What is the role of putrescine accumulated under potassium deficiency?

Jing Cui[1], Igor Pottosin[2], Emmanuelle Lamade[3] & Guillaume Tcherkez[1*]

1. Research School of Biology, ANU Joint College of Sciences, Australian National University, 2601 Canberra ACT, Australia.
2. Biomedical Centre, University of Colima, 28045, Colima, Mexico.
3. UPR34 Performance des systèmes de culture des plantes pérennes, Département PERSYST, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), 34398 Montpellier, France.

*Contact author to whom correspondence should be addressed: guillaume.tcherkez@anu.edu.au

Short title: Putrescine roles under K^+ deficiency

Keywords: polyamines, potassium, deficiency, putrescine, ion balance

Twitter account: @IsoSeed

ORCID account: G. Tcherkez: https://orcid.org/0000-0002-3339-956X
Corresponding author
Guillaume Tcherkez
Research School of Biological Sciences
Australian National University
2601 Canberra ACT, Australia
guillaume.tcherkez@anu.edu.au

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pce.13740

Abstract (178 words)

Biomarker metabolites are of increasing interest in crops since they open avenues for precision agriculture, whereby nutritional needs and stresses can be monitored optimally. Putrescine has the potential to be a useful biomarker to reveal potassium (K^+) deficiency. In fact, although this diamine has also been observed to increase during other stresses such as drought, cold or heavy metals, respective changes are comparably low. Due to its multifaceted biochemical properties, several roles for putrescine under K^+ deficiency have been suggested, such as cation balance, antioxidant, reactive oxygen species (ROS) mediated signaling, osmolyte, or pH regulator. However, the specific association of putrescine build-up with low K^+ availability in plants remains poorly understood, and possible regulatory roles must be consistent with putrescine concentration found in plant tissues. We hypothesize that massive increase of putrescine upon K^+ starvation plays an adaptive role. A distinction of putrescine function from that of other polyamines (spermine, spermidine) may be based either on its specificity or (which is probably more relevant under K^+ deficiency) on a very high attainable concentration of putrescine, which far exceeds those for spermidine and spermine. Excessive putrescine and its catabolites appear to possess a strong potential in controlling cellular K^+ and Ca^{2+}, and mitochondria and chloroplasts bioenergetics under K^+ stress.

Introduction

Putrescine, spermine and spermidine are dominant polyamine species, naturally found in all organisms. It is now more than 65 years since putrescine was found to accumulate under K^+ deficiency in plants (Richards & Coleman, 1952; Coleman & Richards, 1956; Coleman & Hegartv, 1957). In fact, when K^+ availability is low or very low in the nutrient solution or in soil, putrescine accumulates in several parts of the plant, particularly in leaves, to levels that can be up to 150 times higher than the normal content under K^+-sufficient conditions. As such, putrescine is one of the first metabolic biomarker that has been discovered in the history of plant physiology.

Biomarker metabolites that are tractable using metabolomics are of potential importance in crop management, not only to follow developmental stages but also to monitor disease progression, nutritional needs or abiotic stresses (for a recent review, see (Alexandersson *et al.*, 2014)). Here, putrescine is an interesting candidate to detect K^+ deficiency situations, as suggested back in the 80s (Smith, 1984). Leaf metabolic biomarkers would be extremely useful to adjust cropping practices and in particular, K^+ fertilization. In effect, the simple measurement of K^+ levels in leaves can be insufficient to characterize the ion status of crops and thus to detect K^+ deficiency. This is typically the case in oil palm (*Elaeis guineensis*, a high K^+-demanding species) where variations in leaf potassium elemental content are relatively small even though K^+ availability may vary widely.

Putrescine is synthesized from ornithine, either via the direct route involving ornithine decarboxylase (ODC) or the indirect route that involves arginine decarboxylase (ADC) (Fig. 1) (Slocum, 2005). Metabolomics studies on *Arabidopsis* have suggested that putrescine and ornithine are positively correlated with growth (Meyer *et al.*, 2007) and ADC activity is essential for root growth (Watson *et al.*, 1998). In tobacco, the ODC pathway is believed to be related to growth and proliferation, whereas the ADC pathway seems to be associated with morphogenesis and stress response (Masgrau *et al.*, 1997). However, the putrescine biosynthetic pathway depends on the plant species. For example, *Arabidopsis* lacks ornithine decarboxylase and thus synthesize putrescine from arginine only (Hanfrey *et al.*, 2001). Most other species have both enzymes, with varying proportions of the biochemical route used. For example, in oil palm there is a quantitative decrease in ornithine with no appearance of intermediates (like arginine) when putrescine accumulates (Fig. S1a), suggesting that the direct route is used. Similarly, in sunflower leaves, both ornithine and putrescine accumulate under K^+ deficiency (Fig. S1b) and putrescine generally anticorrelates with arginine (Fig. S2), suggesting a competition between arginine and putrescine synthesis from ornithine and therefore, the direct route of putrescine biosynthesis. In response to low K^+ conditions, Poaceae generally synthesize putrescine via arginine decarboxylase (see, e.g., (Young & Galston, 1984)).

Despite its widespread accumulation (Table 1), the precise role played by putrescine under K^+ deficiency remains somewhat enigmatic. There are several reasons to explain this limitation. First, several biochemical roles are in principle possible (described below). Second, putrescine (as other polyamines) has been found to accumulate (although to a lower extent and less systematically) under stress conditions other than K^+ deficiency, suggesting it

is part of a more general stress response (Table 1). Third, putrescine accumulation is metabolically "expensive" because it requires ATP, redox power (NADPH) and assimilated nitrogen (that might be limiting under K^+ deficiency because of altered nitrate circulation). Using the direct route, the overall equation gives:

$$2 \text{ glutamate} + \text{ATP} + \text{NADPH} \rightarrow \text{putrescine} + CO_2 + 2\text{OG} + \text{NADP} + \text{ADP} + \text{Pi}$$

where 2OG stands for 2-oxoglutarate. The overall equation with the indirect route is even more expensive in terms of consumed ATP, as follows (assuming that fumarate is recycled via NAD-dependent malate dehydrogenase and that carbamoyl phosphate is synthesized de novo):

$$2 \text{ glutamate} + 4 \text{ ATP} + \text{NADPH} + \text{NAD} \rightarrow \text{putrescine} + CO_2 + 2\text{OG} + \text{NADP} + \text{NADH} + 4 (\text{ADP} + \text{Pi})$$

Considering such an energy requirement, the function of putrescine should be of considerable importance. In this short review, we briefly describe possible roles of putrescine, and summarize data that help defining most likely and specific roles of putrescine under K^+ deficiency.

Is putrescine a versatile biomarker of K deficiency?

Putrescine accumulates under K^+ deficiency up to the 1-10 mM range, with an increase up to by two orders of magnitude as compared to its level at optimal K^+ (Table 1). For instance, in oil palm putrescine concentration is *ca*. 60 µM at high K^+ and 1.8 mM at low K^+ (i.e., ≈ 7 µmol g DW^{-1}; Fig. S1) (Cui *et al.*, 2019b). Conversely, high (>10 mM) external K^+ causes a decrease in putrescine content, which is converted to "higher polyamines" (this term refers to higher molecular weight polyamines synthesized from putrescine, such as spermine and spermidine) (Aurisano *et al.*, 1993; Reggiani *et al.*, 1993) and/or putrescine extrusion (Tamai *et al.*, 2000). Thus, putrescine metabolism is sensitive to external K^+, but the underlying mechanism is still unknown. It might be speculated that the increase in putrescine content at low K^+ is caused by the stimulation of ammonium assimilation (see high NH_4^+ conditions in Table 1), which has indeed been observed in *Arabidopsis* (Armengaud *et al.*, 2009). Regardless of the underlying metabolic cause for its accumulation, putrescine seems to be a good low-K^+ biomarker in the bio-statistical sense since its increase is highly significant (order of magnitude of the *P*-value far below that of many other metabolites changed by low K^+) and it has a very high weight (loading) in multivariate analyses. Therefore, it might be used as an index for K^+ availability (Cui *et al.*, 2019a; Cui *et al.*, 2019b).

Nevertheless, putrescine also may accumulate under other conditions, such as low pH, anoxia, heavy metals, low Mg^{2+}, cold or osmotic stress. In half of cases, putrescine has been found to decrease under salt stress (Table 1) and to confer no specific advantage for NaCl tolerance when applied exogenously (Ndayiragije & Lutts, 2006). Polyamines other than putrescine (spermine, spermidine) may also accumulate under K^+ deficiency although not to the same extent and can even decrease (for an example in *Arabidopsis*, see (Watson & Malmberg, 1996); see also Fig. S2 where neither spermine nor spermidine appear in significant metabolites). In fact, the biosynthesis of spermine and spermidine requires S-

adenosyl methioninamine (SAE, Fig. 1), which is produced from S-adenosyl methionine (SAM) decarboxylation. SAM synthetase requires K^+ as a cofactor (Takusagawa *et al.*, 1996) and therefore its activity is probably very limited under K^+ deficiency, thereby impacting not only polyamines, but also all cellular reactions that use SAM as a methyl donor. Also, in plants, it is remarkable that putrescine is not an effector of SAM decarboxylase activity (contrary to its mammalian counterpart) (Bennett *et al.*, 2002) thereby allowing putrescine accumulation without stimulation of SAE (and thus spermine and spermidine) production. Phosphate deprivation has also been reported to trigger putrescine build-up (Knobloch & Berlin, 1981; Shih & Kao, 1996). However, in (Shih & Kao, 1996) phosphate abstraction from the medium seems to have been done by withdrawing potassium phosphate from the nutrient solution, meaning that the build-up of putrescine was in fact coupled to K^+ deficiency.

One molecule, too many roles?

Plant polyamines have been studied for a long time and quite understandably, the literature on polyamines in plant physiology is now considerable. Taken as a whole, polyamines are believed to be of importance under stressful conditions and to play a signaling role during plant development (Galston & Sawhney, 1990; Alcázar *et al.*, 2006; Tiburcio *et al.*, 2014). Historically, putrescine has been suggested to play a role of *(i)* a cation to substitute K^+, *(ii)* an antioxidant and/or a ROS-mediated signal (via oxidation), *(iii)* an osmolyte under salt or osmotic stress, *(iv)* a root-shoot transport molecule (either as a nitrogen-containing metabolite or a cation), and *(v)* a cryoprotectant at low temperature. However, ionomics analyses have shown that when compared to other cations, putrescine represents a small pool (< 5%) of positive charges (Fig. 2), so its role in charge balance is minor and the same is true for its role in osmoprotection. The role of antioxidant, although widely supported experimentally, seems to depend on concentration and conditions, since there are examples where polyamine addition may trigger oxidative stress (Mohapatra *et al.*, 2009) and polyamine catabolism is indeed an important source of hydrogen peroxide and other ROS species, especially under stress conditions (Moschou *et al.*, 2008; Pottosin *et al.*, 2014b; Wang *et al.*, 2019) (see also *Concurrent effects of putrescine* below). In the next sections, we focus on roles of putrescine, as compared to higher polyamines, in the regulation of K^+ acquisition and re-distribution, Ca^{2+} signaling, and chloroplast and mitochondrion functions.

