



Review

# ROS-Activated Ion Channels in Plants: Biophysical Characteristics, Physiological Functions and Molecular Nature

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**Abstract:** Ion channels activated by reactive oxygen species (ROS) have been found in the plasma membrane of charophyte *Nitella flexilis*, dicotyledon *Arabidopsis thaliana*, *Pyrus pyrifolia* and *Pisum sativum*, and the monocotyledon *Lilium longiflorum*. Their activities have been reported in charophyte giant internodes, root trichoblasts and atrichoblasts, pollen tubes, and guard cells. Hydrogen peroxide and hydroxyl radicals are major activating species for these channels. Plant ROS-activated ion channels include inwardly-rectifying, outwardly-rectifying, and voltage-independent groups. The inwardly-rectifying ROS-activated ion channels mediate Ca<sup>2+</sup>-influx for growth and development in roots and pollen tubes. The outwardly-rectifying group facilitates K<sup>+</sup> efflux for the regulation of osmotic pressure in guard cells, induction of programmed cell death, and autophagy in roots. The voltage-independent group mediates both Ca<sup>2+</sup> influx and K<sup>+</sup> efflux. Most studies suggest that ROS-activated channels are non-selective cation channels. Single-channel studies revealed activation of 14.5-pS Ca<sup>2+</sup> influx and 16-pS K<sup>+</sup> efflux unitary conductances in response to ROS. The molecular nature of ROS-activated Ca<sup>2+</sup> influx channels remains poorly understood, although annexins and cyclic nucleotide-gated channels have been proposed for this role. The ROS-activated K<sup>+</sup> channels have recently been identified as products of Stellar K<sup>+</sup> Outward Rectifier (*SKOR*) and Guard cell Outwardly Rectifying K<sup>+</sup> channel (*GORK*) genes.

**Keywords:** reactive oxygen species; ROS; ion channels; calcium signaling; potassium ions; electrolyte leakage; copper ions; hydroxyl radicals; plant growth regulation; plant stress physiology

## 1. Introduction

Ion channels are proteinaceous pores in membranes that play indispensable roles in plant physiology. Originally, they were explored as systems for uptake of minerals in ionic form for nutritional and osmotic needs and generation of the electric potential difference across the membrane [1]. Over the last two to three decades, ion channels have been shown to be involved in much more than initially expected [2–5]. New functions included elongation growth of root cells [6] and pollen tube [7], regulation of programmed cell death [8], sophisticated stomatal behavior [9–11], control of stress-induced signaling and electrolyte leakage [12–14], and even the regulation of photosynthesis [15,16]. One of the recent findings is that ion channels are involved in redox-dependent processes and are capable of sensing reactive oxygen species (ROS), which are abundantly synthesized by plant cells during expansive growth, and response to hormones and stresses [10,12,13,17–21].

ROS-activated ionic conductances in plant membranes were originally reported in relation to  $\text{Cu}^{2+}$  toxicity in the charophyte algae *Nitella flexilis* by Demidchik et al. [17,22]. We now know that their functions are much broader. Modern schemes of plant regulatory networks, such as the action of signaling molecules, stress perception, and stimulation of elongation growth have included the ROS-activated channels as key components [23,24]. The interest in these systems is in linking transmembrane fluxes of  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$  and other ions to the production of ROS. Both phenomena are ubiquitous and crucial for plant life. Acting in concert, ROS-generating, and  $\text{Ca}^{2+}$ - and  $\text{K}^{+}$ -transporting systems can control most physiological and pathophysiological reactions. The purpose of this review is to summarize available data on ROS-activated channels in plants with an emphasis on their biophysical properties, physiological functions, and genetic background.

## 2. First Observations of ROS-Activated Ion Fluxes: Electrolyte and $\text{K}^{+}$ Leakage in Response to Stresses

The first evidence for stimulation of ion fluxes in plant tissues treated with ROS was obtained from measurements of so-called electrolyte leakage caused by a transition metal copper ions— $\text{Cu}^{+}/^{2+}$ , which is a free radical capable of donating and accepting electrons from hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), superoxide anion radicals ( $\text{O}_2^{\bullet-}$ ), nitric oxide ( $\bullet\text{NO}$ ) and other ROS and reactive nitrogen species (RNS) [14,25].  $\text{Cu}^{+}$  reduces oxygen in  $\text{H}_2\text{O}_2$  molecule to extremely reactive hydroxyl radical ( $\text{HO}\bullet$ ), in the Haber-Weiss cycle based on Fenton-like reactions [25–29]. McBrien and Hassall [30] treated *Chlorella vulgaris* cells with copper ions and found a dramatic loss of  $\text{K}^{+}$  from these cells, accounting for 94% of all cell  $\text{K}^{+}$  at approximately 0.3 mM  $\text{CuSO}_4$ . Detectable  $\text{K}^{+}$  activities in medium were measured in the few minutes after the addition of copper ions. Very similar rapid  $\text{K}^{+}$  release was found in tests on two species of marine algae, *Dunaliella tertiolecta* and *Phaeodactylum tricorutum* treated with copper ions [31]. Studying the sensitivity of *Chlorella* cells to heavy metal ions and oxidants, De Filippis [32] showed that not only  $\text{Cu}^{2+}$ , but also  $\text{Hg}^{2+}$ , as well as methylmercuric chloride and phenylmercuric acetate, can induce  $\text{K}^{+}$  efflux [32]. All these agents are redox-active and can produce ROS.

