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Mechanisms and physiological roles of K^+ efflux from root cells☆

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SUMMARY

Potassium is the most abundant macronutrient, which is involved in a multitude of physiological processes. Potassium uptake in roots is crucial for plants; however, K^+ efflux can also occur and has important functions. Potassium efflux from roots is mainly induced by stresses, such as pathogens, salinity, freezing, oxidants and heavy metals. Reactive oxygen species (ROS) and exogenous purines also cause this reaction. The depolarisation and activation of cation channels are required for K^+ efflux from plant roots. Potassium channels and nonselective cation channels (NSCCs) are involved in this process. Some of them are 'constitutive', while the others require a chemical agent for activation. In Arabidopsis, there are 77 genes that can potentially encode K^+-permeable channels. Potassium-selective channel genes include 9 Shaker and 6 Tandem-Pore K^+ channels. Genes of NSCCs are more abundant and present by 20 cyclic nucleotide gated channels, 20 ionotropic glutamate receptors, 1 two-pore channel, 10 mechanosensitive-like channels, 2 mechanosensitive 'Mid1-Complementing Activity' channels, 1 mechanosensitive Piezo channel, and 8 annexins. Two Shakers (SKOR and GORK) and several NSCCs are expressed in root cell plasma membranes. SKOR mediates K^+ efflux from xylem parenchyma cells to xylem vessels while GORK is expressed in the epidermis and functions in K^+ release. Both these channels are activated by ROS. The GORK channel activity is stimulated by hydroxyl radicals that are generated in a Ca^{2+}-dependent manner in stress conditions, such as salinity or pathogen attack, resulting in dramatic K^+ efflux from root cells. Potassium loss simulates cytosolic proteases and endonucleases, leading to programmed cell death. Other physiological functions of K^+ efflux channels include repolarisation of the plasma membrane during action potentials and the 'hypothetical' function of a metabolic switch, which provides inhibition of energy-consuming biosyntheses and releasing energy for defence and reparation needs.

Introduction

Potassium (K^+) is crucial for plant growth, development, defence, immunity, signalling and transport processes (Beringer and Troldenier, 1980). The concentration of this element in plant tissues is at least 2% of the dry weight (Bergmann, 1992). Plants accumulate K^+ from soil and create a significant chemical activity gradient of this ion across the plasma membrane. Cytosol, vacuole and organelles contain 50–200 mM K^+ (Leigh and Wyn Jones, 1984); most of it is present in the mobile ionic form (K^+). The potassium content in root epidermal cells of the model plant *Arabidopsis thaliana*, for example, is 70–80 mM (Shabala et al., 2006), while the soil level of K^+ is relatively low (10^{-6} to 10^{-3} M) (Bergmann, 1992). Therefore plants evolved a sophisticated system of passive and active transporters for maintaining adequate K^+ balance (high $K^+_{inside}/K^+_{outside}$ ratio).

The plasma membrane plays a key role in K^+ exchange processes. It is a barrier for K^+ entry into root cells but, at the same time, it prevents loss of accumulated K^+ (for more details on K^+ uptake by plant roots see Nieves-Cordones et al., 2014). It is charged negatively to balance (to hold) the high concentration of positively charged K^+ inside the cell. Typical electric potential differences across the root cell plasma membrane range from −120 to −180 mV (Shabala et al., 2006; Demidchik et al., 2010). According to the Nernst equation, this allows maintaining a two to three orders of magnitude higher K^+ activity in the cytosol than in the extracellular space. Depolarisation triggers K^+ efflux, which causes loss of some K^+ from cells and also repolarises the plasma membrane, returning its negative electric potential. Transport of K^+ through the lipid bilayer of the plasma membrane is catalysed by specialised proteins, which include passive transporters (ion channels; using

Abbreviations: *I–V*, current–voltage; GLRs, glutamate receptors; GORK, guard cell outwardly rectifying K^+ channel; MIFETM, microelectrode ion flux estimation; MSLs, mechanosensitive-like channels; NSCCs, nonselective cation channels; ROS, reactive oxygen species; PCD, programmed cell death; SOS-1, salt-oversensitive 1.