Putrescine and regulation of cation transport and balance

Consequences of K^+ deficiency for ion composition

K^+ deficiency is not associated with a general decrease, but actually leads to a significant increase in cation load (Fig. 2, insets). That is, quite counter-intuitively, K^+ deficiency implies an extra demand in negative charges to reach electro-neutrality, which is met by accumulated organic and amino acids (Armengaud *et al.*, 2009). The excess of positive charges mostly comes from the considerable increase in Ca^{2+} (up to 2-fold increase) and Mg^{2+} (more than 2-fold) in oil palm and sunflower (Cui *et al.*, 2019a; Cui *et al.*, 2019b). Under K^+ deficiency, there is also an increase in the difference between Ca^{2+} and the sum $Mg^{2+} + K^+$ (of about 0.4 mmol positive charges g^{-1} DW in Fig. 2). In general, there is a well-supported negative

relationship between K^+ and Ca^{2+}, which has been documented for nearly 50 years in herbaceous crops (such as sunflower, rapeseed, tobacco, or wheat). This is here examplified in oil palm, cultivated under varying K^+ fertilization (Fig. S3). Similarly, in other species such as castor bean, K deficiency causes an increase in Ca^{2+} and Mg^{2+}, and a slight decrease in Na^+ in leaf lamina, but conversely a considerable increase in Na^+ with little change in Ca^{2+} and Mg^{2+} in petioles and phloem sap, leading to an excess of positive charges (Peuke *et al.*, 2002). In grape, low K^+ is compensated for by Ca^{2+} and Mg^{2+} in leaves and by Na^+ in fruits also suggesting that phloem sap carries more Na^+ (Ruhl, 1989). While these effects reflect the antagonism between K^+, Na^+ and Mg^{2+} absorption and exchange (Diem & Godbold, 1993; Jakobsen, 1993), they also show that K^+ deficiency is associated with more positive charges in the phloem, and thus that putrescine is unlikely to play the role as a cation to substitute K^+ in sap. However, when K^+ deficiency is compensated for by K^+-substitution with Na^+ or Rb^+, putrescine accumulates less, suggesting that there is a link with cations (Richards & Coleman, 1952; Smith, 1984). Quite remarkably, if K^+-deficiency is accompanied by low Ca^{2+} provision, putrescine accumulation is also lower (Richards & Coleman, 1952; Coleman & Richards, 1956). These observations suggest that putrescine is mostly a response to a disequilibrium in cation composition, in which Ca^{2+} would be over-represented. Mg^{2+} deficiency also leads to a modest putrescine accumulation (Table 1), probably because it changes the cation balance in favor of Ca^{2+}, but to a lower extent than K^+ deficiency (due to the naturally lower Mg^{2+} content compared to K^+; for example, see Fig. 2).

Regulation of H^+-ATPases by putrescine

Rather than acting as a charge-balancing cation, putrescine appears to regulate the cation balance (summarized in Fig. 3). Lowering external K^+ concentration causes a rapid (within minutes) membrane hyperpolarization, which stimulates K^+ uptake via inward-rectifying AKT1 channels (Chérel *et al.*, 2013; Wang & Wu, 2013). When K^+ starvation lasts, however, membrane depolarization may occur, which correlates with a marked decrease in cytosolic K^+ concentration (Armengaud *et al.*, 2009). To drive K^+ uptake, the activity of root K^+/H^+ symporter (mainly via HAK5) energized by plasma membrane H^+-ATPase is critical (Wang & Wu, 2013). Potassium ions uncouple ATP hydrolysis from the H^+ extrusion in plasma membrane H^+-ATPase (Buch-Pedersen *et al.*, 2006). Thus, at low cytosolic K^+, ATP/H^+ coupling is probably better and H^+ extrusion is stimulated, thereby favoring ion uptake (Chérel *et al.*, 2013; Wang & Wu, 2013). Then do polyamines and putrescine in particular, influence plasma membrane H^+-ATPase? The answer to this question appears to be species- and tissue-dependent. Suppression of both plasma membrane and vacuolar H^+-ATPase activity was observed in cucumber roots pretreated for 24 h with either putrescine, spermine or spermidine (Janicka-Russak *et al.*, 2010). In that case, the inhibition was caused by a decrease in the expression for an H^+-ATPase isoform and not by a direct (physical) interaction affecting ATPase catalysis. In rice coleoptiles, direct stimulation of plasma membrane H^+-pumps by all polyamines at millimolar (mM) concentration has been reported, while only putrescine may reach such a concentration in physiological situations (Reggiani *et al.*, 1992). In maize roots, plasma membrane H^+-pumping is rapidly stimulated by putrescine (in the elongation zone) and depressed by spermine (in the maturation zone) (Pandolfi *et al.*, 2010).

Similarly, spermine at high concentrations suppresses, whereas putrescine has no direct effect, on H^+-pumping in plasma membrane vesicles isolated from pea roots (Pottosin *et al.*, 2014a). This contrasted effect of putrescine and other polyamines on H^+-ATPases could originate from difference in competing with Mg^{2+} for ATP-binding and/or ATPase phosphorylation. In fact, putrescine does not bind to ATP, but spermine does (Igarashi *et al.*, 1989) while Mg-ATP (and not free ATP) acts as a substrate for H^+-ATPases. In intact roots, both polyamines induced Ca^{2+}-pumping, which in turn stimulated H^+-pumping, most likely via a decrease of H^+-ATPase protein phosphorylation by a Ca^{2+}-dependent kinase (see (Pottosin *et al.*, 2014a) and references therein). Thus, putrescine stimulates H^+-pumping whereas spermine stimulates ATPase at low concentration and suppresses H^+-pumping at high concentration. Taken as a whole, putrescine seems to favor H^+-pumping across the plasma membrane unlike higher polyamines (spermine).

Putrescine, ROS and K^+ transport

Externally applied polyamines at relatively high (0.5-1 mM) concentration inhibit both inward and outward rectifying K^+-selective currents in roots (Zhao *et al.*, 2007; Pottosin, 2015), whereas internal polyamines at 1 mM halved the current mediated by KAT1 in guard cells (Liu *et al.*, 2000). It is not very likely, therefore, that these effects have a huge significance for K^+ absorption and retention. On the other hand, a combination of polyamines with oxidative stress induces a substantial K^+ loss from roots. ROS are produced via the oxidation of putrescine and other polyamines by intrinsic apoplast diamine and polyamine oxidases (DAO and PAO, respectively) (DiTomaso *et al.*, 1989; Zepeda-Jazo *et al.*, 2011; Velarde-Buendía *et al.*, 2012; Pottosin *et al.*, 2014b). The occurrence of DAO and PAO is variable, with DAO being more abundant in Dicots and PAO in Monocots like Poaceae (Moschou *et al.*, 2008). The loss of K^+, especially in specialized zones like the root apex, is not necessarily harmful despite oxidative stress. Instead, low intracellular K^+ may be sensed and induce a metabolic switch to defense responses (Shabala, 2017). Another product of putrescine catabolism, GABA, has recently been shown to improve K^+ retention in *Arabidopsis* roots by a stimulation of plasma membrane H^+-ATPase activity, a decrease of stress-induced ROS production and a decrease in the expression of outward-rectifying K^+ channel, *GORK* (Su *et al.*, 2019).

Putrescine and Ca^{2+} homeostasis

Overall, the cation load as well as total Ca^{2+} increase under K^+ deficiency (e.g. Fig. 2 and S3). Free cytosolic Ca^{2+} may be kept low by (*i*) efficient Ca^{2+} extrusion while as mentioned above, there is a stimulation of plasma membrane Ca^{2+} pumps by polyamines; and (*ii*) vacuolar Ca^{2+} sequestration. The latter is especially important, bearing in mind the observed increase in total Ca^{2+} because in plant cells, total cellular Ca^{2+} mostly reflects vacuolar Ca^{2+}. Ca^{2+} accumulates in vacuoles via CAX-mediated H^+/Ca^{2+} antiport, fueled by the trans-tonoplast H^+ gradient. To ensure efficient vacuolar Ca^{2+} retention, channel-mediated Ca^{2+} loss from the vacuole to the cytosol must be negligible. SV/TPC1 channels are the major routes of vacuolar Ca^{2+} release (Pottosin & Schönknecht, 2007). Consequently, relative expression of *TPC1* and *CAX* is

crucial for vacuolar Ca^{2+} accumulation (Gilliham *et al.*, 2011). Importantly, ionic currents via SV channels are efficiently suppressed by polyamines in their physiological range of concentrations. Albeit this effect is charge-dependent, with putrescine having the lowest affinity (Dobrovinskaya *et al.*, 1999b), it could be compensated for by a very high putrescine concentration under K^+ deprivation.

Putrescine and vacuole-cytosol K^+ balance

Under K^+ deficiency, maintenance of relatively high cytosolic K^+ is achieved at the expense of the vacuolar K^+ (Walker *et al.*, 1996). In the initial phase, vacuole will indeed compensate for the decrease in cytosolic K^+ by K^+-release via selective (TPK) and non-selective monovalent cation FV channels, both marginally sensitive to putrescine at the sub-millimolar range (Brüggemann *et al.*, 1998; Dobrovinskaya *et al.*, 1999a; Hamamoto *et al.*, 2008). Under very strong K^+ deprivation, the electrochemical gradient for K^+ becomes vacuole-directed (Walker *et al.*, 1996). Thus, to minimize passive vacuolar K^+ re-uptake, it is certainly crucial to reduce K^+-transport by K^+-permeable channels. When putrescine reaches millimolar concentration, K^+ transport not only via SV channels but also via FV channels will be suppressed (Brüggemann *et al.*, 1998; Dobrovinskaya *et al.*, 1999a).