The first evidence for ROS-induced ion fluxes in higher plants was probably obtained in the study of electrolyte leakage from  $\text{Cu}^{2+}$ -treated *Agrostis tenuis* plants [33]. In these experiments, root growth inhibition and  $\text{K}^{+}$  efflux were measured and compared in  $\text{Cu}^{2+}$ - and  $\text{Zn}^{2+}$ -treated *Agrostis tenuis* populations with different sensitivities to these heavy metal ions. Experiments demonstrated that  $\text{Zn}^{2+}$  was without effect on the release of  $\text{K}^{+}$  from the root tips, but all the concentrations of  $\text{Cu}^{2+}$  tested (0.01–1 mM) caused dramatic efflux of  $\text{K}^{+}$ , although the effect was less in the case of the Cu-tolerant populations than for the others. Similar results were obtained for  $\text{Cu}^{2+}$ -treated *Silene cucubalus* roots [34] and *Avena sativa* leaves [35]. These tests were additionally accompanied by treatment with SH-oxidising and ROS-generating reagents, such as *N*-ethylmaleimide, cumene hydroperoxide and *p*-chloromercuribenzoate, which all caused effects similar to  $\text{Cu}^{2+}$ , demonstrating that  $\text{Cu}^{2+}$ -induced ROS, but not the specific characteristics of copper, are the reason of  $\text{K}^{+}$  efflux from cells. An involvement of potassium channels in  $\text{K}^{+}$  release was finally demonstrated by Murphy and Taiz [36] and Murphy et al. [37] working on *Arabidopsis thaliana* varieties with different copper sensitivity. They found that  $\text{K}^{+}$  loss from *Arabidopsis thaliana* roots is a good indicator of general sensitivity of plants to  $\text{Cu}^{2+}$ , and that it is inhibited by a specific  $\text{K}^{+}$  channel blocker—tetraethylammonium ions ( $\text{TEA}^{+}$ ). Further studies of  $\text{K}^{+}$  efflux from *Arabidopsis thaliana* root demonstrated that  $\text{Cu}^{2+}$  ions can induce hydroxyl radical production in intact roots which increases upon addition of  $\text{H}_2\text{O}_2$  and ascorbate [12]. Using Microelectrode Ion Flux Estimation™ (MIFE™) technique, Demidchik et al. [12,13] showed that the hydroxyl radical-generating mixture containing  $\text{Cu}^{2+}$ ,  $\text{H}_2\text{O}_2$ , and ascorbate causes massive and reproducible  $\text{TEA}^{+}$ -sensitive  $\text{K}^{+}$  efflux from *Arabidopsis*, pea, corn, spinach, wheat, and clover roots, which was lost in the *Arabidopsis*  $\text{K}^{+}$  channel knockout mutant *Atgork1-1*, lacking  $\text{K}^{+}$  efflux channel AtGORK (*Arabidopsis thaliana* Guard cell Outwardly Rectifying  $\text{K}^{+}$  channel).

ROS generation is ubiquitous in most stresses, such as salinity, freezing, pathogen attack, drought, heavy metals, hypoxia, ultraviolet, ozone, and many others [29]. Intriguingly, cation channel-mediated

electrolyte leakage always accompanies these stresses [14]. It can be speculated that  $K^+$  leakage during stress response is induced by ROS (in addition to commonly observed depolarisation). The idea of an ion channel mechanism of stress-induced electrolyte/ $K^+$  efflux was proposed by Palta et al. (1977) [38], based on studies of freezing tolerance, which has now been shown to be dependent on ROS generation [39]. Atkinson et al. (1985, 1990, 1996) found the sensitivity of pathogen-induced  $K^+$  efflux to cation channel blockers, such as  $La^{3+}$ ,  $Gd^{3+}$ , and  $Co^{2+}$ , and concluded that there was an involvement of cation channels [40–42]. It is becoming clear that stresses caused by pathogens rely on de novo ROS synthesis and sometimes severe oxidative stress [43]. Electrolyte and  $K^+$  leakage induced by high levels of NaCl was observed in the 1960s [44]. Originally, this effect was attributed to the non-specific membrane damage. However, in the 1970s, Nassery (1975, 1979) provided evidence that high levels of NaCl specifically induces efflux of  $K^+$ , but not other ions. Analyzing  $K^+$  efflux from wheat, barley, bean, and chick pea roots, Nassery (1975, 1979) demonstrated that this reaction was sensitive to  $K^+$  channel blockers and that it was not induced by osmotic stress [45,46]. Similar reactions have been found in drought- and heat-treated plants, which demonstrated dramatic electrolyte/ $K^+$  efflux [47,48]. Recent findings showed that NaCl can trigger production of hydroxyl radicals in roots of higher plants, which stimulate  $K^+$  efflux via  $K^+$  channel GORK activation [13].

In parallel to  $K^+$  release studies, Price [49] and Price et al. [50], using *Commelina communis* leaf epidermal strips and aequorin-transformed *Nicotiana plumbaginifolia* seedlings, demonstrated that exogenously applied ROS or ROS-generating chemicals (0.05–1 mM  $H_2O_2$  and 0.1 mM paraquat) can induce influx of  $Ca^{2+}$  from extracellular space to the cytosol transiently increasing cytosolic free  $Ca^{2+}$ . Effects were inhibited by verapamil, a  $Ca^{2+}$  channel antagonist, which is widely used in animal ion channel studies, pointing to involvement of ROS-activated  $Ca^{2+}$ -permeable ion channels. Clayton et al. [51] demonstrated very similar elevation of cytosolic  $Ca^{2+}$  in response to high levels of  $O_3$ , which is a critically important abiotic stress factor and, at the same time, it is a ROS. This increase in cytosolic  $Ca^{2+}$  was inhibited by  $La^{3+}$ , which is a non-specific inhibitor of cation channels in plants. Demidchik et al. [12,18], utilising  $Ca^{2+}$ -aequorin luminometry and MIFE techniques, found that  $HO^\bullet$ -generating mixtures ( $Cu^{2+}$ ,  $H_2O_2$  and ascorbate) induced  $Ca^{2+}$  influx, which was sensitive to the range of cation channel blockers and antioxidants.