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the difference in electrochemical potentials across the membrane) and active transporters (transporters and exchangers that consume metabolic energy, such as ATP). Low soil K^+ or inhibition of plasma membrane K^+ uptake systems (for example, by heavy metals or oxidants) result in nutritional disorders in all major plant families and decrease plant productivity (Bergmann, 1992; Marschner, 2012; see also Zörb et al., 2014).

However, apart from K^+ uptake, there is also K^+ release, occurring under certain physiological conditions and playing important roles in plant's life. Cation efflux channels are responsible for this phenomenon, including depolarisation activated K^+-selective channels, several types of nonselective cation channels (NSCCs) and annexins. The process of K^+ efflux through depolarisation-activated K^+ channels has been investigated in guard cells in detail. Stomatal closure is directly driven by K^+ release through depolarisation activated K^+ efflux channel GORK, which is regulated by abscisic acid, blue light and other factors (Hosy et al., 2003; Kim et al., 2010). K^+ efflux increases the water potential in guard cells, leading to water efflux, guard cell shrinking and stomata closing (for more details on stomatal movement see Blatt et al., 2014). K^+ efflux from root cells is less studied although this process can have very important physiological functions. It is probably involved in root plasma membrane repolarisation, osmoregulation and K^+ loading to the xylem (Maathuis et al., 1997). Recent data show that K^+ efflux is important for sensing of reactive oxygen species (ROS) during stress reactions and induction of programmed cell death (PCD) (Demidchik et al., 2003b, 2010; Demidchik, 2010, 2012a,b). The aim of this paper is to summarise data on mechanisms of K^+ efflux from plant cells and physiological roles of this phenomenon in roots.

The K^+ leakage is usually detected almost instantaneously after the application of a stress factor and lasts from a few minutes to several hours (or even days). Note that it requires the movement of so-called counterions (Cl^-, HPO_4^{2-}, NO^{3-}, $citrate^{3-}$, and $malate^{2-}$) to balance the efflux of positively charged potassium ions (Palta et al., 1977). In the case of NaCl-induced stress, the K^+ efflux is balanced by influx of Na^+ and hypothetically does not require movement of counterions. Moreover, in the case of depolarisation, K^+ efflux directly causes repolarisation of the plasma membrane, which does not require movement of counterions. The focus of this paper is on K^+ efflux; therefore the anion efflux will not be discussed here in details.

K^+ efflux from roots is a common reaction to stress

The leakage of electrolytes from damaged plant tissues, including roots, has been known for almost a century (Osterhaut, 1922; Dexter et al., 1932). It has been routinely used to assess the quantity of dead cells, particularly in the assessment of plant freezing tolerance. However, in 1977, Palta and co-authors reported that cells treated by freezing–thawing cycle do not die, although they release K^+ and organic anions (Palta et al., 1977). Since the membrane permeability to water and other inorganic ions was not changed, these authors concluded that "freezing increased passive permeability of cells to K^+ and possibly to counterion(s)". In the modern sense this means "freezing activated K^+ permeable channels in the cell membrane".

Potassium efflux can also be triggered in root cells under pathogen attack (Atkinson et al., 1985, 1990, 1996; Ebel and Mithofer, 1998; Blatt et al., 1999; Maffei et al., 2007), salinity (Nassery, 1975, 1979; Maathuis and Amtmann, 1999; Shabala et al., 2006; Demidchik et al., 2010), heavy metals (De Vos et al., 1991; Murphy and Taiz, 1997; Demidchik et al., 2003b), oxidative stress (Demidchik et al., 2003b, 2006), high soil acidity (pH < 4) (Marschner et al., 1966), wounding (Nassery, 1971, 1972), waterlogging (Shabala, 2011) and other stresses.