Roles of putrescine in chloroplasts

Possible roles of putrescine on chloroplast metabolism are summarized in Fig. 4. Subcellular fractionation followed by metabolomics analysis has shown that about 40% of cellular putrescine is present in chloroplasts in *Arabidopsis* leaves (Krueger *et al.*, 2011), perhaps reflecting the activity of chloroplastic ADC (Borrell *et al.*, 1995; Bortolotti *et al.*, 2004). Stress-induced stimulation of ADC (Alcázar *et al.*, 2010) might further increase putrescine accumulation in chloroplasts. In chloroplasts, polyamines are believed to regulate different aspects of photosynthesis, with reported differences in action between putrescine and other polyamines. Exogenous putrescine decreases non-photochemical quenching (NPQ) and increases photochemical yield (Ioannidis *et al.*, 2006). Yet, these results have been obtained under non-physiological conditions, with a low-salt medium, to minimize the interference with other cations (such as Mg^{2+}) and therefore, are perhaps not so informative. On the other hand, with more physiological saline buffers, all polyamines stimulate photophosphorylation at low concentrations, whereas spermidine and spermine but not putrescine act as strong uncouplers at high concentration (> 1 mM for spermidine and >0.1 mM for spermine). That is, only putrescine induces a relatively high and stable stimulation of ATP production in chloroplasts (Ioannidis & Kotzabasis, 2007).

Putrescine is a weak base (pK_a 10.8) thus its uncharged form coexist, albeit at a relatively small fraction (0.04%), with the charged species at pH 7.4. Light induces stromal alkalization and thylakoid lumen acidification and this proton gradient can be damped by transport of uncharged putrescine across the thylakoid membrane. This does not affect the electrical potential difference across the thylakoid membrane ($\Delta\Psi$) but dissipates ΔpH and reduces lumen acidification, optimizing photosynthesis under stress conditions where high

ΔpH values lead to NPQ (Ioannidis *et al.*, 2012). Under K^+ deficiency, the decrease in K^+ can be compensated for by an increase in Mg^{2+} (Fig. 2). Mg^{2+} is a charge-balancing cation that can dissipate ΔΨ and facilitate ΔpH built-up across the thylakoid membrane via Mg^{2+}-permeable channels that are present in thylakoid membranes (Pottosin & Schönknecht, 1996). Thus, putrescine can have a role of Mg^{2+} antagonist, whereby it prevents excessive energy dissipation and decreased photosynthesis, which may be due to the excessive lumen acidification even at relatively low light (see (Davis *et al.*, 2017), for further details). It has also been demonstrated that putrescine up-regulates the expression of ATP-synthase and exerts a general protective effect on the photosynthetic membrane and in particular PSII structure (Shu et al., 2015).

Roles of putrescine in mitochondria

Putrescine is synthesized outside mitochondria but can be taken up by them. It is likely exchanged between the cytosol and the mitochondrial matrix via a basic amino acid transporter which is able to carry arginine, citrulline, and ornithine (Hoyos *et al.*, 2003; Palmieri *et al.*, 2006). In animal cells, mitochondrial putrescine uptake has a low affinity ($K_{0.5}$ ≈ 1-4 mM) but a high capacity driven by electrical gradient, i.e., the high negative potential of the mitochondrial matrix (Toninello *et al.*, 1992; Dalla Via *et al.*, 1999). Similarly, in plants, polyamine accumulation in mitochondria depends on membrane potential, but its regulation differs somewhat from that in animals (Pistocchi *et al.*, 1990) and associated molecular mechanisms remain unknown (Fujita & Shinozaki, 2015). Polyamines have diverse effects in mitochondria, typically on metabolism, electron transport and the permeability transition (summarized in Fig. 4).

Putrescine and mitochondrial metabolism

Under stress conditions, putrescine causes a stimulation of the tricarboxylic acid pathway (TCAP) and thus facilitate mitochondrial ATP production (Zhong *et al.*, 2016). So far, this effect has been demonstrated for salt stress, when putrescine was supplied exogenously. This still needs to be tested under K^+ deficiency, based on large amounts of putrescine accumulated naturally. However, metabolomics analyses have suggested that the increased CO_2 release under K^+ deficiency is not associated with a higher ATP production but rather reflects lower efficiency of the TCAP when K^+ is limiting enzymatic activity (Cui *et al.*, 2019a). Also, it should be noted that mitochondrial carbonic anhydrase, which might play an important role in anaplerosis (conversion of catabolic CO_2 into bicarbonate), is inhibited with a high affinity (low K_i) by spermine and spermidine, while putrescine has no effect (Carta *et al.*, 2010).

Interestingly, tobacco mitochondrial complex I mutants, which have a slow growth phenotype, show a significant increase in putrescine, along with related compounds such as GABA (Lothier *et al.*, 2019). At physiologically attainable K^+, higher polyamines inhibit mitochondrial membrane-bound F_0F_1-ATPase in *Vigna* (Peter *et al.*, 1981), which may be partly caused by the fact that higher polyamines (but not putrescine) are able to displace Mg^{2+} from Mg-ATP complexes (Igarashi *et al.*, 1989). That is, putrescine can activate

mitochondrial F_0F_1-ATPases even at low K^+/Na^+ (in contrast to spermine and spermidine, the action of which decreases at low K^+/Na^+) (Peter *et al.*, 1981) thereby allowing ATP production despite low K^+ concentration encountered under potassium deficiency. In addition, enzymatic transglutaminase covalent binding of putrescine to mitochondrial membrane proteins is associated with higher F_0F_1-ATPase activity and tolerance to osmotic stress (Votyakova *et al.*, 1999; Liu & Zhang, 2004). Putrescine, albeit with a 100 times lower affinity compared to higher polyamines (yet with $K_{0.5}$ = 0.3 mM), stimulates the activity of the mitochondrial membrane ATP/ADP exchanger (Krämer *et al.*, 1986). This activation may become significant under K^+ deficiency, when putrescine reaches millimolar levels.

Putrescine and mitochondrial membrane permeability

Polyamines can have an impact on mitochondrial transmembrane potential ($\Delta\Psi$), perhaps mediated by their effect on mitochondrial ATP-sensitive K^+ channels ($^{mito}K_{ATP}$). Both the molecular identity of $^{mito}K_{ATP}$ and their structural similarity with plasma membrane K_{ATP} channels (which are abundant in animal tissues but absent in plants) are still a matter of debate (Szabo & Zoratti, 2014; Trono *et al.*, 2015). Under the assumption that $^{mito}K_{ATP}$ are structurally similar to K^+ inward rectifiers (as animal plasma membrane K_{ATP} channels are), the K^+ current through the channel pore would be modulated in a voltage-dependent manner by cytosolic polyamines. In Mammals, spermine, spermidine and putrescine can regulate the K^+ efflux upon depolarization (Aguilar-Bryan & Bryan, 1999). Unlike their animal counterparts, plant K_{ATP} are not sensitive to Mg^{2+} (Pastore *et al.*, 1999) but to our knowledge, the effect of polyamines has not been documented yet. Mitochondrial depolarization by K^+ influx is believed to reduce ROS production in plants under stress (Trono *et al.*, 2015) and, *vice versa*, hyperpolarization is associated with excessive electron pressure in the mitochondrial electron transfer chain (mETC) and higher ROS production. For example, under osmotic stress, a ROS-mediated activation of K_{ATP}^+ has been found in wheat (Trono *et al.*, 2015). Thus, activation of plant $^{mito}K_{ATP}$ could in principle be efficient to regulate mitochondrial activity, since it not only decreases $\Delta\Psi$ but also impedes ROS generation.

The effect of polyamines and in particular putrescine on mitochondria can also be linked to the control of mitochondrial permeability transition (MPT), which is a massive increase in permeability of the inner mitochondrial membrane, with a collapse of $\Delta\Psi$ and release of pro-apoptotic factors (cytochrome c). In effect, MPT with properties similar to those found in animal MPT, such as activation by Ca^{2+} overload and ROS, and inhibition by Mg^{2+} and low pH, has been reported in plants and shown to promote programmed cell death (Fortes *et al.*, 2001; Arpagaus *et al.*, 2002; Tiwari *et al.*, 2002; Lin *et al.*, 2005; Scott & Logan, 2008). Potentially, polyamines can have an action on MPT via electron pressure on mETC, Ca^{2+} concentration, and ROS generation.

In fact, MPT is stimulated by the increase in Ca^{2+} via ROS generation while polyamines have been found to mitigate ROS generation and inhibit MPT in both plants and animals (Tabor, 1960; Arpagaus *et al.*, 2002; Toninello *et al.*, 2004). While, unlike spermine, putrescine has been shown to be inefficient on cytochrome c release at up to 1 mM in mitochondria isolated from rat heart (Stefanelli *et al.*, 2000). the intermediate of putrescine synthesis, agmatine (Fig. 1), inhibits Ca^{2+}-mediated MPT in Mammals (Battaglia *et al.*, 2010).

Conversely, in yeast, spermine stimulates Ca^{2+} uptake by mitochondria, thereby favoring MPT (Votyakova *et al.*, 1993).

Polyamines at a physiological concentration (0.1 mM) lead to a reduction of $\Delta\Psi$ by 30 and 50%, with putrescine and spermine respectively; this differential effect of putrescine and spermine has been found to correlate with substrate preference of mitochondrial amine oxidase (Maccarrone et al., 2001) but whether this effect is effectively mediated by amine oxidase is not known. In plant mitochondria under low cytosolic cation load (low K^+), putrescine slightly stimulates external NAD(P)H dehydrogenases while at high cation load, it has little effect; this is in contrast with spermidine and spermine, which stimulate NAD(P)H dehydrogenases activity considerably at low cation load (and inhibit dehydrogenases activity at high cation load) (Phelps & McDonald, 1990; Rugolo *et al.*, 1991; Sjölin & Møller, 1991). Therefore, when K^+ concentration is low, spermine and spermidine tend to increase the electron pressure on the mETC and promotes ROS generation, while this effect does not take place with putrescine.