### 3. $Cu^{2+}$ -Activated Non-Selective Cation Conductances in Charophyte Algae

Following the observations of  $Cu^{2+}$ -induced ion leakage from alga cells [30–32], Demidchik et al. [17,22] examined effects of  $Cu^{2+}$  on major types of ionic currents in charophyte *Nitella flexilis* and discovered  $Cu^{2+}$ -activated cation conductances, which were rapidly activated after the addition of  $Cu^{2+}$  to the bathing solution. This microelectrode voltage-clamp study had an advantage in the preservation of the cell wall, where  $H_2O_2$  and ascorbate can catalyse  $HO^\bullet$  generation in the presence of  $Cu^{2+}$  [29], with minimal cell damage since it was impaled just by one microelectrode (compared to patch-clamp techniques). Demidchik et al. [17,22,52] showed that exposure of intact *Nitella flexilis* cells to redox-active transition metals,  $Cu^{2+}$  and  $Fe^{3+}$  (5–100  $\mu M$ ), induced voltage-independent rapidly activating conductance, which showed permeation of different cations, but did not allow passage of anions [17,22,52]. This conductance was blocked by nifedipine ( $Ca^{2+}$  channel blocker) and had temperature coefficient  $Q_{10}$  between 1.2 and 1.6, suggesting the involvement of an ion channel-based mechanism rather than of active transporters, which have higher  $Q_{10}$ .  $Cu^{2+}$ -activated cation conductance in *Nitella flexilis* explained the mechanism of high heavy metal toxicity in green alga. Increase in non-specific cation efflux caused by  $Cu^{2+}$  resulted in irreversible collapse of the electric potential across the plasma membrane and stable loss of cell turgor. Thus alga cells died due to activation of the cation channels by very low concentrations of  $Cu^{2+}$  (10–30  $\mu M$ ). The effect of cell death was prevented by addition to the bathing solution of cation channels blockers within 20–30 min after  $Cu^{2+}$  application. These studies also demonstrated that  $Cu^{2+}$  blocked *Nitella* anion channels and  $H^+$ -ATPase, demonstrating specificity of the effect on the non-selective conductance [17,22].

#### 4. Ca<sup>2+</sup> Influx Activated by H<sub>2</sub>O<sub>2</sub> in Leaves

The first report on ROS-activated cation channel in leaves of higher plants was published in 2000 by Pei et al. [10] who studied Ca<sup>2+</sup>-permeable channels in guard cells in relation to the mechanism of stomata closure. These authors have found that exogenously applied H<sub>2</sub>O<sub>2</sub>, which was probably produced in response to abscisic acid, activated Ca<sup>2+</sup>-permeable non-selective cation channels in the guard cell plasma membrane. The channels were sensitive to phosphorylation status [53]. In *abi2-1* protein phosphatase mutants, the H<sub>2</sub>O<sub>2</sub>-mediated activation of guard cell Ca<sup>2+</sup>-permeable channels was suppressed and was insensitive to abscisic acid [24]. Further biochemical analyses of phosphatase 2C (PP2C) showed that this protein is directly inhibited by H<sub>2</sub>O<sub>2</sub> and could be one of the prime targets for H<sub>2</sub>O<sub>2</sub> in guard cells [54]. Another possible scenario is that PP2C dephosphorylates some intermediate regulators controlling gating of ROS-activated Ca<sup>2+</sup>-permeable channels [55]. Moreover, the phosphatase can block NADPH oxidase activation and ROS production evoked by abscisic acid, resulting in no ROS generation and no channel activation [24].

The function of ROS-activated Ca<sup>2+</sup>-permeable channels in guard cell plasma membrane relates to the need for decreasing the turgor pressure when stomata close under drought stress. Ca<sup>2+</sup> influx increases the cytosolic Ca<sup>2+</sup>, which activates anion channels, efflux of anions and water loss from guard cells needed for stomatal closure [53]. The guard cell ROS-activated Ca<sup>2+</sup>-permeable channels are activated by NADPH oxidase-generated ROS while the NADPH oxidase, in turn, is stimulated by the drought stress phytohormone, abscisic acid (reviewed by Murata et al. [24]). Moreover, salicylic acid can also participate in this reaction but via peroxidase-dependent ROS production, resulting in very similar Ca<sup>2+</sup> influx-mediated phenomena (reviewed by Sierla et al. [56]). Intriguingly, Wu et al. [57] have recently shown that leaf mesophyll ROS production for activation of K<sup>+</sup> efflux can be catalyzed by disruption of electron flow in the chloroplast electron transport chains. This suggests a different pattern of regulation comparing to the guard cells.

#### 5. ROS-Activated Cation Channels in Roots

In 2003, Demidchik et al. [12] and Foreman et al. [6] provided the first evidence that ROS-activated cation channels exist in roots of higher plants. The idea that, similar to charophytes, Cu<sup>2+</sup> and hydroxyl radicals can activate ionic conductances in the plasma membrane of higher plants was tested using *Arabidopsis thaliana* roots [6,12,13,18,58]. The protoplasts, which are used in patch-clamp studies, lack apoplastic H<sub>2</sub>O<sub>2</sub> and ascorbate (because they do not contain cell walls), which can be required for hydroxyl radical production by Cu<sup>2+</sup> [59]. Therefore, Cu<sup>2+</sup> was applied to root epidermal protoplasts together with ascorbate (Cu/Asc) or as three-component mixture of Cu<sup>2+</sup>, ascorbate and H<sub>2</sub>O<sub>2</sub> (Cu/Asc/H<sub>2</sub>O<sub>2</sub>). These mixtures are used by animal physiologists studying ROS-activated conductances [60]. Demidchik et al. [12] demonstrated that Cu/Asc activated two types of conductances in protoplasts isolated from root mature epidermis, including Ca<sup>2+</sup>-permeable inwardly-directed and K<sup>+</sup>-selective outwardly-directed conductances, respectively. The Ca<sup>2+</sup>-permeable conductance was voltage-independent and non-selective, showing the following permeability series: K<sup>+</sup> (1.00) ≈ NH<sub>4</sub><sup>+</sup> (0.91) ≈ Na<sup>+</sup> (0.71) ≈ Cs<sup>+</sup> (0.67) > Ba<sup>2+</sup> (0.32) ≈ Ca<sup>2+</sup> (0.24) > TEA<sup>+</sup> (0.09). The K<sup>+</sup> efflux conductance demonstrated typical Shaker-like outward rectification with relatively high selectivity for K<sup>+</sup>: K<sup>+</sup> (1.00) > Na<sup>+</sup> (0.31) >> Ba<sup>2+</sup> (0.06) > TEA<sup>+</sup> (0.05). These selectivity series were typical for *Arabidopsis thaliana* 'K<sup>+</sup> outward rectifiers' (KOR) reported by [61]. Surprisingly, the kinetics of activation over time for *Arabidopsis* cation currents after Cu/Asc addition was similar to the time-dependence of Cu<sup>2+</sup>-induced conductances in *Nitella*, suggesting the involvement of a similar activation mechanism. Interestingly, Cu<sup>2+</sup> added without ascorbate did not activate conductances in protoplasts [6,12] although it caused elevation of cytosolic free Ca<sup>2+</sup> in intact roots [12] and induced currents in intact *Nitella* cells [17]. This indicates that apoplastic ascorbate and H<sub>2</sub>O<sub>2</sub> are probably required for HO• generation via the Haber-Weiss cycle in the cell wall. Hydrogen peroxide for these reactions is likely to be produced by NADPH oxidases because, when cell wall peroxidases were removed by the protoplast isolation procedure, Cu/Asc was still capable of activating currents [6].

