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Chapter

Ion Homeostasis Response to Nutrient-Deficiency Stress in Plants

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Abstract

A crucial feature of plant performance is its strong dependence on the availability of essential mineral nutrients, affecting multiple vital functions. Indeed, mineral-nutrient deficiency is one of the major stress factors affecting plant growth and development. Thereby, nitrogen and potassium represent the most abundant mineral contributors, critical for plant survival. While studying plant responses to nutrient deficiency, one should keep in mind that mineral nutrients, along with their specific metabolic roles, are directly involved in maintaining cell ion homeostasis, which relies on a finely tuned equilibrium between cytosolic and vacuolar ion pools. Therefore, in this chapter we briefly summarize the role of the ion homeostasis system in cell responses to environmental deficiency of nitrate and potassium ions. Special attention is paid to the implementation of plant responses via NO_3^- and K^+ root transport and regulation of ion distribution in cell compartments. These responses are strongly dependent on plant species, as well as severity and duration of nutrient deficiency.

Keywords: nutrient deficiency, ion homeostasis, nitrate, potassium, ion transport mechanisms, vacuolar and cytosolic ion pools

1. Introduction

Plant growth and development often depend on various biotic and abiotic stressors. In particular, alterations in environmental conditions have a direct impact on the nutrient uptake and assimilation in plants [1]. In this context, a surplus and even more so a deficiency of essential macronutrients in soils represents one of the most common stress types, with nitrogen (N), potassium (K) and phosphorous (P) being the most relevant ones. These macronutrients are directly involved in multiple metabolic pathways and physiological responses, acting as structural constituents of vital metabolites, playing a key role in osmotic regulation and cellular permeability, being critical for proper growth and development [2]. Currently, it is often assumed that due to the existence of fast recycling mechanisms, macronutrient availability does not limit plant growth in natural, uncultivated systems. However, due to a widely spread overexploitation

of soils, it might be the case in the modern agricultural practice [3]. In particular, deficiency of nitrogen (N) and potassium (K) is quite common in developing and least developed countries, especially in rice and wheat production in Asia, Africa and Central and South America [4]. Such nutrient deficiency eventually leads to decrease of plant productivity and losses of crop yields. Visual manifestations of stress caused by a deficiency of individual macro- and microelements are well documented [5]. The underlying key physiological processes, affected by mineral deficiencies, are well characterized and include photosynthesis, protein synthesis, primary and secondary metabolism and carbohydrate distribution between source and sink tissues [6–8].

The methods of biochemistry and molecular biology proved to be efficient in disclosing the fine regulatory mechanisms behind ion homeostasis in plants [8]. Thus, the emergence of RNA microarray technology tremendously contributed to the investigation of rapid transcriptional changes associated with mineral imbalance [9–13]. Most of the studies addressing plant responses of ion-transporting systems to deficiencies of K^+ and NO_3^- rely on *Arabidopsis thaliana* [9, 11–13]. The same is true for phosphorus [14, 15], not further detailed here. However, experiments with such crop plants as wheat [16], tomato [17], rice [18], barley, pepper as well as *Mesembryanthemum crystallinum* [19] revealed a pronounced increase in abundance of K^+ transporter transcripts in response to potassium starvation. Similarly, expression of nitrate transporters in wheat roots and leaves [20, 21], sorghum [22] and rice [23, 24] seedlings was enhanced in response to NO_3^- starvation.

The fact, that all mineral nutrients enter the plant in ionic form and, along with involvement in metabolic processes, are crucial for maintaining the cell ion homeostasis seems to be underestimated. Thereby, the existence of cytosolic and vacuolar ion pools needs to be taken into account. These pools are maintained by numerous membrane ion transporters, representing an integrated part of a complex cellular regulatory network [25]. Hence, the specificity of plant responses to nutritional stress may imply a relevant adjustment of systems involved in absorption, transport, distribution, accumulation and remobilization of mineral ions.

Nitrogen and potassium are the most abundant mineral elements in plant nutrition. The principal features of their reception and distribution in plant organs and cells are well studied to date [2]. On the other hand, a lack of these nutrients in soils (especially in the form of NO_3^- and K^+) is quite a common phenomenon [8] that appears to be an appropriate argument for a more thorough analysis of plant responses to NO_3^- and K^+ deficiency given the role of ion homeostasis system in their development.

2. The role of ion homeostasis in the mechanisms of plant responses to nitrate deficiency

2.1 Nitrate deficiency and its effects on plants

Nitrogen is not only the most abundant mineral element in plants [2], but it also contributes signaling [26] and often plays the role of the limiting nutrient for plant growth [27]. However, in aerobic soils, nitrate (NO_3^-) often represents the predominant source of inorganic nitrogen [28, 29]. However, due to its high solubility in soil water, NO_3^- is a highly mobile ion [30, 31]. Moreover, its distribution in soils is heterogeneous; thereby in temperate regions the concentrations of nitrate in soil solution can vary from a few micromoles in nonagricultural soils to several millimoles after fertilization [32, 33].

Nitrogen is known to be one of the major elements contributing to the structure of organic molecules, including proteins, nucleic acids, cofactors and metabolites [2, 13]. Therefore, a sustainable supply of plants with NO_3^- is critically important for plant survival. Indeed, any limitation of nitrate availability affects plant function at different levels—from cellular metabolism to resource allocation, growth and development [2, 13, 34, 35]. The most pronounced visual sign of nitrogen deficiency is chlorosis of older leaves. Since more than half of the total leaf nitrogen is allocated to the photosynthetic apparatus, nitrate shortage results in a marked decrease in the plant photosynthesis [36]. Due to the activity of nitrogen-fixing and nitrifying bacteria, the complete absence of nitrate in soil is rather unlikely. However, this scenario can be simulated in aqueous culture experiments by exclusion of nitrate or other nitrogen sources transformable to nitrate (e.g. urea and ammonium) from the nutrient solution for several days with subsequent re-supplementation [33, 34]. Such experiments proved to be useful to reveal a wide range of responses and multiple mechanisms of plant adaptation to nitrate stress.