Surprisingly, although polyamines can inhibit MPT at relatively high concentration, they may also favor Ca^{2+} accumulation in the mitochondrial matrix, which normally acts as a MPT inducer (reviewed in (Toninello *et al.*, 2004)). Thus, under K^+ deficiency, high putrescine concentration with higher Ca^{2+} load (MPT promoter) and high Mg^{2+} (MPT opposer) may either stimulate or down-regulate MPT, depending on whether the change in mitochondrial Ca^{2+} predominates over Mg^{2+} change, ROS limitation and electron pressure mitigation. Alternatively, one might speculate that a brief MPT event may have a protective role, releasing excess ROS and Ca^{2+} from the matrix and restoring normal mitochondrial ATP production. However, the release of ROS and Ca^{2+} may become self-propagative, causing Ca^{2+}-induced Ca^{2+} release and ROS-induced ROS release (Zorov *et al.*, 2014) and ultimately cell death. It is thus more likely that putrescine accumulation under K^+ deficiency is beneficial due to its combination of physiological effects, that is, simultaneous limitation of Ca^{2+} release in the cytosol (see above, *Putrescine and Ca^{2+} homeostasis*) and down-regulation of MPT.

Side effects of putrescine

The beneficial effects of putrescine in particular on cation balance (see above) probably explain why the addition of exogenous putrescine or the production of endogenous putrescine in transgenics has often been described as being advantageous to improve stress tolerance and mitigate oxidative stress (Öztürk & Demir, 2003; Verma & Mishra, 2005; Ndayiragije & Lutts, 2006). However, overexpression of *ADC2* in *Arabidopsis* induces dwarfism and late flowering (Alcázar *et al.*, 2005). Also, overexpression of oat *ADC* in tobacco leads to short internodes, thin stems and leaves, leaf chlorosis and necrosis, and reduces root growth (Masgrau *et al.*, 1997), which mimics to some extent the symptoms of some stresses like K^+ deficiency or osmotic shock. Conversely, inhibiting putrescine synthesis using D-arginine under phosphorus deficiency appears to be beneficial for total biomass in cultured rice cells (Shih & Kao, 1996). It should be recognized that adding putrescine or boosting putrescine synthesis changes nitrogen metabolism and promotes putrescine recycling. In fact, putrescine is believed to be easily recycled via diamine oxidase to GABA (Shelp *et al.*, 2012) and

importantly, putrescine oxidation can be a source of ROS (see above), signaling a stress response and leading to changes in gene expression (Minocha *et al.*, 2014; Gupta *et al.*, 2016). Putrescine can thus be occasionally detrimental in terms of oxidative stress or net photosynthesis (Mohapatra *et al.*, 2009; Pál *et al.*, 2018). Whenever the pro-oxidant effect predominates over the anti-oxidant function of putrescine, the suppression of arginine formation and ADC activity (along with a decrease in putrescine and concomitant decrease of ROS production) may be beneficiary for plant performance under stress (for example, the decrease in putrescine synthesis by metasilicic acid (H_2SiO_3) application can alleviate some effects of K^+ deficiency (Chen *et al.*, 2016)). However, such a situation nevertheless seems unlikely under K^+ deficiency since putrescine accumulates to very high levels, certainly reflecting an adaptive trait of plant metabolism.

Conclusions and perspectives

Putrescine has specific biochemical properties that differ from other polyamines and this probably explains why K^+ deficiency appears to be closely associated with putrescine rather than spermine or spermidine. Putrescine accumulation under K^+ deficiency is perhaps advantageous via its concerted action on several cellular processes including cation balance, ultimately down-regulating MPT. To better understand stress responses where putrescine is involved, a difference should be made between endogenous, natural putrescine production under K^+ deficiency and artificial putrescine provision. To definitely appreciate the adaptive role of putrescine under K^+ deficiency, it will be necessary to use plant lines with altered putrescine content such as *ADC* overexpression or knock-out lines and at the same time, verify putrescine subcellular distribution, measure both K^+ and Ca^{2+} content, and monitor mitochondrial activity (ATP synthesis, transmembrane potential, ROS production). Also, a possible venue would be to examine further the roles of putrescine in chloroplasts (its major site of production via the ADC pathway) and in particular, to check its effect on ion and pH homeostasis, electrochemical gradient across the thylakoid membrane, and ultimately optimization of photosynthesis. It should be kept in mind that aside from examples of positive effects of *ADC* overexpression (increase in tolerance to drought, cold, or salinity in *Arabidopsis*, or rice (Alcázar *et al.*, 2010; Wang *et al.*, 2011)), toxic effects of putrescine over-production have been observed (see above). It is possible that deleterious effects were caused by enhanced DAO activity and excessive ROS production. Therefore, one might hypothesize that engineering plants with simultaneous overexpression of ADC and knock-down of DAO could be beneficial. In the field, the putrescine content in crops could be used as a component of the metabolomics signature of K^+ nutrition or a marker to detect K^+-responsive varieties, because it reflects several processes (described above) triggered by intracellular K^+ scarcity. In the near future, it might then be amongst biomarkers used by precision agriculture.

Acknowledgements

G.T. thanks the financial support of the Région Pays de la Loire and Angers Loire Métropole via the Connect Talent grant *Isoseed*. J.C. was supported by an Australia Awards PhD Scholarship.

References

Adams DO, Franke KE, Christensen LP. 1990. Elevated putrescine levels in grapevine leaves that display symptoms of potassium deficiency. *American Journal of Enology and Viticulture* **41**: 121-125.

Aguilar-Bryan L, Bryan J. 1999. Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocrine Reviews* **20**: 101-135.

Alcázar R, Bitrián M, Bartels D, Koncz C, Altabella T, Tiburcio AF. 2011. Polyamine metabolic canalization in response to drought stress in *Arabidopsis* and the resurrection plant *Craterostigma plantagineum*. *Plant Signaling and Behavior* **6**: 243-250.

Alcázar R, García-Martínez JL, Cuevas JC, Tiburcio AF, Altabella T. 2005. Overexpression of ADC2 in *Arabidopsis* induces dwarfism and late-flowering through GA deficiency. *The Plant Journal* **43**: 425-436.

Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T. 2006. Involvement of polyamines in plant response to abiotic stress. *Biotechnology Letters* **28**: 1867-1876.

Alcázar R, Planas J, Saxena T, Zarza X, Bortolotti C, Cuevas J, Bitrián M, Tiburcio AF, Altabella T. 2010. Putrescine accumulation confers drought tolerance in transgenic *Arabidopsis* plants over-expressing the homologous Arginine decarboxylase 2 gene. *Plant Physiology and Biochemistry* **48**: 547-552.

Alet AI, Sánchez DH, Cuevas JC, Marina M, Carrasco P, Altabella T, Tiburcio AF, Ruiz OA. 2012. New insights into the role of spermine in *Arabidopsis thaliana* under long-term salt stress. *Plant Science* **182**: 94-100.

Alexandersson E, Jacobson D, Vivier MA, Weckwerth W, Andreasson E. 2014. Field-omics—understanding large-scale molecular data from field crops. *Frontiers in Plant Science* **5**: Article 286.

Armengaud P, Sulpice R, Miller AJ, Stitt M, Amtmann A, Gibon Y. 2009. Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in *Arabidopsis* roots. *Plant Physiology* **150**: 772-785.

Arpagaus S, Rawyler A, Braendle R. 2002. Occurrence and characteristics of the mitochondrial permeability transition in plants. *Journal of Biological Chemistry* **277**: 1780-1787.

Aurisano N, Bertani A, Mattana M, Reggiani R. 1993. Abscisic acid induced stress-like polyamine pattern in wheat seedlings, and its reversal by potassium ions. *Physiologia Plantarum* **89**: 687-692.

Aziz A, Martin-Tanguy J, Larher F. 1998. Stress-induced changes in polyamine and tyramine levels can regulate proline accumulation in tomato leaf discs treated with sodium chloride. *Physiologia Plantarum* **104**: 195-202.

Bagni N, Ruiz-Carrasco K, Franceschetti M, Fornalè S, Fornasiero RB, Tassoni A. 2006. Polyamine metabolism and biosynthetic gene expression in *Arabidopsis thaliana* under salt stress. *Plant Physiology and Biochemistry* **44**: 776-786.

Balestrasse KB, Gallego SM, Benavides MP, Tomaro ML. 2005. Polyamines and proline are affected by cadmium stress in nodules and roots of soybean plants. *Plant and Soil* **270**: 343-353.

Basso LC, Smith TA. 1974. Effect of mineral deficiency on amine formation in higher plants. *Phytochemistry* **13**: 875-883.

Basu R, Ghosh B. 1991. Polyamines in various rice (*Oryza sativa*) genotypes with respect to sodium chloride salinity. *Physiologia Plantarum* **82**: 575-581.

Basu R, Maitra N, Ghosh B. 1988. Salinity results in polyamine accumulation in early rice (*Oryza sativa* L.) seedlings. *Functional Plant Biology* **15**: 777-786.

Battaglia V, Grancara S, Satriano J, Saccoccio S, Agostinelli E, Toninello A. 2010. Agmatine prevents the Ca^{2+}-dependent induction of permeability transition in rat brain mitochondria. *Amino Acids* **38**: 431-437.

Benavides MP, Aizencang G, Tomaro ML. 1997. Polyamines in *Helianthus annuus* L. during germination under salt stress. *Journal of Plant Growth Regulation* **16**: 205-211.

Bennett EM, Ekstrom JL, Pegg AE, Ealick SE. 2002. Monomeric S-adenosylmethionine decarboxylase from plants provides an alternative to putrescine stimulation. *Biochemistry* **41**: 14509-14517.

Borrell A, Culianez-Macia FA, Altabella T, Besford RT, Flores D, Tiburcio AF. 1995. Arginine decarboxylase is localized in chloroplasts. *Plant Physiology* **109**: 771-776.

Bortolotti C, Cordeiro A, Alcázar R, Borrell A, Culiañez-Macià FA, Tiburcio AF, Altabella T. 2004. Localization of arginine decarboxylase in tobacco plants. *Physiologia Plantarum* **120**: 84-92.

Brüggemann LI, Pottosin II, Schönknecht G. 1998. Cytoplasmic polyamines block the fast-activating vacuolar cation channel. *The Plant Journal* **16**: 101-105.

Buch-Pedersen MJ, Rudashevskaya EL, Berner TS, Venema K, Palmgren MG. 2006. Potassium as an intrinsic uncoupler of the plasma membrane H^+-ATPase. *Journal of Biological Chemistry* **281**: 38285-38292.

Camacho-Cristóbal JJ, Maldonado JM, González-Fontes A. 2005. Boron deficiency increases putrescine levels in tobacco plants. *Journal of plant physiology* **162**: 921-928.

Capell T, Bassie L, Christou P. 2004. Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 9909-9914.

Carta F, Temperini C, Innocenti A, Scozzafava A, Kaila K, Supuran CT. 2010. Polyamines inhibit carbonic anhydrases by anchoring to the zinc-coordinated water molecule. *Journal of medicinal chemistry* **53**: 5511-5522.