Moreover, knockout mutants lacking NADPH oxidase RBOHC, were deficient in  $\text{Ca}^{2+}$  flux needed for root cell elongation [6]. Peroxidase and oxidase sources of ROS in the apoplastic space can be important during stress responses, but the evidence that this source is involved in activation of ion channels is still missing [29].

Based on the higher ROS-activated  $\text{Ca}^{2+}$  conductance in growing cells compared to mature non-growing cells, and inhibition of root hair growth by cation channel antagonists, a mechanism of cell expansive and polar growth, has been proposed by [6]. According to this mechanism, the localised elevation of ROS-activated  $\text{Ca}^{2+}$  influx in growing cell parts leads to up to 10 times higher  $\text{Ca}^{2+}$  activity in the cytosol, which stimulates exocytosis and the delivery of new cell structural material [6,62–64]. NADPH oxidase provides most ROS for this  $\text{Ca}^{2+}$  loading [6,62]. This mechanism has been confirmed in other species, for example in young roots of *Mesembryanthemum crystallinum* [65] and *Salix nigra* [66]. ROS-activated  $\text{Ca}^{2+}$  influx in growing root cells is up-regulated by auxin (a major hormone inducing root growth) via stimulation of expression of NADPH oxidases and peroxidases by transcription factor RSL4, which in turn is transcriptionally regulated by auxin through several auxin response factors [67]. Apart from stimulation of root cell elongation, this mechanism is also involved in formation the lysigenous aerenchyma in rice roots in hypoxia conditions [68]. NADPH oxidases may not be the only system for ROS generation in root cell growth. Peroxidases can also participate in this process [69].

Outer layers of cells in different plants organs, such as guard cells, leaf and root epidermis, and particularly in the root tip, are the first to sense new environments. While the root elongates, the root tip explores new areas providing first contact with stresses as well. Internal tissues, such as the pericycle or vascular system, do not have contact with the soil and may not be involved in primary stress signalling. Supporting this,  $\text{HO}^{\bullet}$ -activated  $\text{Ca}^{2+}$  influx currents in root elongation zone and tips of root hairs was larger than in other tissues [12]. For example, it was 10 to 12 times larger in elongation zone cells than in cells of pericycle [12]. This indicates that  $\text{Ca}^{2+}$ -permeable conductances responding to  $\text{HO}^{\bullet}$  are necessary for both growth and sensing a new environment. Sidedness of ROS application, developmental stage of cells and potential source of generation are major factors altering activation of cation-permeable conductances in the root cell plasma membrane [5,18]. In contrast to the guard cells,  $\text{H}_2\text{O}_2$  was unable to activate currents in mature *Arabidopsis thaliana* root epidermal cells when it was applied inside and outside the pipette in a whole-cell patch-clamp configuration or added outside in excised outside-out patches [12,18]. However,  $\text{H}_2\text{O}_2$  activated  $\text{Ca}^{2+}$ -permeable channels when it was applied to excised outside-out patches at the cytoplasmic side. This shows that  $\text{H}_2\text{O}_2$  should be delivered directly to the channel inside mature epidermal cells. Nevertheless, in protoplasts from young cells, exogenously applied  $\text{H}_2\text{O}_2$  was capable of activating  $\text{Ca}^{2+}$  currents [18]. This suggests that ROS-activated channels in different tissues/cells may be encoded by different genes or that younger root cells (and guard cells) may have a higher density of  $\text{H}_2\text{O}_2$ -permeable aquaporins which facilitate  $\text{H}_2\text{O}_2$  delivery to the cytosol [70–73]. Hypothetically, elongating root cells and guard cells can have higher catalytic activities of transition metal ions converting  $\text{H}_2\text{O}_2$  to hydroxyl radicals than mature root epidermal cells.

Apart from *Arabidopsis thaliana*, ROS-activated  $\text{Ca}^{2+}$ -permeable channels were investigated in detail in *Pisum sativum* mature root cells [21]. In patch-clamped root protoplasts of this species, hydroxyl radicals but not  $\text{H}_2\text{O}_2$  activated a  $\text{Ca}^{2+}$ -permeable nonselective conductance, which was sensitive to cation channel blockers ( $\text{Gd}^{3+}$ , nifedipine and verapamil) as well as to anion channel blockers (5-nitro-2(3-phenylpropylamino)-benzoate and niflumate). Interestingly, the same pharmacology was seen on  $\text{K}^+$  efflux and  $\text{Ca}^{2+}$  influx of  $\text{HO}^{\bullet}$ -treated intact pea roots (analysed by MIFE system). These authors also tested effect of polyamines on  $\text{HO}^{\bullet}$ -activated  $\text{K}^+$  and  $\text{Ca}^{2+}$  conductances. Polyamines are key endogenous stress protectants in higher plants. Their levels increase under stress conditions, such as salinity or drought and facilitate adaptation [74]. Intriguingly, polyamines (spermine, spermidine, and putrescine) stimulated the  $\text{HO}^{\bullet}$ -induced  $\text{Ca}^{2+}$  and  $\text{K}^+$  currents and fluxes in pea roots [21]. Authors of this study suggested that polyamines directly (chemically) interact with ROS increasing their reactivity. Hypothetically, polyamines can interact with ROS-activated channels

by penetrating the pore and keeping it in open state that can be important for ROS-induced activation. Recent data showed that ROS-sensing groups in  $K^+$  channels activated by ROS become accessible to extracellular substances only when the channel is activated by depolarisation [75]. In the case of ROS-activated  $Ca^{2+}$ -permeable channels reported by Zepeda-Jazo et al. [21], the accessibility of ROS-sensing group probably increases, when polyamines interact with the pore of the channel complex. Moreover, it should also be considered that polyamine radicals could have a longer half-life than  $HO^\bullet$ .