Adaptation of plants to the prevailing soil nitrate conditions rely on systemic response mechanisms, finely tuned during millions years of terrestrial plant evolution [37]. According to Miller and Cramer [38], plants respond to nitrate stimuli by adjusting expression of the genes, involved in nitrate transport and assimilation—that is, nitrate transporters and assimilatory enzymes, in parallel with post-translational modifications of these proteins. The simplest model, explaining nitrate sensing in plant root cells, assumes alteration of cytosolic nitrate concentrations in agreement with its external supply [33]. The authors propose that the cytosol represents a finely regulated ionic environment with precise nitrate homeostasis, that is, even minor changes in cytosolic nitrate levels in response to external alterations in nutrient supply might essentially impact on cell signaling [33].

It is well known that nitrate deficiency is closely related to the plant primary metabolism. For example, in *Arabidopsis* leaves, the plant response to nitrate starvation is manifested with the accumulation of sugars and allocation of carbon to roots. This, in turn, results in enhancement of root growth and triggering branching of the lateral roots [8, 13, 39]. This response is important for establishment of root morphology and is accompanied with an increase of root to shoot biomass ratio and in root absorption capacity [40, 41]. As was shown by the RNA microarray techniques, the observed metabolic shifts were underlied by clearly detectable transcriptional changes, which could be mapped to pathways resulting in accumulation of mono- and oligosaccharides, and starch in shoots [8]. For example, enhanced translocation of sucrose from shoots to the roots might indicate an increase in plant root to shoot biomass ratio [8]. Along with the above mentioned fast transcriptional changes, associated with mineral imbalance, the relationship between the cell genome, metabolome and ionome has also become a subject of a special interest [13, 42]. The ionome is usually defined as the mineral nutrient and trace element composition of an organism and represents the inorganic component of cells and organisms [42]. Significant progress has been made in the field of ionomics in the last decade, in which high-throughput elemental profiling is combined with genetics to identify the genes that control the ionome [43]. Since sugars exert metabolite feedback regulation and affect multiple genes involved in photosynthesis, it can be assumed that photosynthesis suppression in nitrogen-deficient plants represents a direct consequence of sugar accumulation in leaves [8]. On the other hand, it needs to be taken into account that primary metabolites play a crucial role in expression regulation of several genes involved in nitrogen consumption and metabolism [44]. Not less important, nitrate itself is involved in regulation of multiple genes assigned to sugar metabolism [9].

2.2 Regulation of NO_3^- transport and mobilization of NO_3^- storage pools in plant responses to nitrate deficiency

Importantly, root growth and branching in response to nitrate deficiency are accompanied with an increase in the number of nitrate transporters in plant roots [31]. It results in enhancement of nitrate influx due to up-regulation of nitrate transporters in the membranes of root cells. Not less important might be improvement of their biochemical properties and increase of nitrogen utilization efficiency within the plant [31, 45].

During the last two decades, multiple nitrate transporters/channels were identified and comprehensively characterized [31, 41, 46]. These proteins belong to at least four different families: nitrate transporter 1/peptide transporter family (NRT1/NPF), nitrate transporter 2 (NRT2) family, chloride channel (CLC) family and slow anion channel-associated homologs (SLAC/SLAH) [31, 46]. Thereby, the complex processes, underlying NO_3^- uptake, translocation and storage in plants, are controlled by fine regulation and crosstalk between these four main types of transporters [47]. This crosstalk gives plants access to sufficient amounts of bioavailable nitrate under changing levels of environmental nitrogen. Among the listed NO_3^- transporters, NRT1.1 (NPF6.3) is one of the best characterized [47]. It was shown to be a dual-affinity transporter [48] with sensing functions [41, 49]. The activity of this protein is regulated via phosphorylation at the threonine T101 by calcineurin B-like (CBL)-interacting protein kinase (CIPK23) [48]. This phosphorylation results in a shift from a low affinity to a high-affinity NO_3^- uptake kinetics [50] with a K_m of approximately 4 mmol/L for the low-affinity phase of uptake and 50 μmol/L for the high-affinity one.

As far as NRT2 is concerned, it functions as a high-affinity nitrate transporter in association with a smaller protein NAR 2 (NRT3), which facilitates targeting of NRT2 to the plasma membrane [31, 32]. Under a limited access to exogenic nitrate, altogether four NRT2 transporters (NRT2.1, NRT2.2, NRT2.4 and NRT2.5) take up approximately 95% of the total amount of this anion, with NRT2.1 and NRT2.2 being the main contributors [51]. In durum wheat plants, NRT2.5 was shown to be mostly up-regulated under the conditions of NO3$^-$ starvation [20]. In sorghum, N stress caused higher abundance of NRT2.2, NRT2.3, NRT2.5 and NRT2.6 transcripts [22].

It is well known that nitrate, as the principal nitrogen source, can be accumulated in leaves and stems of some crop plants, specifically in the plants belonging to the families Chenopodiaceae, Brassicaceae and Asteraceae where nitrate contents can exceed 300 mmol/kg fresh weight or 17–24% of the plant dry weight [52]. Such a strong accumulation of nitrate can be explained by the existence of a small "metabolic" pool of nitrate accessible to nitrate reductase (NR) in cytosol and a large "storage" pool, compartmentally separated from the sites of its metabolism [53]. Later, the predominant identity of this storage compartment was confirmed as the central vacuole of the leaf cell [54]. Further evaluation of cytosol nitrate concentrations using compartmental analysis and cell fractionation techniques provided a solid evidence for the existence of nitrate homeostasis [55] and supported existence of a variable nitrate pool [56]. It was shown that a nitrate/proton ($2NO_3^-/1H^+$) antiporter AtCLCa, localized in the tonoplast, is expressed in both shoots and roots and mediates nitrate accumulation in the vacuole [57] as the most important requirement for maintaining nitrate homeostasis in cells. The major mechanisms prospectively involved in plant responses to nitrate deficiency are summarized in **Figure 1**.