Chen CT, Kao CH. 1993. Osmotic stress and water stress have opposite effects on putrescine and proline production in excised rice leaves. *Plant Growth Regulation* **13**: 197-202.

Chen D, Cao B, Qi L, Yin L, Wang S, Deng X. 2016. Silicon-moderated K-deficiency-induced leaf chlorosis by decreasing putrescine accumulation in sorghum. *Annals of Botany* **118**: 305-315.

Chérel I, Lefoulon C, Boeglin M, Sentenac H. 2013. Molecular mechanisms involved in plant adaptation to low K^+ availability. *Journal of Experimental Botany* **65**: 833-848.

Coleman R, Hegartv M. 1957. Metabolism of DL-ornithine-2-^{14}C in normal and potassium-deficient barley. *Nature* **179**: 376-377.

Coleman R, Richards F. 1956. Physiological studies in plant nutrition: XVIII. Some aspects of nitrogen metabolism in barley and other plants in relation to potassium deficiency. *Annals of Botany* **20**: 393-409.

Corey K, Barker A. 1989. Ethylene evolution and polyamine accumulation by tomato subjected to interactive stresses of ammonium toxicity and potassium deficiency. *Journal of the American Society for Horticultural Science (USA)* **114**: 651-655.

Cowley T, Walters DR. 2005. Local and systemic changes in arginine decarboxylase activity, putrescine levels and putrescine catabolism in wounded oilseed rape. *New Phytologist* **165**: 807-811.

Crocomo O, Basso L. 1974. Accumulation of putrescine and related amino acids in potassium deficient Sesamum. *Phytochemistry* **13**: 2659-2665.

Cuevas JC, López-Cobollo R, Alcázar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio AF, Ferrando A. 2008. Putrescine is involved in Arabidopsis freezing tolerance and cold acclimation by regulating abscisic acid levels in response to low temperature. *Plant Physiology* **148**: 1094-1105.

Cui J, Abadie C, Carroll A, Lamade E, Tcherkez G. 2019a. Responses to K deficiency and waterlogging interact via respiratory and nitrogen metabolism. *Plant Cell and Environment* **42**: 647-658.

Cui J, Davanture M, Zivy M, Lamade E, Tcherkez G. 2019b. Metabolic responses to potassium availability and waterlogging reshape respiration and carbon use efficiency in oil palm. *New Phytologist* **223**: 310-322.

Dalla Via L, Di Noto V, Toninello A. 1999. Binding of spermidine and putrescine to energized liver mitochondria. *Archives of biochemistry and biophysics* **365**: 231-238.

Davis GA, Rutherford AW, Kramer DM. 2017. Hacking the thylakoid proton motive force for improved photosynthesis: modulating ion flux rates that control proton motive force partitioning into $\Delta\psi$ and ΔpH. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**: Article 20160381.

Diem B, Godbold D. 1993. Potassium, calcium and magnesium antagonism in clones of *Populus trichocarpa*. *Plant and Soil* **155**: 411-414.

DiTomaso JM, Shaff JE, Kochian LV. 1989. Putrescine-induced wounding and its effects on membrane integrity and ion transport processes in roots of intact corn seedlings. *Plant Physiology* **90**: 988-995.

Dobrovinskaya O, Muniz J, Pottosin I. 1999a. Inhibition of vacuolar ion channels by polyamines. *The Journal of membrane biology* **167**: 127-140.

Dobrovinskaya O, Muñiz J, Pottosin II. 1999b. Asymmetric block of the plant vacuolar Ca^{2+}-permeable channel by organic cations. *European Biophysics Journal* **28**: 552-563.

Erdei L, Trivedi S, Takeda K, Matsumoto H. 1990. Effects of osmotic and salt stresses on the accumulation of polyamines in leaf segments from wheat varieties differing in salt and drought tolerance. *Journal of plant physiology* **137**: 165-168.

Escribano MI, Aguado P, Reguera RM, Merodio C. 1996. Conjugated polyamine levels and putrescine synthesis in cherimoya fruit during storage at different temperatures. *Journal of plant physiology* **147**: 736-742.

Feirer RP, Hocking KL, Woods PJ. 1998. Involvement of arginine decarboxylase in the response of *Arabidopsis thaliana* to osmotic stress. *Journal of plant physiology* **153**: 733-738.

Feng J, Barker AV. 1993. Polyamine concentration and ethylene evolution in tomato plants under nutritional stress. *HortScience* **28**: 109-110.

Flores HE, Galston AW. 1982. Polyamines and plant stress: activation of putrescine biosynthesis by osmotic shock. *Science* **217**: 1259-1261.

Flores HE, Galston AW. 1984. Osmotic stress-induced polyamine accumulation in cereal leaves. *Plant Physiology* **75**: 102-109.

Fortes F, Castilho RF, Catisti R, Carnieri EGS, Vercesi AE. 2001. Ca2+ induces a cyclosporin A-insensitive permeability transition pore in isolated potato tuber mitochondria mediated by reactive oxygen species. *Journal of Bioenergetics and Biomembranes* **33**: 43-51.

Foster SA, Walters DR. 1991. Polyamine concentrations and arginine decarboxylase activity in wheat exposed to osmotic stress. *Physiologia Plantarum* **82**: 185-190.

Friedman Ra, Altman A, Levin N. 1989. The effect of salt stress on polyamine biosynthesis and content in mung bean plants and in halophytes. *Physiologia Plantarum* **76**: 295-302.

Friedman RA, Levin N, Altman A. 1986. Presence and identification of polyamines in xylem and phloem exudates of plants. *Plant Physiology* **82**: 1154-1157.

Fujita M, Shinozaki K 2015. Polyamine transport systems in plants. In: Kusano T, Suzuki H eds. *Polyamines*. Tokyo: Springer, 179-185.

Galston AW, Sawhney RK. 1990. Polyamines in plant physiology. *Plant Physiology* **94**: 406-410.

Gilliham M, Athman A, Tyerman S, Conn S. 2011. Cell-specific compartmentation of mineral nutrients is an essential mechanism for optimal plant productivity - another role for TPC1? *Plant Signaling and Behavior* **6**: 16656-11661.

Groppa MD, Ianuzzo MP, Tomaro ML, Benavides MP. 2007. Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. *Amino Acids* **32**: 265-275.

Gupta K, Sengupta A, Chakraborty M, Gupta B. 2016. Hydrogen peroxide and polyamines act as double edged swords in plant abiotic stress responses. *Frontiers in Plant Science* **7**: Article 01343.

Hamamoto S, Marui J, Matsuoka K, Higashi K, Igarashi K, Nakagawa T, Kuroda T, Mori Y, Murata Y, Nakanishi Y. 2008. Characterization of a tobacco TPK-type K+ channel as a novel tonoplast K^+ channel using yeast tonoplasts. *Journal of Biological Chemistry* **283**: 1911-1920.

Hanfrey C, Sommer S, Mayer MJ, Burtin D, Michael AJ. 2001. Arabidopsis polyamine biosynthesis: absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity. *The Plant Journal* **27**: 551-560.

Hauschild MZ. 1993. Putrescine (1,4-diaminobutane) as an indicator of pollution-induced stress in higher plants: barley and rape stressed with Cr(III) or Cr(VI). *Ecotoxicology and Environmental Safety* **26**: 228-247.

Houdusse F, Garnica M, Zamarreño AM, Yvin JC, García-Mina J. 2008. Possible mechanism of the nitrate action regulating free-putrescine accumulation in ammonium fed plants. *Plant Science* **175**: 731-739.

Houman F, Godbold DL, Majcherczyk A, Shasheng W, Hüttermann A. 1991. Polyamines in leaves and roots of *Populus maximowiczii* grown in differing levels of potassium and phosphorus. *Canadian journal of forest research* **21**: 1748-1751.

Hoyos ME, Palmieri L, Wertin T, Arrigoni R, Polacco JC, Palmieri F. 2003. Identification of a mitochondrial transporter for basic amino acids in *Arabidopsis thaliana* by functional reconstitution into liposomes and complementation in yeast. *The Plant Journal* **33**: 1027-1035.

Igarashi K, Kashiwagi K, Kobayashi H, Ohnishi R, Kakegawa T, Nagasu A, Hirose S. 1989. Effect of polyamines on mitochondrial F_1-ATPase catalyzed reactions. *The Journal of Biochemistry* **106**: 294-298.

Ioannidis NE, Cruz JA, Kotzabasis K, Kramer DM. 2012. Evidence that putrescine modulates the higher plant photosynthetic proton circuit. *PLOS ONE* **7**: e29864.

Ioannidis NE, Kotzabasis K. 2007. Effects of polyamines on the functionality of photosynthetic membrane in vivo and in vitro. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1767**: 1372-1382.

Ioannidis NE, Sfichi L, Kotzabasis K. 2006. Putrescine stimulates chemiosmotic ATP synthesis. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1757**: 821-828.

Jakobsen ST. 1993. Interaction between plant nutrients: III. antagonism between potassium, magnesium and calcium. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* **43**: 1-5.

Janicka-Russak M, Kabała K, Młodzińska E, Kłobus G. 2010. The role of polyamines in the regulation of the plasma membrane and the tonoplast proton pumps under salt stress. *Journal of plant physiology* **167**: 261-269.

Katiyar S, Dubey R. 1990. Changes in polyamine titer in rice seedlings following NaCl salinity stress. *Journal of Agronomy and Crop Science* **165**: 19-27.

Klein H, Priebe A, Jäger H-J. 1979. Putrescine and spermidine in peas: effects of nitrogen source and potassium supply. *Physiologia Plantarum* **45**: 497-499.

Knobloch KH, Berlin J. 1981. Phosphate mediated regulation of cinnamoyl putrescine biosynthesis in cell suspension cultures of *Nicotiana tabacum*. *Planta Medica* **42**: 167-172.

Kotakis C, Theodoropoulou E, Tassis K, Oustamanolakis C, Ioannidis NE, Kotzabasis K. 2014. Putrescine, a fast-acting switch for tolerance against osmotic stress. *Journal of plant physiology* **171**: 48-51.

Krämer R, Mayr U, Heberger C, Tsompanidou S. 1986. Activation of the ADP/ATP carrier from mitochondria by cationic effectors. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **855**: 201-210.

Krueger S, Giavalisco P, Krall L, Steinhauser M-C, Büssis D, Usadel B, Flügge U-I, Fernie AR, Willmitzer L, Steinhauser D. 2011. A topological map of the compartmentalized *Arabidopsis thaliana* leaf metabolome. *PLOS ONE* **6**: Article e17806.

