showed more severe phenotypes compared with single mutants under both low- and high-Mg conditions, indicating that these two MGT-type transporters play additive roles in controlling plant Mg^{2+} homeostasis under a wide range of external Mg concentrations (Yan *et al.*, 2018). Transgenic *A. thaliana* plants overexpressing *ZmMGT10* exhibited longer root length, higher shoot weight, chlorophyll content and Mg^{2+} uptake under low Mg conditions, thus suggesting that ZmMGT10 is essential for plant growth and development (H. Li *et al.*, 2017).

After uptake from the soil, Mg^{2+} is loaded into the xylem for long-distance transport to the shoots. Mg^{2+} is also mobile in the phloem and therefore easily translocated to fruits, seeds and tubers, as well as remobilised from old to young tissues, the latter promoting reutilisation under Mg-limited conditions. The phloem mobility also implies continuous recycling of Mg^{2+} between shoots and roots. Relevant transporters for these processes have not been identified. AtMGT2 and AtMGT3 are assumed to be involved in Mg^{2+} transport into vacuoles and may be assisted by AtMHX, which is an Mg^{2+}/H^+ exchanger (Tang & Luan, 2017).

In order for Mg to fulfil its role as a central atom in chlorophyll, it needs to be transported into the chloroplasts. In *A. thaliana*, MGT10 is localised in the envelope of chloroplasts and expressed mainly in vascular bundles, where it is required for the transport of Mg^{2+} into the stroma, particularly under high light conditions (Sun *et al.*, 2017). In the chloroplasts, the first step of chlorophyll biosynthesis involves insertion of Mg^{2+} into the porphyrin ring, a process catalysed by Mg chelatase. The Mg-dechelatases NYE1/SGR1 and NYE2/SGR2 (NON-YELLOWING/STAY-GREEN) are responsible for the breakdown of chlorophyll and release of Mg^{2+} (Shimoda *et al.*, 2016). Loss of function of both *NYE1* and *NYE2* resulted in a nearly complete retention of chlorophyll during leaf senescence while at the same time enhancing photooxidative damage to maturing seeds in *A. thaliana* and soybean (Z. Li *et al.*,