Due to a pronounced success in isolation of pure fractions of barley leaf vacuole vesicles, the storage nitrate pools were found to range from 58% [58] to 99% of the total cellular nitrate content [54]. Indeed, over 90% volume of a mature plant cell is occupied by the vacuole, while the nitrate concentrations in cytoplasm and vacuole

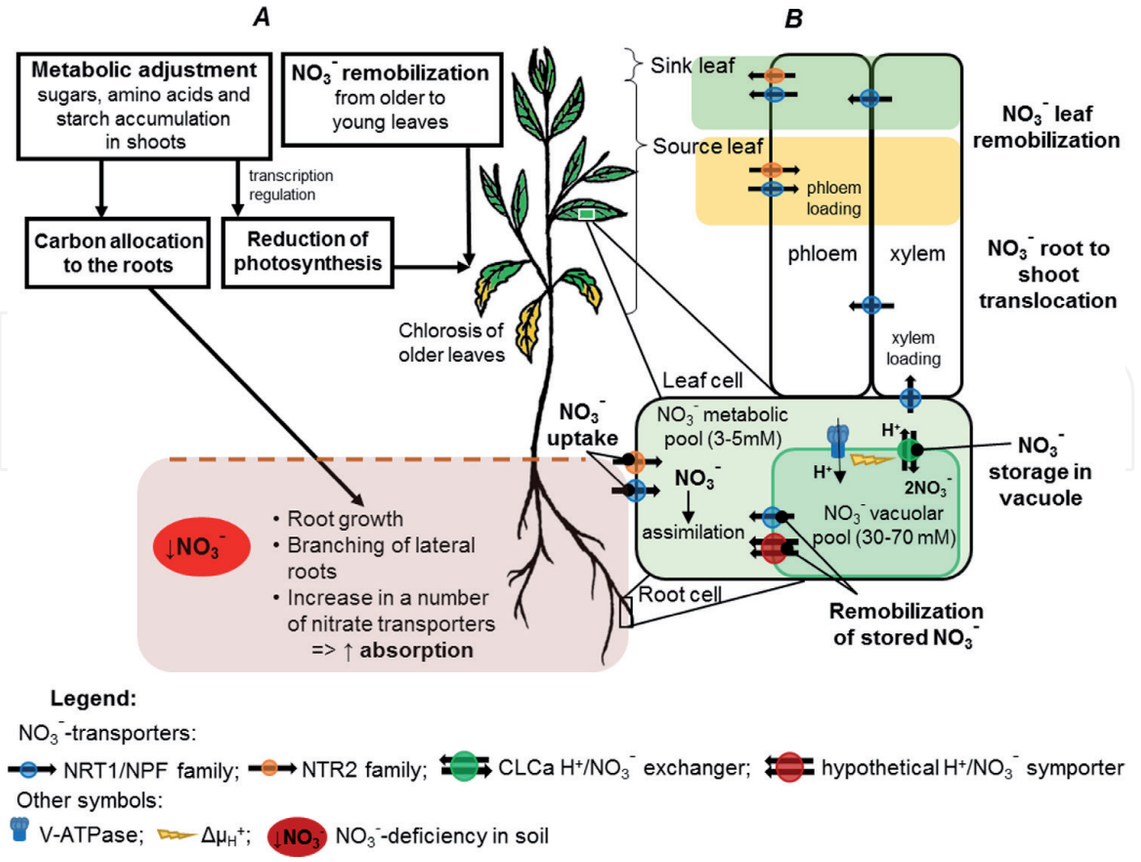

Figure 1.
Schematic view of plant responses to nitrate deficiency. (A) The effect of NO_3^- deficiency on carbon metabolism in plants. The carbon flow from shoots to roots is triggered in response to NO_3^- deficiency in soil, and is underlied by the mechanisms of metabolic adjustment and NO_3^- remobilization. This carbon flow supports root growth and branching, sustaining their adsorption capacity and provides young (sink) leaves with NO_3^-, whereas older (source) leaves undergo chlorophyll degradation. Chlorosis of leaves serves as a key indicator of NO_3^- deficiency. (B) The major transport systems, involved in plant response to NO_3^- deficiency. NO_3^- deficiency results in activation of the plant nitrate transport systems, involved in NO_3^- uptake, accumulation, remobilization in root cell, root cell-to-cell distribution, root to shoot and source to sink leaves translocations.

are generally in the range of 3–5 and 30–70 mmol/L, respectively [52, 59, 60]. For example, when Arabidopsis plants were grown in modified Hoagland solution, containing 4.25 mmol/L NO_3^-, nitrate concentrations in the cytosol of leaf mesophyll cells were 2.8 mmol/L, whereas in vacuoles of the same cells, this value reached 31.8 mmol/L [61]. It is important to notice that nitrate, stored in the vacuole, may be available for assimilation, serving thereby as a reservoir supporting growth for longer periods when external nitrogen supply is limited to only several days [62].

Van der Leij et al. [63] were the first to study the remobilization of vacuolar nitrate in the root cells of barley seedlings during 24 h of nitrate deprivation using double-barrelled nitrate-selective microelectrodes. Their measurements showed much slower remobilization from the vacuoles of cortical cells than those in epidermal cells. The cytosolic nitrate concentrations (activities) in both roots and shoots slowly decreased from 3.7 to 2.9 mmol/L during the first 24 h of the nitrate deprivation, whereas the vacuolar and xylem nitrate activities gradually decreased from 69.6 to 40.2 mmol/L in the same period. In this regard, the authors proposed that the remobilization of vacuolar nitrate can be treated as short term, that is, lasting not more than a few days [63]. It was shown in later studies that nitrate, accumulating in the vacuole, cannot be rapidly transported into the cytoplasm and the transport rates depend on genotypes and differ between individual cultivars [23, 29]. The reuse of NO_3^- in plant tissues to a great extent depends on its transport from the vacuole to the cytoplasm and vice versa [64, 65]. According to Glass et al.