Lee T-M, Shieh Y-J, Chou C-H. 1996. Role of putrescine in enhancing shoot elongation in *Scirpus mucronatus* under submergence. *Physiologia Plantarum* **96**: 419-424.

Lin C, Kao CH. 1999. Excess copper induces an accumulation of putrescine in rice leaves. *Botanical Bulletin Academia Sinica* **40**: 213-218.

Lin CC, Kao CH. 2002. NaCl-induced changes in putrescine content and diamine oxidase activity in roots of rice seedlings. *Biologia Plantarum* **45**: 633-636.

Lin J, Wang Y, Wang G. 2005. Salt stress-induced programmed cell death via Ca^{2+}-mediated mitochondrial permeability transition in tobacco protoplasts. *Plant Growth Regulation* **45**: 243-250.

Liu J, Zhang Y-y. 2004. Relationship between ATPase activity and conjugated polyamines in mitochondrial membrane from wheat seedling roots under osmotic stress. *Journal of Environmental Sciences* **16**: 712-716.

Liu K, Fu H, Bei Q, Luan S. 2000. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. *Plant Physiology* **124**: 1315-1326.

Lothier J, De Paepe R, Tcherkez G. 2019. Mitochondrial complex I dysfunction increases CO_2 efflux and reconfigures metabolic fluxes of day respiration in tobacco leaves. *New Phytologist* **221**: 750-763.

Masgrau C, Altabella T, Farrás R, Flores D, Thompson AJ, Besford RT, Tiburcio AF. 1997. Inducible overexpression of oat arginine decarboxylase in transgenic tobacco plants. *The Plant Journal* **11**: 465-473.

McDonald RE, Kushad MM. 1986. Accumulation of putrescine during chilling injury of fruits. *Plant Physiology* **82**: 324-326.

Meyer RC, Steinfath M, Lisec J, Becher M, Witucka-Wall H, Törjék O, Fiehn O, Eckardt Ä, Willmitzer L, Selbig J, et al. 2007. The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* **104**: 4759-4764.

Minocha R, Majumdar R, Minocha SC. 2014. Polyamines and abiotic stress in plants: a complex relationship. *Frontiers in Plant Science* **5**: Article 00175.

Mohapatra S, Minocha R, Long S, Minocha SC. 2009. Putrescine overproduction negatively impacts the oxidative state of poplar cells in culture. *Plant Physiology and Biochemistry* **47**: 262-271.

Moschou PN, Paschalidis KA, Roubelakis-Angelakis KA. 2008. Plant polyamine catabolism: the state of the art. *Plant Signaling and Behavior* **3**: 1061-1066.

Murty KS, Smith TA, Bould C. 1971. The relation between the putrescine content and potassium status of black currant leaves. *Annals of Botany* **35**: 687-695.

Mutlu F, Bozcuk S. 2007. Relationship between salt stress and levels of free and bound polyamines in sunflower plants. *Plant Biosystems* **141**: 31-39.

Naka Y, Watanabe K, Sagor G, Niitsu M, Pillai MA, Kusano T, Takahashi Y. 2010. Quantitative analysis of plant polyamines including thermospermine during growth and salinity stress. *Plant Physiology and Biochemistry* **48**: 527-533.

Ndayiragije A, Lutts S. 2006. Do exogenous polyamines have an impact on the response of a salt-sensitive rice cultivar to NaCl? *Journal of plant physiology* **163**: 506-516.

Öztürk L, Demir Y. 2003. Effects of putrescine and ethephon on some oxidative stress enzyme activities and proline content in salt stressed spinach leaves. *Plant Growth Regulation* **40**: 89-95.

Pál M, Tajti J, Szalai G, Peeva V, Végh B, Janda T. 2018. Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. *Scientific Reports* **8**: Article 12839.

Palmieri L, Todd CD, Arrigoni R, Hoyos ME, Santoro A, Polacco JC, Palmieri F. 2006. *Arabidopsis* mitochondria have two basic amino acid transporters with partially overlapping specificities and differential expression in seedling development. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1757**: 1277-1283.

Pandolfi C, Pottosin I, Cuin T, Mancuso S, Shabala S. 2010. Specificity of polyamine effects on NaCl-induced ion flux kinetics and salt stress amelioration in plants. *Plant and Cell Physiology* **51**: 422-434.

Pastore D, Stoppelli MC, Di Fonzo N, Passarella S. 1999. The existence of the K^+ channel in plant mitochondria. *Journal of Biological Chemistry* **274**: 26683-26690.

Peter HW, Pinheiro MR, Lima MS. 1981. Regulation of the F1-ATPase from mitochondria of *Vigna sinensis* (L.) Savi cv. Pitiuba by spermine, spermidine, putrescine, Mg^{2+}, Na^+, and K^+. *Canadian journal of biochemistry* **59**: 60-66.

Peuke AD, Jeschke WD, Hartung W. 2002. Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation. *Journal of Experimental Botany* **53**: 241-250.

Phelps DC, McDonald RE. 1990. Inhibition of electron transport activities in mitochondria from avocado and pepper fruit by naturally occurring polyamines. *Physiologia Plantarum* **78**: 15-21.

Pistocchi R, Antognoni F, Bagni N, Zannoni D. 1990. Spermidine uptake by mitochondria of *Helianthus tuberosus*. *Plant Physiology* **92**: 690-695.

Pottosin I 2015. Polyamine action on plant ion channels and pumps. In: Kusano T, Suzuki H eds. *Polyamines*. Tokyo: Springer, 229-241.

Pottosin I, Schönknecht G. 1996. Ion channel permeable for divalent and monovalent cations in native spinach thylakoid membranes. *The Journal of membrane biology* **152**: 223-233.

Pottosin I, Schönknecht G. 2007. Vacuolar calcium channels. *Journal of Experimental Botany* **58**: 1559-1569.

Pottosin I, Velarde-Buendía AM, Bose J, Fuglsang AT, Shabala S. 2014a. Polyamines cause plasma membrane depolarization, activate Ca^{2+}-, and modulate H^+-ATPase pump activity in pea roots. *Journal of Experimental Botany* **65**: 2463-2472.

Pottosin I, Velarde-Buendía AM, Bose J, Zepeda-Jazo I, Shabala S, Dobrovinskaya O. 2014b. Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. *Journal of Experimental Botany* **65**: 1271-1283.

Priebe A, Jäger HJ. 1978. Effect of NaCl on the levels of putrescine and related polyamines in plants differing in salt tolerance. *Plant Science Letters* **12**: 365-369.

Reggiani R, Aurisano N, Mattana M, Bertani A. 1993. Influence of K^+ ions on polyamine level in wheat seedlings. *Journal of plant physiology* **141**: 136-140.

Reggiani R, Giussani P, Bertani A. 1990. Relationship between the accumulation of putrescine and the tolerance to oxygen-deficit stress in Gramineae seedlings. *Plant and Cell Physiology* **31**: 489-494.

Reggiani R, Hochkoeppler A, Bertani A. 1989. Polyamines in rice seedlings under oxygen-deficit stress. *Plant Physiology* **91**: 1197-1201.

Reggiani R, Zaina S, Bertani A. 1992. Plasmalemma ATPase in rice coleoptiles; Stimulation by putrescine and polyamines. *Phytochemistry* **31**: 417-419.

Richards F, Coleman R. 1952. Occurrence of putrescine in potassium-deficient barley. *Nature* **170**: 460-462.

Rugolo M, Antognoni F, Flamigni A, Zannoni D. 1991. Effects of polyamines on the oxidation of exogenous NADH by Jerusalem artichoke (*Helianthus tuberosus*) mitochondria. *Plant Physiology* **95**: 157-163.

Ruhl E. 1989. Effect of potassium and nitrogen supply on the distribution of minerals and organic acids and the composition of grape juice of Sultana vines. *Australian Journal of Experimental Agriculture* **29**: 133-137.

Sarjala T. 1996. Growth, potassium and polyamine concentrations of Scots pine seedlings in relation to potassium availability under controlled growth conditions. *Journal of plant physiology* **147**: 593-598.

Sarjala T, Kaunisto S. 1993. Needle polyamine concentrations and potassium nutrition in Scots pine. *Tree Physiology* **13**: 87-96.

Scaramagli S, Biondi S, Leone A, Grillo S, Torrigiani P. 2000. Acclimation to low water potential in potato cell suspension cultures leads to changes in putrescine metabolism. *Plant Physiology and Biochemistry* **38**: 345-351.

Scott I, Logan DC. 2008. Mitochondrial morphology transition is an early indicator of subsequent cell death in *Arabidopsis*. *New Phytologist* **177**: 90-101.

Shabala S. 2017. Signalling by potassium: another second messenger to add to the list? *Journal of Experimental Botany* **68**: 4003-4007.

Shelp BJ, Bozzo GG, Trobacher CP, Zarei A, Deyman KL, Brikis CJ. 2012. Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. *Plant Science* **193**: 130-135.

Shih CY, Kao CH. 1996. Growth inhibition in suspension-cultured rice cells under phosphate deprivation is mediated through putrescine accumulation. *Plant Physiology* **111**: 721-724.

Sinclair C. 1969. The level and distribution of amines in barley as affected by potassium nutrition, arginine level, temperature fluctuation and mildew infection. *Plant and Soil* **30**: 423-438.

Sjölin A, Møller I. 1991. The effect of polyamines and other cations on NADH oxidation on the inner surface of the inner mitochondrial membrane. *Plant Physiology and Biochemistry* **29**: 607-613.

Slocum RD. 2005. Genes, enzymes and regulation of arginine biosynthesis in plants. *Plant Physiology and Biochemistry* **43**: 729-745.

Smith GS, Lauren DR, Cornforth IS, Agnew MP. 1982. Evaluation of putrescine as a biochemical indicator of the potassium requirements of lucerne. *New Phytologist* **91**: 419-428.

Smith T 1984. Putrescine and inorganic ions. In: Timmermann B, C S, Loewus F eds. *Phytochemical adaptations to stress*. Boston: Springer, 7-54.

Smith TA, Richards FJ. 1962. The biosynthesis of putrescine in higher plants and its relation to potassium nutrition. *The Biochemical journal* **84**: 292-294.

Stefanelli C, Maddalena Z, Bonavita F, Flamigni F, Zambonin L, Landi L, Pignatti C, Guarnieri C, Caldarera CM. 2000. Polyamines directly induce release of cytochrome c from heart mitochondria. *Biochemical Journal* **347**: 875-880.