Single-channel characteristics of ROS-activated cation channels have been reported twice [13,18]. Hydrogen peroxide-activated  $Ca^{2+}$  influx unitary conductance was 14.5 pS (bath: 20 mM  $CaCl_2$ ; pipette: 25 mM Kgluconate, 5 mM KCl, 1 mM  $H_2O_2$ ) [18]. The ROS-activated  $K^+$  channel unitary conductance was 16 pS (bath: 1 mM KCl, 0.3 mM  $CaCl_2$ ; pipette: 70 mM Kgluconate, 10 mM KCl) [13]. These data indicate that ROS activated one  $Ca^{2+}$ -permeable channel and one  $K^+$ -permeable channel. The gene of  $K^+$  channel activated by ROS was identified as *GORK*.

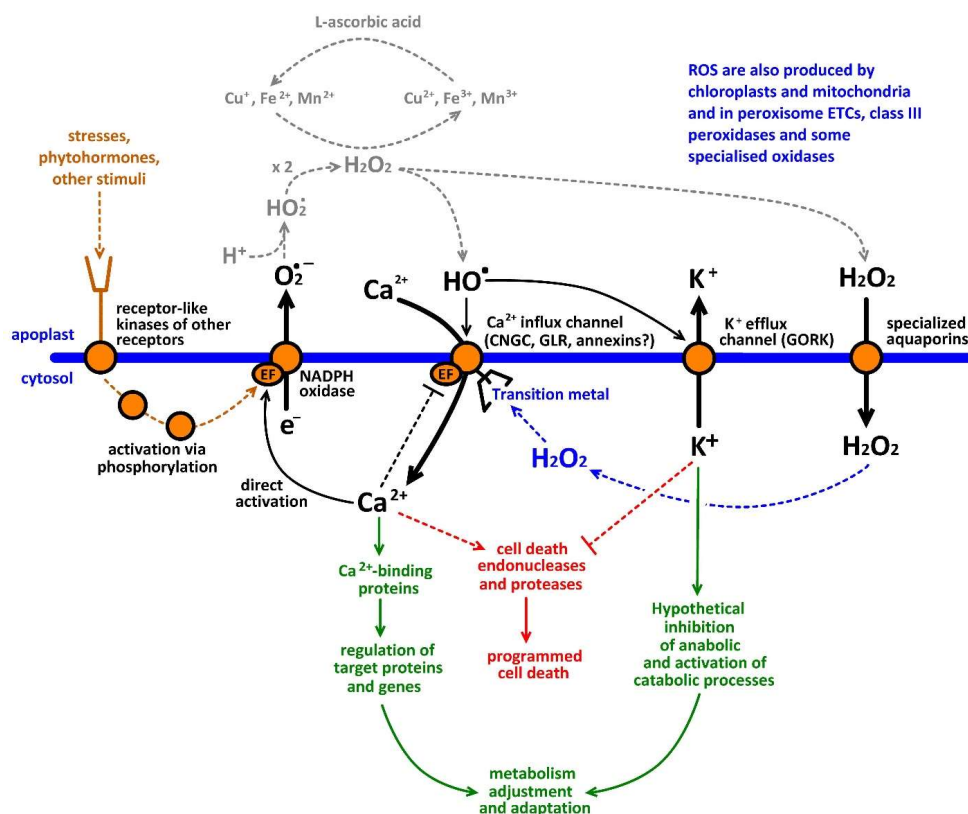
## 6. Structure and Function of ROS-Activated $K^+$ Efflux Channels

Although major focus of ROS signaling in relation to ion channel activities has always been the  $Ca^{2+}$  influx, which is involved in growth, signalling and stress responses, another group of ROS-evoked events, which are related to ROS-activated  $K^+$  efflux channels, may be as important. The activation of  $K^+$  efflux channels by  $HO^\bullet$  was demonstrated under salt stress conditions and pathogen attack [13]. Tests with electron paramagnetic resonance spectroscopy and the range of electrophysiological techniques showed that treatment of intact roots by high (NaCl) (100 and 250 mM) stimulated  $HO^\bullet$  production leading to  $K^+$  channel activation and  $K^+$  efflux from roots [13]. Hydroxyl radicals and NaCl caused programmed cell death (PCD) and collapse of membrane potential in root cells of *Arabidopsis thaliana* in a  $K^+$ -dependent manner (blocked by  $TEA^+$ ). These effects were delayed in plants, lacking functional  $K^+$  efflux channel *AtGORK* (*Atgork1-1*). *Atgork1-1* plants showed no  $K^+$  efflux (measured by MIFE), nor  $K^+$  outwardly-directed currents (measure by patch-clamp) in response to  $HO^\bullet$ . They also demonstrated much smaller  $K^+$  efflux after exposure to high NaCl levels, pathogen elicitors, hypoxia and other treatments [13,76]. *GORK* transcription was up-regulated upon onset of drought, salt stress and cold [77]. This effect was probably related to ROS production caused by stresses, because the level of *GORK* channel transcript significantly increased in the presence of  $O_2^{\bullet-}$  leading to an increased activity of this channel [78]. Identification of root  $HO^\bullet$ -activated  $K^+$  efflux channels (*GORK*) has linked stress induced ROS generation and electrolyte/ $K^+$  leakage [12,13]. Electrolyte leakage is a hallmark of stress in plant physiology [14]. However, until recently the mechanism of this phenomenon was not understood. According to the recently proposed metabolic adjustment hypothesis,  $K^+$  leakage mediated by *GORK* could also play the role of a 'metabolic switch', which decreases the rate of anabolic reactions and stimulates catabolic processes, causing the release of energy for adaptation and repair needs [14].