[66], low concentrations of NO_3^- in the cytosol are sufficient to ensure NR activity, whereas relatively high amounts of nitrate in the vacuole only minimally influence the NR activity [66].

Based on the ability to sustain nitrate deficiency, tolerant and sensitive genotypes can be distinguished [51]. At least to some extent, this difference might depend on the rates of nitrate transport across the tonoplast from vacuole into cytoplasm during nitrate starvation and on the ability to restore the vacuolar pool under normal nitrate supply afterwards [52]. Thereby, the tonoplast proton pumps (V-ATPase and V-PPase) play a key role in the distribution of NO_3^- between vacuole and cytoplasm [59] by building an electrochemical transmembrane proton gradient [52, 67]. It was proposed that the NO_3^- transport from the cytoplasm to the vacuole is mainly mediated by the vacuole H^+/ NO_3^- antiport system [68]. Later, it was demonstrated that AtCLCa operates as a NO_3^-/H^+ exchanger with a 2:1 nitrate-proton stoichiometry, building an up to 1:50 nitrate gradient between the two compartments [57]. In this context, the nitrate/proton symport system might be responsible for the remobilization of the vacuolar nitrate [69]. However, the mechanisms behind nitrate efflux from vacuoles to cytoplasm are mostly unknown so far. The tonoplast efflux NO_3^- transporters were not identified as well [31]. However, three members of the NRT1/NPF family, localized in the tonoplast—NPF5.11, NPF5.12 and NPF5.16—have been proposed to be the vacuolar nitrate efflux transporters in Arabidopsis [67]. They are predominantly expressed in the cells of root pericycle and xylem parenchyma, thus acting as important players in modulation of nitrate allocation between roots and shoots and being crucial for plant adaptation to the changing environment [67]. Generally, the vacuole is not only a storage location for nitrate but also impacts on turgor maintenance and osmotic balance [33, 50, 70].

Usually, the highest concentrations of nitrate are found in stems and petioles, whereas the contents of nitrate are lower in leaf blades [50]. According to our own observations, in lettuce and Chinese cabbage, the corresponding differences in nitrate concentrations can reach two- to eightfold under high nitrate supply [71]. In response to a 7-day long nitrate exclusion from the nutrient solution, the nitrate contents decreased primarily in vascular tissues. Essentially this reduction in nitrate contents was accompanied with a pronounced increase in the levels of chloride and anions of organic acids, obviously acting to compensate for nitrate to maintain the required turgor in the vacuoles.

It is logical to assume that the ability of different plants to generate, maintain and mobilize the reserve nitrate pools in vascular tissues is one of the strategies for their survival under adverse conditions of nitrogen deficiency. In particular, nitrate storage pools enable survival of winter cultivars during the periods of nitrate depletion in soils due to intensive rains [32]. This strategy is in agreement with the plant inherited nitrate mobilization and transport from older (source) to younger (sink) leaves during plant ontogenesis or when the external nitrate availability is decreasing [50, 72]. This was confirmed by the results of gene expression analysis. Thus, it was shown that the expression of the AtNRT1.7 gene is induced by nitrogen starvation in the sieve elements/companion cells of the leaf minor veins and facilitates phloem loading in older leaves [73]. Analogously, AtNRT2.4 and AtNRT2.5 are induced by nitrogen starvation and repressed by nitrogen resupply [74]. NRT2.4 is expressed in the phloem of leaf major veins and might retrieve nitrate under nitrogen starvation towards minor veins [75]. In turn, NRT2.5 is expressed in minor veins and, together with NRT2.4, may affect leaf remobilization and phloem transport of nitrate [51]. Moreover, NRT1.11 and NRT1.12, localized in the companion cells of the leaf major veins, were shown to impact on phloem loading, besides playing a role in xylem-to-phloem transfer [76].

Thus, it can be concluded that activation and induction of nitrate transport and mobilization of nitrate from its reserve pools likely are an important component of the cell response to nitrate deficiency.

3. The role of ion homeostasis in the mechanisms of plant responses to potassium deficiency

3.1 Potassium deficiency and its effects on plants

Potassium is the second (after nitrogen) most abundant mineral element in plants [2]. It is the main cation (K^+) in plant cells [77] and is essential for plant growth and adaptation to the environment [78]. In contrast to nitrogen, potassium is not involved in metabolism and remains in ionic form to execute its specific functions in plant cells [79]. Potassium is associated with or directly involved in several physiological processes supporting plant growth and development—photosynthesis, protein and starch biosynthesis; transport of sugars and nutrients; and stomatal closure [77, 80]. Moreover, K^+ was shown to be essential in the activation of 50–60 key enzymes, involved in critical metabolic processes [81, 82], including photosynthesis, oxidative metabolism and protein synthesis [2]. Thus, this cation might be involved in the regulation of metabolite patterns and their relative abundances in higher plants [83].

As mentioned above, the lack of potassium might suppress various enzymatic activities [81, 82], but this can occur only when potassium cytosolic contents decrease due to prolonged K deficiency [81]. Hence, cytosolic K^+ homeostasis is crucial for the central cell metabolism [84], plant growth and adaptation to the environment and must, therefore, be finely controlled [78]. In addition, K^+ plays a crucial role in the establishment of cell turgor and osmoregulation, neutralization/scavenging of anions (e.g. those of organic and inorganic acids), control of cytosolic pH, ion homeostasis and electrical membrane potential [77, 78, 83, 85].