Pathogen-induced K^+ efflux

Thousands of reports have been published on the problem of electrolyte leakage from plant tissues, induced by pathogens, as bacteria, viruses, fungi and insects. In most cases, the major 'leaking' electrolyte species was K^+ (Ebel and Mithofer, 1998; Maffei et al., 2007). The story has begun in the 1980s, when Atkinson and co-authors have found that pathogen elicitors induce K^+ efflux from suspension-culture cells derived from tobacco and soybean plants (Atkinson et al., 1985, 1990, 1996). This reaction activated in few minutes and lasted approximately one hour. Elicitor activated K^+ efflux was accompanied by Ca^{2+} influx, H^+ efflux and generation of ROS and was followed by development of programmed cell death (PCD) symptoms. These reactions delayed in the presence of non-specific cation channel antagonists (La^{3+}, Gd^{3+} and Co^{2+}), which can block all types of cation channels.

Nowadays, generation of ROS and influx of Ca^{2+} are believed to be a major reason of plant PCD during so-called 'hypersensitive response' to pathogens. Some data suggest that K^+ efflux is tightly linked to ROS production and may also be involved in PCD (Demidchik et al., 2010). Both production of $^{\bullet}OH$ and K^+ leakage has been observed in plants treated with different pathogenic elicitors, originating, for example, from *Alternaria alternate* (Jennings et al., 2002), *Botrytis cinerea* (Govrin et al., 2006), *Cladosporium fulvum* (Veraestrella et al., 1992; Blatt et al., 1999) and *Magnaporthe grisea* (Pasechnik et al., 1998). Some of these elicitors (*Cladosporium fulvum*) have been shown to activate the plasma membrane outwardly directed K^+ conductance (Blatt et al., 1999). In root cells, TEA^+-sensitive K^+ efflux has been found in *Arabidopsis thaliana* treated with the *Trichoderma viride* elicitor cellulysin (Demidchik et al., 2010). ROS scavengers or inhibitors of ROS-producing enzymes, cation channel antagonists as well as overexpression of antioxidant systems and specific defence proteins can prevent or significantly delay plant responses to pathogens, including K^+ efflux (Ebel and Mithofer, 1998; De Gara et al., 2003; Pike et al., 2005; Shetty et al., 2008; Demidchik et al., 2010). The $^{\bullet}OH$ activated K^+ outwardly rectifying channel, which has recently been identified in Arabidopsis roots, provides a direct link between pathogen induced ROS generation and K^+ release (Demidchik et al., 2003b, 2010).

Nevertheless, the activation of K^+ efflux by pathogen elicitors is not ubiquitous. Errakhi et al. (2008) have shown that thaxtomin A isolated from the pathogenic streptomycete bacterium *Streptomyces scabie*, which damages roots, induces PCD in *Arabidopsis thaliana* culture cells through cation channel dependent mechanism. However, this elicitor inhibited K^+-selective outwardly rectifying channels (although it activated Ca^{2+} influx channels). Zhang et al. (2008) have obtained similar data on Arabidopsis guard cell K^+ efflux channels treated with the bacterial flg22 elicitor. Both K^+ influx and K^+ efflux channels were inhibited by flg22 in a G-protein dependent manner; the reaction was not observed in *gpa-1* plants lacking the G-protein α subunit. Thus, the modulation of K^+ efflux by elicitors is a complex phenomenon, which includes both activation and inhibition, depending on a given type of the plant–pathogen interaction.

Potassium efflux in response to salinity

Nassery (1975, 1979) has shown that barley, wheat, chick pea and bean roots that were treated by acute NaCl stress (>50 mM) rapidly release K^+. This reaction was inhibited by addition of millimolar [Ca^{2+}] to the external solution and stimulated by respiratory drugs. K^+ release was not induced by equimolar concentrations of mannitol; therefore, the author concluded that this reaction was not related to osmotic stress. Nowadays, K^+ efflux in response to