The  $HO^\bullet$ -activated  $K^+$  efflux mediated by *GORK* channels also explains how stress-induced ROS generation leading to  $K^+$ /electrolyte leakage triggers PCD and autophagy. Demidchik et al. [13] demonstrated that  $K^+$  loss by root cells dramatically enhances the activities of endonucleases and proteases. This was accompanied by a set of cytological PCD symptoms. In animal cells,  $K^+$ -dependent caspases and endonucleases are major players in PCD [79]. They are directly inhibited by high cytosolic  $K^+$  concentrations, but  $K^+$  loss through  $K^+$ -permeable ion channels can release their activities [80]. Similar to plants, the cytoplasmic  $K^+/Na^+$  ratio is a major parameter regulating animal PCD [81,82]. Sodium ions cannot substitute for  $K^+$  in its protease inhibition reaction therefore high  $Na^+$  levels ultimately lead to onset of PCD [80,81]. Various death factors can stimulate animal  $K^+$  efflux channels promoting increased protease and endonuclease activities [83]. Identification of ROS-activated  $K^+$  efflux channels in plants and characterisation of its role in PCD demonstrates that a similar mechanism of hydrolase activation exists in plants.  $K^+$ -modulated PCD is also shared by fungi [84,85].

## 7. Pollen Tube ROS-Activated Channels

Pollen tubes elongate using similar ion channel-mediated mechanism as root hairs (Figure 1). This mechanism is based on polar (apical) ROS-activated loading of  $\text{Ca}^{2+}$ , which induces cytoskeleton rearrangement facilitating exocytosis and delivery of new cell wall material to the growing part of the cell [2,67,86]. Interestingly, growth-related gene expression is shared between pollen tubes and root hairs [77]. Similar to root hairs, a steep  $\text{Ca}^{2+}$  activity gradient in the tip of elongating pollen tubes is regulated via ROS produced by the NADPH oxidase [87]. Wu et al. [57] demonstrated that exogenous  $\text{H}_2\text{O}_2$  rapidly activated plasma membrane inwardly-rectifying  $\text{Ca}^{2+}$ -permeable conductance in pear pollen tube protoplasts. Breygina et al. [88] found very similar  $\text{H}_2\text{O}_2$ -activated conductances in lily pollen grain protoplasts. Breygina et al. [88] also demonstrated activation of weak  $\text{K}^+$  efflux current by  $\text{H}_2\text{O}_2$  in lily pollen tube, also similar to root cells. Among the putative  $\text{Ca}^{2+}$ -permeable ion channels, cyclic nucleotide-gated channels (CNGC7, CNGC8, CNGC16, and CNGC18), ionotropic glutamate receptors (GLR1.2, GLR1.4, GLR3.4, GLR3.7), 'Reduced hyperosmolarity-induced  $[\text{Ca}^{2+}]_i$  increase 1' (OSCA1) and 'Mechanosensitive channel of small conductance-like 8' (MSL8) are expressed in pollen tube [86]. Moreover, a number of  $\text{K}^+$  channels, potentially including stellar  $\text{K}^+$  outward rectifier (SKOR), which can be activated by ROS, are expressed in the pollen tube [86]. Nevertheless, a direct link between gene and function is still missing in the case of ROS-activated ion channels in this important model. Recent studies showed that the mechanosensitive OSCA1 channel, which is responsible for  $\text{Ca}^{2+}$  entry to the cytosol due to osmolarity changes, is insensitive to  $\text{H}_2\text{O}_2$  [89].



**Figure 1.** The concept of reactive oxygen species (ROS)- $\text{Ca}^{2+}$ -hub in the plasma membrane of higher plants. Stresses and regulatory stimuli can react with cell surface receptors leading to the activation of NADPH-oxidases (or directly activate ion channels). NADPH oxidases produce superoxide ( $\text{O}_2^{\bullet-}$ ), which binds  $\text{H}^+$  and forms hydroperoxyl radicals ( $\text{HO}_2^{\bullet}$ ), which undergo dismutation reaction forming  $\text{H}_2\text{O}_2$ .  $\text{H}_2\text{O}_2$  can be reduced in Haber-Weiss cycle using electrons from transition metals, which can be reduced by L-ascorbic acid.  $\text{H}_2\text{O}_2$  reduction leads for formation of  $\text{HO}^{\bullet}$  in the close proximity (clusters) to ROS-activated  $\text{Ca}^{2+}$ -permeable cation channels. Cell wall transition metals are required for Haber-Weiss

cycle in the apoplast. Channels are activated in response to increased  $\text{HO}^\bullet$  production and mediate  $\text{Ca}^{2+}$  influx and  $\text{K}^+$  efflux. Hydrogen peroxide can hypothetically activate channels from inside via reaction with transition metal binding sites (via generation of  $\text{HO}^\bullet$ ). ROS-induced cytosolic  $\text{Ca}^{2+}$  elevation results in activation of signaling and regulatory cascades, stimulation of exocytosis and growth as well as induction of programmed cell death. ROS-activated efflux of  $\text{K}^+$  can lead to stimulation of programmed cell death, autophagy or metabolic adjustment, which is required for changing plant metabolism during stress responses and releasing energy for reparation needs [14].

## 8. Annexins as Potential ROS-Activated Ion Channels

Annexins are proteins, which are normally located in the cytosol. However, in the presence of  $\text{Ca}^{2+}$ , they are capable of association with membrane phospholipids and changing their characteristics of biomembranes. Eight putative genes encoding annexins have been identified in *Arabidopsis thaliana*, and 11 and 25 genes were found in barley and wheat, respectively [90]. Plant annexins consist of repeated annexin domains with a conserved endonexin fold binding  $\text{Ca}^{2+}$ . Their structure significantly differs from animal annexins. Some authors proposed that annexins can form  $\text{Ca}^{2+}$  influx channels, which are regulated by ROS [91–93]. This is based on the observation that an addition of purified plant annexin protein induced elevation of cytosolic free  $\text{Ca}^{2+}$  in *Arabidopsis* root epidermal protoplasts, indicative of formation of ion channel-like  $\text{Ca}^{2+}$ -permeable pores [91]. Laohavisit et al. [92] demonstrated that  $\text{Ca}^{2+}$  influx current activated by  $\text{HO}^\bullet$  were lost in annexin KO mutants (*ann1*) lacking functional annexin. However, it should be noted that *ann1* plants are dwarfs with dramatically changed morphology and physiology. It is expected that characteristics of ion channels can be different in these plants compared to the wild type.