Potassium availability in soil is the main factor, affecting supply of terrestrial plants with this element. Indeed, although potassium is the seventh most abundant element in the Earth crust [86], only a small part of the whole soil potassium pool is present in a form readily available for plants, whereas most of the soil potassium constitutes hardly soluble minerals [87]. Thus, due to the low rates of their solubilization, the concentration of biologically available K^+ in soil solution is rather low and varies between 0.1 and 1.0 mmol/L [82, 88]. Moreover, due to a rapid local depletion at the root surface, in reality these values can be even lower, and supply of the plant with potassium is highly dependent on the rates of its liberation from minerals and transport in soil solution [89]. Both rates are typically relatively slow: growing plants can deplete soil solution to yield potassium concentrations between 1 and 2 μmol/L. On the other hand, this might result in enhancement of potassium release and mobilization in soil [89]. Obviously, the amounts of soil water (i.e. soil water contents) essentially affect the gradients of potassium concentrations on the root surface. Thus, diffusion of K^+ is essentially restricted in dry soil but is significantly increased upon re-supplementation of soil with water [89].

According to the available literature data, under sufficient nutritional supply, the average potassium contents in the most of the plant species vary between 4 and 8% (w/w) in dry matter [84]. However, for multiple other species, the optimal potassium contents are essentially lower and lie in the range of 0.5–2.0% (w/w) [80]. Thus, a comparative study of potassium contents in 14 hydroponically grown plant species (1 mmol/L K^+ in the nutritional solution) revealed potassium contents in the range of 153–274 mmol/kg fresh weight with a drop to 15–53 mmol/kg under a 1000-fold lower potassium supply (1 μmol/L) [90].

In most arable fields, potassium deficiency becomes a limiting factor for sustainable plant growth and development [91]. Therefore, the effects of potassium deficiency on crop plants are intensively studied under hydroponic conditions, which allow reliable defining precise potassium concentrations in nutrient solutions [92]. These experiments revealed the major visual symptoms of potassium deficiency as brown scorching and curling of leaf tips, as well as interveinal chlorosis caused by early chlorophyll degradation induced by ROS generation [92]. At the metabolic level, potassium deficit leads to the accumulation of carbohydrates (mainly sucrose) in leaves [8]. Most likely, this disaccharide plays the role of an osmoprotector, maintaining cell turgor under stressed conditions in plants [6], although some researchers attribute this effect to the enhancement of sucrose export from K^+-deficient leaves [8]. Importantly, in contrast to the conditions of nitrogen deprivation, potassium starvation does not result in any increase of root biomass in terms of an acclimation response to the restriction of potassium supply.

3.2 Regulation of K^+ transport systems and mobilization of vacuolar K^+ pools in plant responses to potassium deficiency

As was unambiguously proven by various analytical techniques, potassium is unequally distributed between different cell compartments, strongly dominating in cytosol and vacuole [80]. Thereby, cytosol and vacuole act as the major depots of potassium in plant cells [93]. This fact is essential for understanding the role of the ion homeostasis system in the mechanisms, underlying cell responses to potassium deficiency. Thus, in barley, the concentrations (activities) of K^+ in cytosol of both root and leaf cells measured with triple-barrelled microelectrodes (recording K^+ activity, pH and membrane potential) typically lie in the range of 100–200 mmol/L [93, 94]. Other methods, like K^+ efflux analysis, X-ray microanalysis or application of fluorescent dyes (reviewed by Britto and Kronzucker [95]) showed a larger range of values (30–320 mmol/L) which was probably due to a strong variation in the supply of potassium in cited experiments. Importantly, in cytosol, K^+ cannot be replaced by other cations, for example, Na^+ [78], that is, it is a specific cytosolic cation. In contrast to the cytosolic pool, the concentration of vacuolar potassium can vary between 10 and 500 mmol/L (i.e. 50-fold), depending on the plant species, cell type and potassium availability in soil [2, 80]. For example, in most glycophytes, it is ranging from approximately 120 mmol/L in root cell vacuoles [93] to 230 mmol/L in the vacuoles of leaf mesophyll cells [94]. In contrast to the cytosol, vacuolar K^+ can be, at least to some extent, replaced by other osmotica (i.e. sucrose, Na^+ or Mg^{2+}) [70, 96]. Vacuolar potassium contents are in a good agreement with the K^+ concentrations in the apoplast, which vary between 10 and 200 mmol/L, sometimes reaching up to 500 mmol/L [83, 97].

The responses of vacuolar and cytosolic K^+ pools to potassium deficiency were intensively studied since the 1980s, when the depletion of vacuolar K^+, accompanied by the accumulation of replacing cations (Na^+ and Mg^{2+}) in the vacuole, was proposed to be the earliest response of the plant cell to potassium starvation [80]. Remarkably, these alterations were not accompanied with significant changes in cytosolic potassium levels, that is, K^+-dependent processes in cytosol remain mostly unaffected. Further, it was proposed that the vacuolar potassium pool can be depleted only to a certain minimal value (10–20 mmol/L) [80]. However, subsequent determination of potassium contents in barley, relying on the measurements with triple-barrelled microelectrodes and a 14-day exposure of plants to 2 μmol/L K^+ in nutrient solution, revealed quite different responses of the vacuolar and cytosolic potassium pools in two types of root cells [93]. On the one hand, potassium concentrations (expressed as activities) demonstrated a concerted

decrease in the vacuole and cytosol from 122 to 124 mmol/L to 10 and 18 mmol/L, respectively. On the other hand, cytosolic concentrations in general showed less of a decline, more pronounced, however, in epidermal (from 81 to 45 mmol/L) than the cortical cells (from 83 to only 67 mmol/L) [93, 98].