Su GX, Bai X. 2008. Contribution of putrescine degradation to proline accumulation in soybean leaves under salinity. *Biologia Plantarum* **52**: 796-801.

Su N, Wu Q, Chen J, Shabala L, Mithöfer A, Wang H, Qu M, Yu M, Cui J, Shabala S. 2019. GABA operates upstream of H^+-ATPase and improves salinity tolerance in Arabidopsis by enabling cytosolic K^+ retention and Na^+ exclusion. *Journal of Experimental Botany* **70**: 6349-6361.

Sung H-I, Liu L-F, Kao CH. 1994. Putrescine accumulation is associated with growth inhibition in suspension-cultured rice cells under potassium deficiency. *Plant and Cell Physiology* **35**: 313-316.

Sung J, Lee S, Lee Y, Ha S, Song B, Kim T, Waters BM, Krishnan HB. 2015. Metabolomic profiling from leaves and roots of tomato (*Solanum lycopersicum* L.) plants grown under nitrogen, phosphorus or potassium-deficient condition. *Plant Science* **241**: 55-64.

Szabo I, Zoratti M. 2014. Mitochondrial channels: ion fluxes and more. *Physiological reviews* **94**: 519-608.

Tabor CW. 1960. The stabilizing effect of spermine and related amines on mitochondria and protoplasts. *Biochemical and Biophysical Research Communications* **2**: 117-120.

Tachimoto M, Fukutomi M, Matsushiro H, Kobayashi M, Takahashi E. 1992. Role of putrescine in *Lemna* plants under potassium deficiency. *Soil Science and Plant Nutrition* **38**: 307-313.

Takahashi H, Imamura T, Miyagi A, Uchimiya H. 2012. Comparative metabolomics of developmental alterations caused by mineral deficiency during in vitro culture of *Gentiana triflora*. *Metabolomics* **8**: 154-163.

Takusagawa F, Kamitori S, Markham GD. 1996. Structure and function of S-adenosylmethionine synthetase: crystal structures of S-adenosylmethionine synthetase with ADP, BrADP, and PPi at 2.8 Å resolution. *Biochemistry* **35**: 2586-2596.

Tamai T, Shimada Y, Sugimoto T, Shiraishi N, Oji Y. 2000. Potassium stimulates the efflux of putrescine in roots of barley seedlings. *Journal of plant physiology* **157**: 619-626.

Tassoni A, Franceschetti M, Bagni N. 2008. Polyamines and salt stress response and tolerance in *Arabidopsis thaliana* flowers. *Plant Physiology and Biochemistry* **46**: 607-613.

Tattini M, Heimler D, Traversi ML, Pieroni A. 1993. Polyamine analysis in salt stressed plants of olive (*Olea europaea* L.). *Journal of Horticultural Science* **68**: 613-617.

Tiburcio AF, Altabella T, Bitrián M, Alcázar R. 2014. The roles of polyamines during the lifespan of plants: from development to stress. *Planta* **240**: 1-18.

Tiwari BS, Belenghi B, Levine A. 2002. Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. *Plant Physiology* **128**: 1271-1281.

Toninello A, Dalla Via L, Siliprandi D, Garlid KD. 1992. Evidence that spermine, spermidine, and putrescine are transported electrophoretically in mitochondria by a specific polyamine uniporter. *Journal of Biological Chemistry* **267**: 18393-18397.

Toninello A, Salvi M, Mondov B. 2004. Interaction of biologically active amines with mitochondria and their roles in the mitochondrial mediated pathway of apoptosis. *Current Medicinal Chemistry* **11**: 2349-2374.

Trono D, Laus MN, Soccio M, Alfarano M, Pastore D. 2015. Modulation of potassium channel activity in the balance of ROS and ATP Production by durum wheat mitochondria—an amazing defense tool against hyperosmotic stress. *Frontiers in Plant Science* **6**: Article 1072.

Turner LB, Steward GR. 1986. The effect of water stress upon polyamine levels in barley (*Hordeum vulgare* L.) leaves. *Journal of Experimental Botany* **37**: 170-177.

Turner LB, Stewart GR. 1988. Factors affecting polyamine accumulation in barley (*Hordeum vulgare* L.) leaf sections during osmotic stress. *Journal of Experimental Botany* **39**: 311-316.

Urano K, Yoshiba Y, Nanjo T, Ito T, Yamaguchi-Shinozaki K, Shinozaki K. 2004. *Arabidopsis* stress-inducible gene for arginine decarboxylase AtADC2 is required for accumulation of putrescine in salt tolerance. *Biochemical and Biophysical Research Communications* **313**: 369-375.

Velarde-Buendía AM, Shabala S, Cvikrova M, Dobrovinskaya O, Pottosin I. 2012. Salt-sensitive and salt-tolerant barley varieties differ in the extent of potentiation of the ROS-induced K^+ efflux by polyamines. *Plant Physiology and Biochemistry* **61**: 18-23.

Verma S, Mishra SN. 2005. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. *Journal of plant physiology* **162**: 669-677.

Votyakova TV, Bazhenova EN, Zvjagilskaya RA. 1993. Yeast mitochondrial calcium uptake: regulation by polyamines and magnesium ions. *Journal of Bioenergetics and Biomembranes* **25**: 569-574.

Votyakova TV, Wallace H, Dunbar B, Wilson SB. 1999. The covalent attachment of polyamines to proteins in plant mitochondria. *European Journal of Biochemistry* **260**: 250-257.

Walker DJ, Leigh RA, Miller AJ. 1996. Potassium homeostasis in vacuolate plant cells. *Proceedings of the National Academy of Sciences* **93**: 10510-10514.

Wang B-Q, Zhang Q-F, Liu J-H, Li G-H. 2011. Overexpression of PtADC confers enhanced dehydration and drought tolerance in transgenic tobacco and tomato: effect on ROS elimination. *Biochemical and Biophysical Research Communications* **413**: 10-16.

Wang CY. 1987. Changes of polyamines and ethylene in cucumber seedlings in response to chilling stress. *Physiologia Plantarum* **69**: 253-257.

Wang J-W, Kao CH. 2006. Aluminum-inhibited root growth of rice seedlings is mediated through putrescine accumulation. *Plant and Soil* **288**: 373-381.

Wang Y, Wu W-H. 2013. Potassium transport and signaling in higher plants. *Annual review of plant biology* **64**: 451-476.

Wang Z, Wang Y, Shi J, Zheng Q, Gao L, Wang Q, Zuo J. 2019. Effects of putrescine on the postharvest physiology characteristics in cowpea. *Food Science and Nutrition* **7**: 395-403.

Watson MB, Emory KK, Piatak RM, Malmberg RL. 1998. Arginine decarboxylase (polyamine synthesis) mutants of *Arabidopsis thaliana* exhibit altered root growth. *The Plant Journal* **13**: 231-239.

Watson MB, Malmberg RL. 1996. Regulation of *Arabidopsis thaliana* (L.) Heynh arginine decarboxylase by potassium deficiency stress. *Plant Physiology* **111**: 1077-1083.

Weinstein LH, Kaur-Sawhney R, Rajam MV, Wettlaufer SH, Galston AW. 1986. Cadmium-induced accumulation of putrescine in oat and bean leaves. *Plant Physiology* **82**: 641-645.

Yoshida D. 1969. Formation of putrescine from ornithine and arginine in tobacco plants. *Plant and Cell Physiology* **10**: 393-397.

Young ND, Galston AW. 1983. Putrescine and acid stress: induction of arginine decarboxylase activity and putrescine accumulation by low pH. *Plant Physiology* **71**: 767-771.

Young ND, Galston AW. 1984. Physiological control of arginine decarboxylase activity in K-deficient oat shoots. *Plant Physiology* **76**: 331-335.

Yoza K-I, Takeda Y, Sekiya K, Nogata Y, Ohta H. 1996. Putrescine accumulation in wounded green banana fruit. *Phytochemistry* **42**: 331-334.

Zaidan HA, Broetto F, de Oliveira ET, Gallo LA, Crocomo OJ. 1999. Influence of potassium nutrition and the nitrate/ammonium ratio on the putrescine and spermidine contents in banana vitroplants. *Journal of plant nutrition* **22**: 1123-1140.

Zapata PJ, Serrano Ma, Pretel MT, Amorós A, Botella MÁ. 2004. Polyamines and ethylene changes during germination of different plant species under salinity. *Plant Science* **167**: 781-788.

Zepeda-Jazo I, Velarde-Buendía AM, Enríquez-Figueroa R, Bose J, Shabala S, Muñiz-Murguía J, Pottosin II. 2011. Polyamines interact with hydroxyl radicals in activating Ca^{2+} and K^{+} transport across the root epidermal plasma membranes. *Plant Physiology* **157**: 2167-2180.

Zhang G-w, Xu S-c, Hu Q-z, Mao W-h, Gong Y-m. 2014. Putrescine plays a positive role in salt-tolerance mechanisms by reducing oxidative damage in roots of vegetable soybean. *Journal of Integrative Agriculture* **13**: 349-357.

Zhao F, Song C-P, He J, Zhu H. 2007. Polyamines improve K^{+}/Na^{+} homeostasis in barley seedlings by regulating root ion channel activities. *Plant Physiology* **145**: 1061-1072.

Zhong M, Yuan Y, Shu S, Sun J, Guo S, Yuan R, Tang Y. 2016. Effects of exogenous putrescine on glycolysis and Krebs cycle metabolism in cucumber leaves subjected to salt stress. *Plant Growth Regulation* **79**: 319-330.

Zorov DB, Juhaszova M, Sollott SJ. 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological reviews* **94**: 909-950.

Table 1. Summary and list of abiotic stress situations where putrescine quantity varies in plants. When putrescine decreases or does not change rather than increase, it is mentioned in italics. When the reference cited also include mutants, data tabulated here only refer to wild-type plants.