In animals, annexins are peripheral membrane proteins interacting with membranes in a  $\text{Ca}^{2+}$ -dependent manner [94]. Ion channel activity of various animal annexins has been shown in artificial lipid bilayers while in vivo evidence for this is still missing. Animal annexin monomers cannot expand the lipid bilayer of the membrane. Animal annexins A7 and A5 trigger  $\text{Ca}^{2+}$  influx activity in the plasma membrane and endomembranes. One hypothetical mechanism suggests that annexins can assemble to a seven- or four-domain structure forming a transmembrane hydrophilic cation-permeable channel. At the same time, an alternative hypothesis proposed that a membrane destabilisation and electroporation as well as membrane flippage and resealing due to peripheral interaction with phospholipids underlie annexin function [94,95]. Annexins are usually associated with the onset of  $\text{Ca}^{2+}$ -dependent apoptosis in different animal tissues. However recent studies showed that annexin action on apoptosis is induced by inhibition of  $\text{K}^+$  efflux channels [96]. In resting conditions, annexin blocked  $\text{Ca}^{2+}$ -activated cytoplasmic site of  $\text{K}^+$  efflux channels, but increased cytosolic  $\text{Ca}^{2+}$  causes relocation of annexin to the extracellular side triggering  $\text{K}^+$  efflux channel activation by cytosolic  $\text{Ca}^{2+}$ .

Wang et al. (2015) have recently found that Annexin1 was expressed in vesicles derived from the endoplasmic reticulum [97]. This expression was rapidly up-regulated by heat shock. KO plants lacking microsomal annexin showed abolished heat stress-induced  $[\text{Ca}^{2+}]_{\text{cyt}}$  elevation and an increased heat sensitivity. Consistent with this, some animal annexins act as scaffolding proteins in microsomes. They anchor other proteins to the cell membrane and make connections between membranes. Plant annexins probably share this function and can hypothetically mediate intracellular trafficking of  $\text{Ca}^{2+}$ -permeable channels [98]. Annexin knock-out lines may have less channel subunits delivered to the plasma membrane, demonstrating compromised  $\text{Ca}^{2+}$  signals [98]. Supporting this hypothesis, annexin knock-out mutants are dwarfed, having stunted growth and dramatically modified morphology [99]. Qiao et al. (2015) recently demonstrated that plant annexins, which have peroxidase domain, also generated  $\text{H}_2\text{O}_2$  inside the cell and reacted with  $\text{Ca}^{2+}$ -dependent protein kinases, promoting heat stress tolerance [100]. Thus, apart from the proposed direct insertion



into the plasma membrane [91,92,101], intracellular annexins are likely affecting the delivery of  $\text{Ca}^{2+}$ -permeable channels to the plasma membrane [98].

### 9. Mechanisms of Ion Channel Activation by ROS

The study by Garcia-Mata et al. [75] has shed light on the molecular mechanism of ROS-induced activation of  $\text{K}^+$  channels. Using heterologous expression systems (HEK293 cells and *Xenopus* oocytes), these authors demonstrated that the  $\text{K}^+$  channel SKOR, which has very similar structure to GORK, is activated by  $\text{H}_2\text{O}_2$  via targeted oxidation of Cys168 at the S3  $\alpha$ -helix within channel's voltage sensor. This residue is exposed to the outside when the GORK channel is in the open conformation. Substitution of this amino acid abolished SKOR's sensitivity to  $\text{H}_2\text{O}_2$ . A corresponding Cys residue exists in GORK [75].

A mechanism of  $\text{HO}^\bullet$  generation from  $\text{H}_2\text{O}_2$  in the cation channel (leading to the channel activation) was proposed by Demidchik et al. [14]. Using Metal Detector ver. 2.0 software (Universities of Florence and Trento, Florence, Italy) the putative Cu/Fe binding sites in CNGC19 and CNGC20 were identified. Cys 102, 107 and 110 of CNGC19 and Cys 133, 138 and 141 of CNGC20 can co-ordinate transition metals assembling into the metal-binding sites with a probability close to 100%. These sites potentially generate  $\text{HO}^\bullet$  from  $\text{H}_2\text{O}_2$  within a channel complex, which is crucial, considering that  $\text{HO}^\bullet$  is extremely short-lived and can therefore act at a distance not more than 1 nm from the point of its generation. Notably, cysteine residues have been shown to be responsible for direct  $\text{HO}^\bullet$ -induced activation of animal  $\text{Ca}^{2+}$ -permeable channels [102] and transcription factors [103].

### 10. The Hypothesis of a ROS- $\text{Ca}^{2+}$ Hub for Amplification of Redox and $\text{Ca}^{2+}$ Signals at the Plant-Environment Interface

ROS are critically important for stress and hormonal signalling, polar and gravitropic growth, autophagy, PCD and other functions of higher plants [29,104]. ROS and cytosolic  $\text{Ca}^{2+}$  signals simultaneously control the same physiological processes and cross-talk via reciprocal stimulation— $\text{Ca}^{2+}$ -dependent activation of NADPH oxidase and ROS activation of  $\text{Ca}^{2+}$ -permeable channels (Table 1).

The hypothesis of a 'ROS- $\text{Ca}^{2+}$  hub' (Figure 1) assumes that the  $\text{Ca}^{2+}$ -activated NADPH oxidases (encoded by RBOH genes) act together with ROS-activated  $\text{Ca}^{2+}$ -permeable channels to amplify stress-induced  $\text{Ca}^{2+}$  and ROS signals [2,12,18,29,104]. Elevation of cytosolic  $\text{Ca}^{2+}$  level causes an increase in superoxide production and vice versa, superoxide, via generation  $\text{HO}^\bullet$ , activates ROS-activated  $\text{Ca}^{2+}$  influx channels (Figure 1). This forms a self-amplification positive feedback loop stimulating duration and amplitude of initially weak signals and transforming them into the large-scale responses [58]. Moreover, this also involves higher activation of GORK-mediated  $\text{K}^+$  efflux [13]. The analysis of recent literature demonstrated that ROS- $\text{Ca}^{2+}$  hub is involved in root and pollen tube growth, hypersensitive response to pathogen, transduction of hormonal and other regulatory chemical signals, responses to abiotic stresses, water balance, autophagy, programmed cell death and mineral nutrition [5]. The ROS- $\text{Ca}^{2+}$  has a sophisticated system for control and regulation in plants, which include self-inactivation of  $\text{Ca}^{2+}$ -permeable channels, induction of  $\text{Ca}^{2+}$ -ATPase through  $\text{Ca}^{2+}$ -calmodulin binding to specific binding sites of these enzymes, suppression of Rac/Rop GTPases, and  $\text{Ca}^{2+}$ -Dependent Protein Kinase (CDPK)-catalysed phosphorylation of ion channels or Botrytis-Induced Kinase (BIK)-catalysed phosphorylation of NADPH oxidase [5,104–112].