Thermodynamic calculations of the cellular potassium homeostasis, performed to explain this phenomenon in cells severely depleted in K^+, clearly indicated the existence of an active transport mechanism for the translocation of K^+ from the vacuole to the cytosol which might rely on a 1:1 H^+:K^+ symport [93]. One also needs to take into account that cortical cells might have a higher capacity for the activation of K^+ influx into the cytosol via the high-affinity K^+ transporter HKT1, which is mostly associated with root cortical cells [16]. This might result in a higher ability of cortical cells to maintain a suitable potassium cytosolic concentration under nutrient starvation conditions. On the other hand, the observed difference can be explained by higher potassium losses via outward-rectifying K^+ channels in epidermal cells [93].

It is well known that cytosolic potassium homeostasis relies on the activities of multiple transport mechanisms, localized in cellular and organelle membranes [75]. The sophisticated network of potassium transport systems, involved in potassium absorption by roots, transport to shoots and further allocation within organs and cells, is the result of millions years of evolution of terrestrial plants [84]. Multiple elements of the potassium transport network were identified as K^+ channels, transporters and their regulators, comprehensively characterized during the last two decades [77, 78, 84, 99–104].

Thus, in Arabidopsis, seven major families of K^+ channels and transporters, comprising in total 75 genes, are known [78, 84]. The most studied and well-characterized families of plant potassium transport systems are (i) Shaker K^+ channel family, represented by voltage-gated channels; (ii) the tandem-pore K^+ (TPK) channels; and (iii) high-affinity K^+/K^+ uptake/K^+ transporter (KUP/HAK/KT) family of high-affinity K^+ transporters [103]. Among the Shaker K^+ channels, Arabidopsis K^+ transporter 1 (AKT1), K^+ Arabidopsis transporters 1 and 2 (KAT1, KAT2) and *Arabidopsis thaliana* K^+ channel 1 (AtKC1) are K^+ inwardly rectifying (Kin) channels. They are activated by membrane hyperpolarization and mediate potassium uptake, whereas stellar K^+ outward-rectifying (SKOR) and guard cell outward-rectifying (GORK) K^+ channels form Kout channels activated by membrane depolarization and mediate potassium release [78, 99, 103]. Finally, *Arabidopsis* K^+ transporter AKT2/3 is a weakly rectifying channel, switching from an inwardly rectifying to a non-rectifying state, mediating both potassium uptake and release depending on the local potassium electrochemical gradient [99, 103]. The Shaker channels are present in all plant organs [102] and ubiquitously expressed in various tissues. Being the main contributors in the potassium membrane fluxes [100, 101], they give access to fast and fine adjustments in K^+ transport in plant cells and for the redistribution of K^+ between distinct plant sections and cellular compartments to match plant demands under challenging environmental conditions [84]. AKT1, expressed predominantly in root epidermal cells [88], has been identified in Arabidopsis as a key K^+ channel protein, involved in potassium uptake under low (nearly 10 μmol/L) soil K^+ concentration [105]. The AtKC1, expressed in root cortex, epidermis and root hairs [106], is thought to be rather a regulatory subunit that does not form an own functional channel but interacts with AKT1 to form a functional heterotetrameric channel, which prevents AKT1-mediated K^+ loss under potassium starvation conditions [107]. It was shown that SKOR mediates the transfer of potassium to xylem sap for further transport to shoots [78]. Analogously, AKT2 substantially contributes to the phloem K^+ loading and unloading for long-distance potassium transport from sources to sinks [78].

The biological roles of the plant TPK K^+-selective channels are much less understood than those of the Shakers [78]. The representatives of the TPK family differ by their intracellular localization, while TPK4 is targeted to the plasma membrane; the other family members—TPK1, 2, 3 and 5—are located in organelle membranes [108]. Among them, due to its high selectivity, TPK1 appears to be mostly involved in the response to K^+ deficiency [109]. This protein is ubiquitously expressed in the tonoplast of guard and mesophyll cells and seems to impact on the vacuolar release of K^+ and on intracellular K^+ homeostasis [110].

The high-affinity K^+ transporters from the KUP/HAK/KT family are directly involved in maintaining the constant influx of K^+ in plant roots under severe potassium deficiency [78, 102]. The best characterized transporters of this group are AtHAK5 from Arabidopsis and its homologs in other species, which are assumed to be involved in K^+ uptake from very dilute potassium soil solutions, in co-transport with protons [103, 111, 112]. Besides this, AtKUP1 is described as a dual-affinity transporter in the plasma membrane of root cells and assumed to be essential in K^+ uptake [113], whereas AtKUP 2, 4, 6 and 8 are supposed to mediate K^+ efflux in root cells [78]. Similarly to HAK5, its homologs in other plant species (rice, barley, pepper, tomato) are highly up-regulated by potassium starvation [103]. It is generally recognized that HAK5 and AKT1 are the two main players in K^+ uptake from the soils, characterized with potassium shortage [102, 114, 115]. Indeed, the studies with T-DNA insertion lines clearly demonstrated that AtHAK5 is the only system mediating K^+ uptake at external potassium concentrations below 10 μmol/L [116], whereas both AtHAK5 and AKT1 systems contribute to K^+ absorption, when potassium concentrations are in the range of 10–200 μmol/L [117]. In particular, the uptake of K^+ in AtHAK5 AKT1 double mutant plants under potassium starvation conditions was reduced by 85% in comparison to the wild-type plants [115].

KUPs are found in plasma membrane and organelle membranes, and, in addition to K^+ uptake from soil, they are involved in K^+ homeostasis, long-distance K^+ transport, cell elongation, response to osmotic stress and even in the regulation of auxin transport [111]. Recently AtKUP7 in Arabidopsis roots was shown to contribute to K^+ uptake and K^+ efflux to the xylem especially under limited access to potassium [118]. The schematic view of the mechanisms behind the plant responses to potassium deficiency is shown in **Figure 2**. Less studied are high-affinity K^+ and Na^+ transporters from the HKT family. These proteins are expressed exclusively in the tonoplast. However, K^+-transporting members of the family seem to be present only in monocots [104]. This family is poorly characterized with regard to post-translational regulation, although some of its representatives are definitely involved in the control of K^+ homeostasis [78, 119].