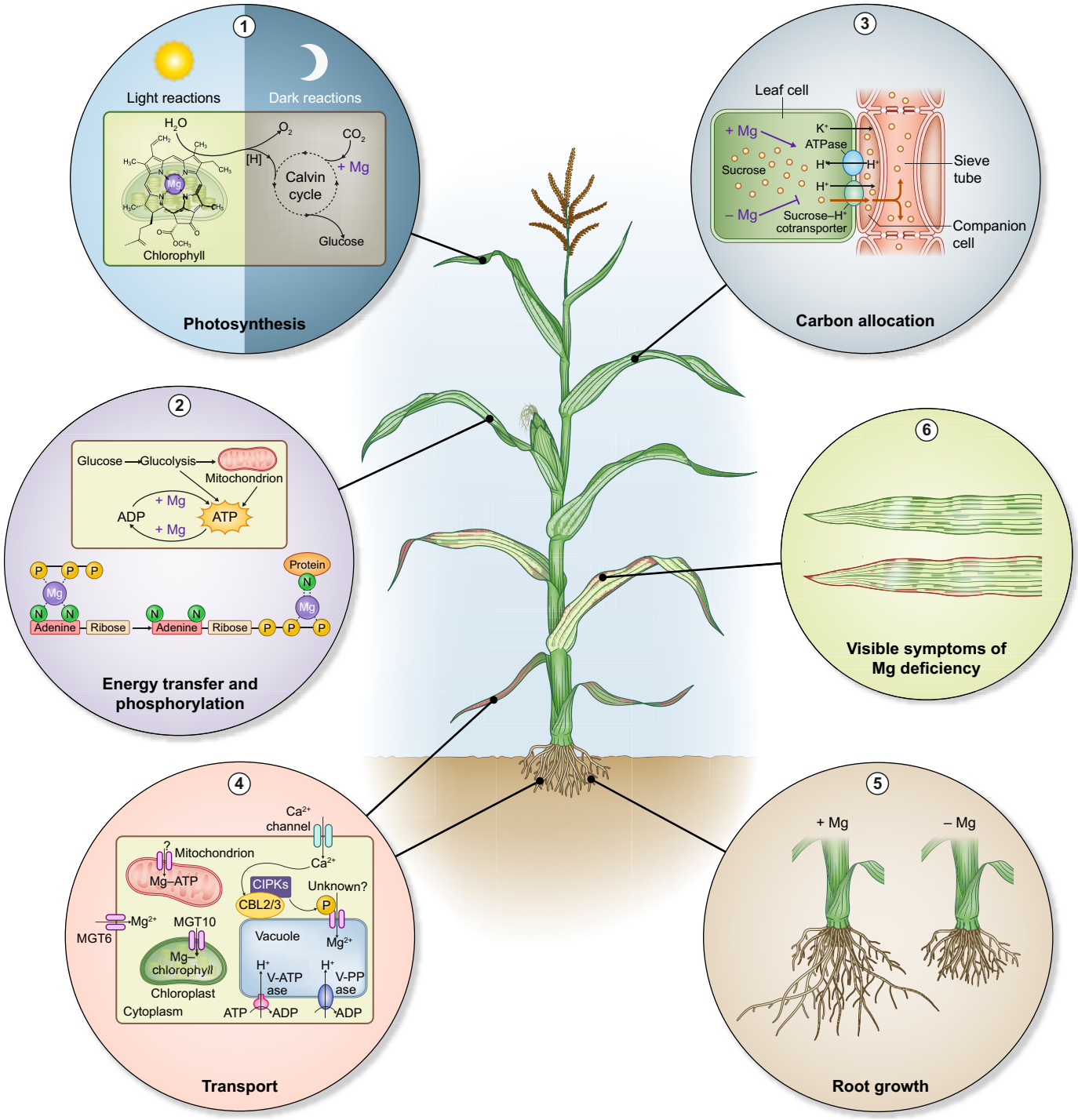


Fig. 11 Schematic model showing how magnesium (Mg) affects plant growth and physiology. (1) Mg is a constituent of chlorophyll and also plays an important role in activation of enzymes, especially RuBisCo, catalysing CO_2 fixation in the Calvin cycle. (2) Mg is involved in a range of phosphorylation processes via creating a ternary complex between ATP, substrate and enzyme. Mg also bridges ADP and ATP synthase, leading to synthesis of ATP. (3) Phloem loading of sucrose is mediated by proton-sucrose transport where Mg couples ATP and the H^+-ATPase. Physiological consequences of Mg deficiency include reduced CO_2 fixation and increased accumulation of sucrose and starch leading to increased production of reactive oxygen species and photo-oxidation of the photosynthetic apparatus. (4) Mg^{2+} transport proteins include homologues of the bacterial CorA Mg^{2+} transporters, the so-called MGTs. In maize, ZmMGT10, a homologue of Arabidopsis AtMGT6, appears to mediate high-affinity Mg^{2+} uptake and is upregulated under Mg limited conditions. Cations such as K^+, NH_4^+, Ca^{2+}, Mn^{2+}, Al^{3+}, H^+ and Na^+ may severely inhibit root uptake of Mg^{2+}, thus leading to deficiency. Adapted from Tang & Luan (2017). (5) Mg deficiency leads to strong inhibition of root growth due to the reduced translocation of photosynthates. (6) Mg is highly phloem mobile and is remobilised from old to young tissues, thus promoting reutilisation under Mg-limited conditions. Visible symptoms of Mg deficiency include chlorosis gradually developing from the tip of fully expanded older leaves. Patches of intervenous chlorosis may appear in different patterns, for example light, irregular transverse bands on the leaves of cereal species, eventually accompanied by necrosis between the leaf veins.

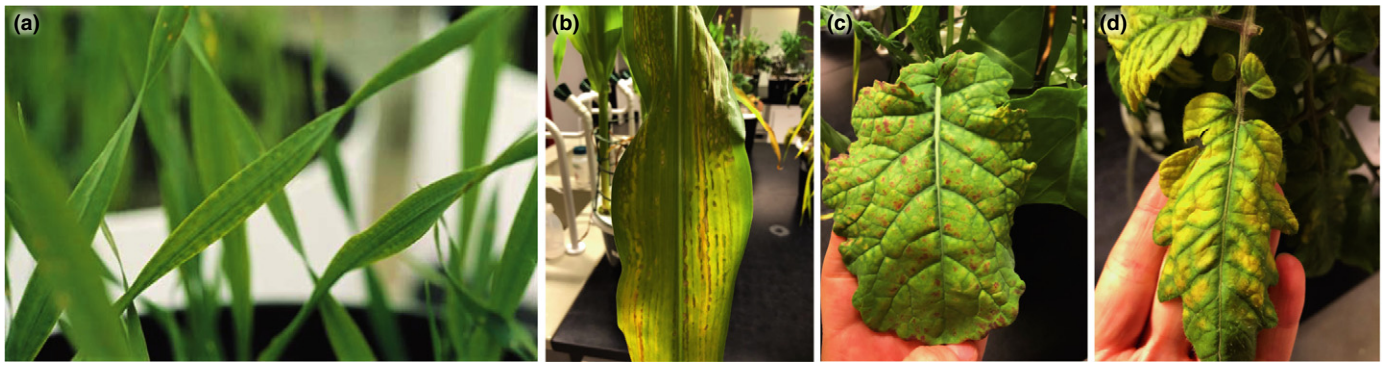


Fig. 12 Magnesium (Mg) deficiency always appears on oldest leaves first and initially as interveinal chlorosis which slowly develops into necrotic plaques. (a) Barley. (b) Maize. (c) Oilseed rape. (d) Tomato.