Tabulated summary:

Stress	Do other polyamine accumulate?	Change in putrescine content
K deficiency	No in most cases	×3 to ×150
Osmotic shock	Variable	×2 to ×14
Drought	No, except in drought-tolerant plants?	≈ ×2
Salinity (NaCl)	Yes in most cases	Generally decreases
Other stresses	Generally yes if mineral nutrition also impacted (heavy metals, N, etc.)	×2 to ×10

Full table (with references):

Stress	Species and tissue	Do other polyamines accumulate?	Observed fold change in putrescine	Reference
K deficiency				
	Various	Unknown	Unknown (based on colorimetric assays at that time)	(Richards & Coleman, 1952; Coleman & Richards, 1956; Smith & Richards, 1962)
	Various	No (except in radish)	Up to 8	(Basso & Smith, 1974)
	Arabidopsis	No	5	(Watson & Malmberg, 1996)
	Pea	Yes (spermidine, slightly)	Up to 27 (depending on NH_4^+ nutrition)	(Klein *et al.*, 1979)
	Blackcurrant leaves	Unknown	Very high (undetectable at high K)	(Murty *et al.*, 1971)
	Tobacco leaves	Unknown	Up to 11 (radioactivity upon isotopic arginine feeding)	(Yoshida, 1969)
	Grapevine leaves	No	5.5	(Adams *et al.*, 1990)
	Lucerne	Unknown	Up to 150	(Smith *et al.*, 1982)
	Scots pine needles	No	Up to 100	(Sarjala & Kaunisto, 1993)
	Lemna species	Unknown	≈10 or 100 (depends on species)	(Tachimoto *et al.*, 1992)
	Scots pine seedlings	No change (roots) or decline (needles)	Up to 9	(Sarjala, 1996)
	Tomato leaves	No	5?	(Corey & Barker, 1989)
	Poplar roots and leaves	No (spermine declines)	25 (leaves), 80 (roots)	(Houman *et al.*, 1991)

	Banana vitroplants	Slightly (spermidine)	Up to 30	(Zaidan *et al.*, 1999)
	Barley leaves	Slightly (agmatine)	Up to 30	(Sinclair, 1969)
	Gentiana shoots	Transiently (spermidine)	56	(Takahashi *et al.*, 2012)
	Tomato roots	No	13	(Sung *et al.*, 2015)
	Oil palm leaves	No	10	(Cui *et al.*, 2019b)
	Sunflower leaves	No	35	(Cui *et al.*, 2019a)
	Rice cells	No (decrease)	3	(Sung *et al.*, 1994)
	Sesame leaves	Unknown (citrulline and ornithine also increase)	9	(Crocomo & Basso, 1974)
Osmotic shock				
Sorbitol	Cereal leaves	No	2 to 10	(Flores & Galston, 1982)
	Rice leaves	Unknown	2.5	(Chen & Kao, 1993)
	Oat leaves	No (slight decrease)	Up to 4 (no change if turgor maintained)	(Turner & Stewart, 1988)
	Arabidopsis leaves	Yes (spermine)	Up to 3	(Feirer *et al.*, 1998)
	Wheat leaves	Yes (spermidine)	Up to 3	(Erdei *et al.*, 1990)
Polyethylene glycol	Tobacco leaves	Unknown	2	(Kotakis *et al.*, 2014)
	Potato cultured cells	No	14 (only insoluble conjugated putrescine)	(Scaramagli *et al.*, 2000)
Various osmotica	Oat leaves	No	Up to 5	(Flores & Galston, 1984)
Mannitol	Wheat	Yes (cadaverine, spermine)	Up to 3 (in leaves)	(Foster & Walters, 1991)
Drought/water deficit				
	Barley leaves	No (spermidine decreases)	2	(Turner & Steward, 1986)
	Rice leaves	No	1.5	(Capell *et al.*, 2004)
	Arabidopsis	No	*Does not change*	(Alcázar *et al.*, 2010)
		Transient increase in spermidine and then declines.	1.7 (transient increase)	(Alcázar *et al.*, 2011)
	Resurrection plant	Yes	Up to 3	(Alcázar *et al.*, 2011)
Salt stress (NaCl)				
	Soybean leaves	Yes (spermine). Spermidine decreases.	*Decreases*	(Su & Bai, 2008)
	Olive tree roots	Yes	≈1.3	(Tattini *et al.*, 1993)
	Soybean roots	Yes	*Decreases*	(Zhang *et al.*, 2014)
	Arabidopsis flowers	Yes (spermidine)	*Decreases*	(Tassoni *et al.*, 2008)
	Tomato leaves	Yes (spermine). No change in	*Decreases*	(Aziz *et al.*, 1998)

		spermidine.		
	Rice seedling roots	Unknown	*Decreases*	(Lin & Kao, 2002)
	Sunflower xylem sap	Yes (spermidine)	Up to 2.5	(Friedman *et al.*, 1986)
	Arabidopsis	Yes (both)	*Does not change*	(Alet *et al.*, 2012)
	Rice shoots	Variable	Up to 1.5	(Katiyar & Dubey, 1990)
	Various seedlings	Yes	*Decreases*	(Zapata *et al.*, 2004)
	Sunflower seedlings	No (decrease)	*Decreases*	(Benavides *et al.*, 1997)
	Arabidopsis	Yes (spermidine). Spermine decreases.	*Decreases*	(Bagni *et al.*, 2006; Naka *et al.*, 2010)
	Rice seedlings	Yes	Up to 3.5	(Basu & Ghosh, 1991)
		Yes (spermidine). Very small change in spermine.	2	(Basu *et al.*, 1988)
	Sunflower shoots	Yes (spermine). Spermidine decreases.	*Decreases or does not change (depends on variety)*	(Mutlu & Bozcuk, 2007)
	Wheat leaves	Yes	*Does not change*	(Erdei *et al.*, 1990)
	Arabidopsis	Yes (spermine). No change in spermidine.	2	(Urano *et al.*, 2004)
	Various	No (decrease)	*Decrease*	(Priebe & Jäger, 1978)
	Mung bean	Yes (spermidine). Spermine content not measured.	Up to 4 (decrease in roots)	(Friedman *et al.*, 1989)
Other stresses				
Magnesium deficiency	Various	No (except in radish)	Up to 7.3	(Basso & Smith, 1974)
Phosphate deprivation (along with K^+)	Rice cells	No (decrease)	≈2	(Shih & Kao, 1996)
Heavy metals:				
Aluminium (Al^{3+})	Rice roots	No (tend to decline)	3	(Wang & Kao, 2006)
Cadmium (Cd^{2+})	Oat and bean leaves	Spermine increases, spermidine does not change	Up to 10	(Weinstein *et al.*, 1986)
	Soybean nodules and roots	Yes (spermine)	2.5 (nodules), 1.5 (roots)	(Balestrasse *et al.*, 2005)
	Sunflower shoots	Yes	2.7	(Groppa *et al.*, 2007)
Chromium (Cr^{3+}, Cr^{6+})	Barley and rape seedlings	No	Up to 10	(Hauschild, 1993)
Copper (Cu^{2+})	Rice leaves	Unknown	Up to 4	(Lin & Kao, 1999)
	Sunflower shoots	Yes	1.6	(Groppa *et al.*, 2007)

Anoxia/hypoxia/submergence	Cereal seedlings	Slightly (but numerical data not reported)	Up to 2	(Reggiani *et al.*, 1990)
	Rice coleoptile	Slightly	2 to 14	(Reggiani *et al.*, 1989; Reggiani *et al.*, 1992)
	Scirpus shoots	No (decrease)	6	(Lee *et al.*, 1996)
Cold	Arabidopsis seedlings	Spermidine stays constant, spermine decreases	Up to 5	(Cuevas *et al.*, 2008)
	Diverse fruits	Unknown, or decrease	Up to 2.5	(McDonald & Kushad, 1986; Escribano *et al.*, 1996)
	Cucumber seedlings	Yes (spermidine)	*Does not change*	(Wang, 1987)
Boron deficiency	Tobacco leaves and roots	Yes	Up to 2 (leaves) and 5 (roots)	(Camacho-Cristóbal *et al.*, 2005)
Change from nitrate to NH_4^+	Tomato	No	≈3	(Feng & Barker, 1993)
	Pepper and wheat leaves	Yes (pepper), No (wheat)	Up to 20	(Houdusse *et al.*, 2008)
Mechanical wounding	Rapeseed leaves	No	2	(Cowley & Walters, 2005)
	Bananas	No	Up to 5	(Yoza *et al.*, 1996)
Low pH (< 5)	Oat and pea leaves	Unknown	2 to 8	(Young & Galston, 1983)

Figure legends :

Fig. 1. Simplified metabolic pathway of putrescine synthesis and utilization. (a) Chemical structure of putrescine. Note that it contains two N atoms and four C atoms, that all come from glutamate. (b) Pathways showing the direct route starting from glutamate via ornithine (black), putrescine synthesis via arginine (gray) and other polyamines synthesis (blue). Cofactors and other compounds involved in reactions are shown in green or light turquoise. The alternative use of N-acetylornithine as an acetyl donor is shown in dashed green. The recycling of fumarate via the Krebs cycle and aspartate synthesis, and the recycling of ammonium by carbamoyl phosphate synthase are shown in dotted light turquoise. Abbreviations: 2OG, 2-oxoglutarate; ADC, arginine decarboxylase (chloroplastic); CP, carbamoyl phosphate; NAG, N-acetyl glutamate; NAGSA, N-acetyl glutamate semialdehyde; ODC, ornithine decarboxylase (cytosolic); P-NAG, phospho-N-acetyl glutamate; SAE, S-adenosyl methioninamine; SAM, S-adenosyl methionine; SMTA, S-methyl thioadenosine.

Fig. 2. Leaf cation balance under normal or low potassium availability in oil palm (a) and sunflower (b). In each panel, the inset shows the sum of cations, also in µmol positive charges g^{-1} DW (dry weight). Abbreviations: Min, other minor cations (Zn^{2+}, Cu^{2+}, Mn^{2+}, and H^{+} calculated assuming a pH value of 7); Put, putrescine (carrying two positive charges); Orn, ornithine (carrying one positive charge). From source data in Cui *et al*. (2019a, 2019b). Asterisks stand for a significant K-availability effect (in sunflower, there is a significant increase in putrescine although it remains very small in terms of positive charge load).

Fig. 3. **Summary of possible roles of putrescine on cellular cation balance under K^{+} deficiency**. Two main roles are highlighted here, via ions channels (orange, left) and H^{+}-ATPases (green, right). See main text for further details. Abbreviations: DAO, diamine oxidase; GABA, γ-aminobutyrate; ROS, reactive oxygen species.

Fig. 4. Summary of possible roles of putrescine on organelles under K^{+} deficiency. Putrescine has a general positive effect on ATP synthesis in both mitochondria and chloroplasts via a number of mechanisms, including mitigation of mitochondrial permeability transition (MPT) and non-photochemical quenching (NPQ), respectively. Abbreviations: NDHs, NAD(P)H dehydrogenases; TCAP, tricarboxylic acid pathway.