Recently, elucidation of the mechanisms underlying the regulation of potassium transport in response to potassium starvation stress became a new focus of research, especially those acting both on the transcription and post-transcription levels [77, 102]. Various signaling cascades, enhancing transport of K^+, triggered by potassium starvation, might rely on reactive oxygen species, phytohormones, calcium and phosphatidic acid [8, 77]. Among the mentioned regulatory pathways, involved in the response to potassium deficiency, due to its spatial and temporal specificity, calcium signaling seems to be the most important one [77, 120]. In response to K^+ deprivation, the intracellular calcium levels change that affects peptide calcium sensors CBL1 and CBL9, localized in the plasma membrane (PM) [102]. The affected peptides interact with the cytoplasm-localized protein kinase CIPK23, and the formed complex is recruited to the root cell plasma membrane where it activates the AKT1 channel protein via phosphorylation [77, 121, 122] that results in enhancement of AKT1-mediated root uptake of K^+. Besides, AtKC1 as a channel regulatory subunit interacts with AtAKT1, forming an AtAKT1-AtKC1

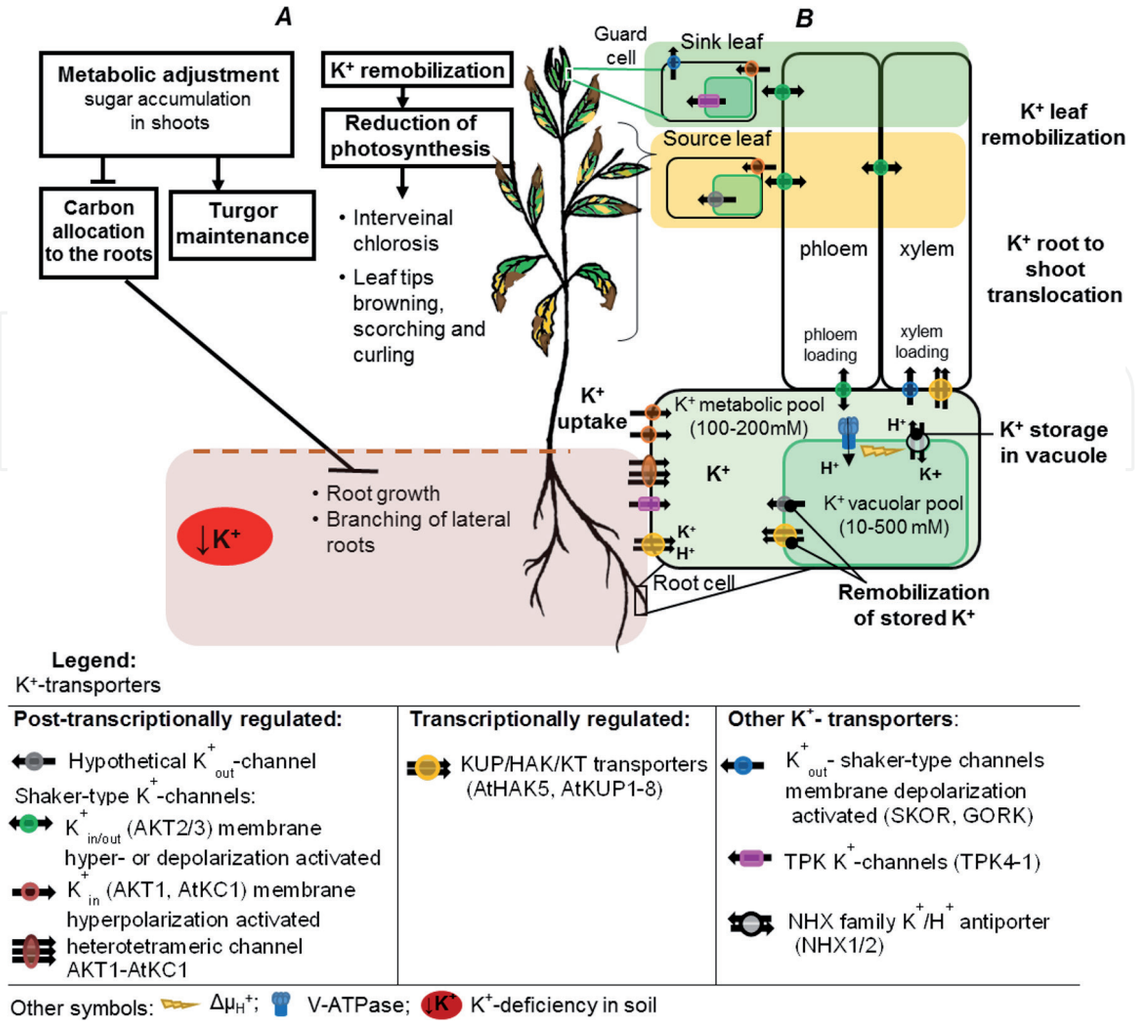

Figure 2.
Schematic view of plant responses to potassium deficiency. (A) The effect of K^+ deficiency on carbon metabolism in plants. K^+ deficiency in soil triggers metabolic adjustment in plant tissues to maintain cell turgor. However, in contrast to the NO_3^- deficiency response, this adjustment does not affect the carbon flow from shoots to roots and therefore does not influence root growth and branching. In source leaves, remobilization of K^+ results in suppression of photosynthesis, inter-veinal chlorosis and tip browning of leaves. Scorching and curling serve as the most prominent indicators of K^+ deficiency. (B) The major transport systems, involved in plant response to K^+ deficiency, that is, contributing in K^+ uptake, accumulation and remobilization in root cell, as well as distribution between root cells, translocation from root to shoot and from source to sink leaves, control of stomata guard cells.

heterotetrameric channel [123]. The regulatory subunit modulates the activity of AtAKT1 together with AtCIPK23 in a synergistic way, coordinating AtAKT1-mediated low-potassium stress responses [123, 124]. Another proposed mechanism relies on the calcium sensor CBL4, acting together with the protein kinase CIPK6, modulating activity and PM localization of the weak inward-rectifying K^+ channel AKT2 in Arabidopsis [125]. Thereby, CBL4 mediates translocation from the endoplasmic reticulum membrane to PM and enhances AKT2 activity.