2017). The Mg dechelatase gene *STAY-GREEN (OsSGR)* is involved in chlorophyll degradation and internal remobilisation of Mg in rice. Under Mg-limited conditions, the expression of *OsSGR* is feedback regulated by ROS, fine tuning chlorophyll degradation in mid-aged leaves with relatively high Mg content and photosynthetic capacity, thereby contributing to photooxidative protection (Peng *et al.*, 2019).

In Mg-deficient plants, photoassimilates accumulate in source leaves before photosynthesis is affected by Mg deficiency. This results in an excessive accumulation of carbohydrates and enhanced production of ROS in the chloroplasts. The impaired allocation of photoassimilates to roots implies a significant reduction of root growth under Mg deficiency (Fig. 11.5). Besides the depletion of carbohydrates, Mg deficiency may increase the production of NO and ethylene in roots, leading to increased root hair development (M. Liu *et al.*, 2017). A recent metabolomics study showed distinct C and N metabolic responses to Mg deficiency in soybean leaves and roots (Yang *et al.*, 2017). Most amino acids showed pronounced accumulation in the leaves, while most organic acids were significantly decreased in the roots.

2. Coupling of visual symptoms and nutrient functionalities

The first visual leaf symptom of Mg deficiency is intervenous chlorosis of fully expanded, older leaves (Table 1; Fig. 12). The chlorotic patches may appear in different patterns, for example forming light, irregular transverse bands on the leaves of cereal species ('tiger striped' leaves). The chlorosis typically develops gradually and intervenously from the tip of fully expanded old leaves and is eventually accompanied by purpling and brown-withering (necrosis) between the leaf nerves (Fig. 11.6). Visual symptoms of Mg deficiency typically first appear on the older leaves (acropetal stratification), reflecting that Mg^{2+} is highly phloem mobile.

At the physiological and biochemical levels, the responses to Mg deficiency include accumulation of sucrose and starch and reduced CO_2 fixation. These changes reflect the essential functions of Mg in chlorophyll biosynthesis (Fig. 11.1), as well as in the export of photosynthates from the chloroplasts and leaf cells (Fig. 11.3). The accumulation of sucrose and starch in the chloroplasts in turn leads to increased production of ROS, photo-oxidation of the photosynthetic apparatus and upregulation of photoprotective mechanisms (Tränkner *et al.*, 2018). An accompanying consequence of the reduced export of photoassimilates is the strong inhibition of root growth (Fig. 11.5). Mg deficiency also causes accumulation of low molecular N compounds, which is due to the essential function of Mg in protein synthesis, where Mg constitutes a bridging element for the aggregation of ribosome subunits.

VIII. Conclusion and perspectives

The macronutrients each have their characteristic deficiency symptoms that can be linked to specific physiological functions. The challenge ahead will be to utilise this mechanistic knowledge to develop sensitive and robust techniques for early diagnosis and correction of nutrient deficiencies. Advancement in imaging technology has greatly accelerated the application of image-based plant nutrition analysis (Li *et al.*, 2020) based on chlorophyll content, foliage reflectance and transmittance (Tan *et al.*, 2018; Zheng *et al.*, 2018). However, interactions between several nutrient deficiencies (Hou *et al*, 2020) and other environmental biotic and abiotic factors may bias imaging techniques. In such cases, diagnostic methods based on a functional role of the nutrient under consideration may potentially be more specific and robust. An example using mechanistic knowledge for diagnostic purposes is the use of time-resolved chlorophyll a fluorescence as a unique tool to monitor bioactive P in plants and to detect latent P deficiency (Frydenvang *et al.*, 2015; Carstensen *et al.*, 2018, 2019). Early detection of nitrogen status in plants using Raman spectroscopy was recently reported by Huang *et al.* (2020). Further developments within these areas may pave the road for a future where crops can be fertilised according to their physiological demand rather than based on the classical practice, where bulk fertilisation often leads to application of either too little or too much, with negative impacts on both environment, climate and the economy of farmers.

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