There are indications that the calcium sensors CBL3 and CIPK9 work together and impact on K^+ homeostasis under low (100 μmol/L) potassium nutritional stress via the regulation of putative outward K^+ channels, localized in tonoplast [97]. However, other authors assume that CIPK9 is more likely involved in K^+ reallocation from older to the younger leaves under the conditions of potassium deficiency [126, 127]. It was also proposed that the AtCBL1/AtCIPK23 complex can phosphorylate the AtHAK5 transporter, which belongs to the KUP/HAK/KT family and expressed mainly in roots [128]. However, in contrast to AKT1, transcriptional regulation of the K^+ transporters seems to be more important for the adaptive response to potassium deficiency than

post-translational modification of K^+ channels [102, 129, 130]. Potassium starvation was shown to increase the abundance of HAK transcripts in a wide variety of plants, including barley, rice, *Arabidopsis thaliana*, *Solanum lycopersicum* and some others [19]. Thereby, the mRNA levels of HAK1-type genes were most remarkably increased [17, 105, 129]. The mechanisms, underlying expressional regulation of the HAK1-type genes might rely on alterations of membrane potential, as well as to reactive oxygen species (ROS) and to hormone-mediated signaling [105]. Recently, it was shown that transcription of AtHAK5 in *A. thaliana* roots can be induced by low-potassium nutritional stress via the transcription factor RAP2.11 that directly binds to the promoter region of *AtHAK5* and may be involved in the low-potassium signaling pathway [88]. The HAK/KUP/KT transporters were proposed to act as K^+-H^+ symporters in the tonoplast and might mobilize K^+ from the vacuole under potassium deficiency conditions [131]. Indeed, in *Arabidopsis thaliana*, OsHAK10 and five members of the KUP family have been found to be localized in the tonoplast [19] that supports the above functional assumption.

Thus, numerous studies in the field of K^+ membrane transport and intracellular potassium distribution in plants with regard to the changes in the availability of potassium in the environment indicate that the mechanisms supporting ion homeostasis of the plant cell might be involved in plant responses to potassium deficiency to ensure stress adaptation.

4. Conclusion

Nutrient deficiency, including moderate or severe shortages of NO_3^- and K^+ in soils, represents a serious challenge to modern agriculture, negatively affecting plant productivity and crop yields. Fortunately, plants possess an array of finely tuned mechanisms of nutritional stress adjustment and maintenance of cell ion homeostasis. Hence, the plant response to nitrate and potassium deficiency relies both on transcriptional and post-transcriptional regulation of high-affinity NO_3^- and K^+ membrane transport mechanisms, impacting on the increase of abundance and activity of transporters. Importantly, this includes not only an increase in the activity of root ion carriers but also mobilization of NO_3^- and K^+ from their storage vacuolar pools and subsequent redistribution to the metabolic cytosolic pool. In general, these data indicate that the ion homeostasis system plays an important role in plant cell responses to nutrient deficiency. Since these responses depend on plant taxonomy and duration of K^+ or NO_3^- shortage, these studies need to be extended to a broad selection of crop plants. To characterize the adaptive potential of these plants, various exposure times need to be addressed.

Currently, proteomics and metabolomics studies, aiming to improve stress tolerance in crop plants, became mainstream in the study of K^+ or NO_3^- starvation. The use of these techniques in research on nutrient stresses seems to be promising. Indeed, these approaches deliver valuable information about the accumulation of important secondary metabolites in plants under different types of environmental stresses.

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Abbreviations

AKT	Arabidopsis K^+ transporter
AtAKT	*Arabidopsis thaliana* Arabidopsis K^+ transporter
AtCLCa	*Arabidopsis thaliana* chloride channel a
AtHAK	*Arabidopsis thaliana* high-affinity K^+ transporter family
AtKC	*Arabidopsis thaliana* K^+ channel
AtKUP	*Arabidopsis thaliana* K^+ uptake transporter family
CBL	calcineurin B-like
CIPK	calcineurin B-like-interacting protein kinase
CLC	chloride channel family
GORK	guard cell outward-rectifying K^+ channel
HAK/KUP/KT	high-affinity K^+/K^+ uptake/K^+ transporter family
HKT	high-affinity K^+ transporter
K	potassium
KAT	K^+ Arabidopsis transporter
K_m	Michaelis-Menten constant
N	nitrogen
NAR	nitrate transporter-activating protein
NO_3^-	nitrate
NR	nitrate reductase
NRT	nitrate transporter
NRT/NPF	nitrate transporter/peptide transporter
OsHAK	*Oryza sativa* high-affinity K^+ transporter family
P	phosphorous
PM	plasma membrane
RNA	ribonucleic acid
ROS	reactive oxygen species
SKOR	stelar K^+ outward-rectifying K^+ channels
SLAC/SLAH	slow anion channel-associated homologs
T-DNA	transfer deoxyribonucleic acid
TPK	tandem-pore K^+ channel
V-ATPase	the vacuolar H^+-translocating adenosine triphosphatase
V-PPase	the vacuolar H^+-pumping pyrophosphatase

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