The concept of ROS- $\text{Ca}^{2+}$  hub allows to predict the ion channel subunits involved in activation by ROS [5]. Most ion channels activated by ROS have not yet been identified genetically or electrophysiologically. However, there is indirect evidence that they function in concert with some specific NADPH oxidases (Table 1). An involvement of both NADPH oxidase and channels points to potential activation of channels by ROS. One of the widely acknowledged examples of 'ROS- $\text{Ca}^{2+}$  hub' function is the loading of  $\text{Ca}^{2+}$  for elongation growth of root cells [6,12] and pollen tubes [87]. This process is driven by extremely high polar NADPH oxidase activity leading to activation of

Ca<sup>2+</sup>-permeable channels. ROS-Ca<sup>2+</sup> hub maintains [Ca<sup>2+</sup>]<sub>cyt</sub> in growing part of the cell, such as tips root hair of pollen tube, at constantly high level, supporting longitudinal cytoskeleton bundles and tip-directed exocytosis [105]. *Arabidopsis* CNGC3 [113], annexins [92] and RbohC [6] probably form the root hair ROS-Ca<sup>2+</sup> hub. Ionotropic glutamate receptors GLR1.2 and GLR3.7 [86] and CNGC18 [114], jointly with RbohH and RbohJ [115] could function as a ROS-Ca<sup>2+</sup> hub in *Arabidopsis* pollen tube.

The ROS-Ca<sup>2+</sup> hub is also involved in plant cell hypersensitive response during pathogen attack, leading to a massive PCD around the infection site and probably preventing spread of the disease [116]. AtrbohD and AtrbohF [117,118] may cross-talk with AtCNGC2, 4, 11 and 12 [119] to form 'ROS-Ca<sup>2+</sup> hub' mediating this reaction. The following hormones are potentially involved in the ROS-Ca<sup>2+</sup> hub: abscisic acid (AtrbohD and AtrbohF cross-talking with AtCNGC5 and AtCNGC6) [53], auxins (AtRbohD/AtCNGC14) [120–122], methyl jasmonate (AtrbohD and AtrbohF/AtCNGC2) [123,124] and salicylic acid (AtrbohD/AtGLR3.3) [125–127]. Drought induces stomata closure via abscisic acid-mediated activation of AtrbohD and AtrbohF/AtCNGC5 and AtCNGC6 couples [128]. Gémes et al. (2016) have shown that NtRbohD and NtRbohF are crucial for NaCl-induced ROS production and activation of ROS-Ca<sup>2+</sup> hub [129], while Guo et al. (2008, 2010) demonstrated that Ca<sup>2+</sup> entry in this case is mediated by AtCNGC10 [130,131]. High temperatures are directly sensed via membrane fluidity changes by CNGC6 [113]. At the same time the heat tolerance is impaired in AtrbohD and AtrbohB KO lines, suggesting a clash with CNGC6 [132]. Overall, these data strongly suggest that the 'ROS-Ca<sup>2+</sup> hub' is an important and ubiquitous mechanism, which is potentially involved in a multitude of physiological processes.

**Table 1.** NADPH oxidases and cation channels, which can potentially function as ROS-Ca<sup>2+</sup> hubs in key physiological reactions (see hypothesis on ROS-Ca<sup>2+</sup> hubs in the text).

Physiological Process	Genes of Cation Channels with Predicted or Demonstrated Ca <sup>2+</sup> Permeability	Genes Encoding NADPH Oxidase Producing ROS for Activation of Ca <sup>2+</sup> -Permeable Channels	References
Growth and development			
Root cell elongation	AtCNGC3	AtRBOHC	[6,113]
Pollen tube elongation	AtCNGC18, AtGLR1.2, AtGLR3.7	AtRBOHH, AtRBOHJ	[86,115,133]
Phytohormonal regulation			
Effects of auxin	AtCNGC14	AtRBOHD	[120–122]
Abscisic acid signalling	AtCNGC5, AtCNGC6	AtRBOHD, AtRBOHF	[7,122]
Methyl jasmonate-induced reactions	AtCNGC2	AtRBOHD, AtRBOHF	[123,124]
Action of salicylic acid	AtGLR3.3	AtRBOHD	[125–127]
Stress responses			
Hypersensitive response (massive PCD around the spot of infection, preventing spread of the disease)	AtCNGC2, AtCNGC4, AtCNGC11, AtCNGC12	AtRBOHD, AtRBOHF	[117–119]
Response to salinity	AtCNGC10	NtRBOHD, NtRBOHF	[128,130]
Drought-induced stomata closure	AtCNGC5, AtCNGC6	AtRBOHD, AtRBOHF	[128]
Response to extreme temperatures	AtCNGC6	AtRBOHD, AtRBOHB	[114,132]

## 11. Summary and Concluding Remarks

Key biophysical and functional properties of ROS-activated ion channels are summarized in Table 2. Conductances mediated by these channels have been measured in the plasma membrane of Charophyte *Nitella flexilis*, three dicotyledonous higher plants, including *Arabidopsis thaliana*, *Pyrus pyrifolia* and *Pisum sativum*, and one monocotyledon *Lilium longiflorum*. They exist in root hairs, root mature and elongation zone epidermal cells, pollen tubes, and guard cells. Activating species include H<sub>2</sub>O<sub>2</sub> and hydroxyl radicals. Prevalent intracellular activating species is H<sub>2</sub>O<sub>2</sub> while, in the extracellular medium, it is the hydroxyl radicals. ROS-activated ion channels can be divided into the following three groups by the shape of current-voltage curves: inwardly-rectifying,

voltage-independent and outwardly-rectifying. Channels of inwardly-rectifying group conducts  $\text{Ca}^{2+}$ -influx currents for growth and signaling needs, while the outwardly-rectifying members transport  $\text{K}^+$  from the cell to extracellular medium to regulate osmotic pressure for guard cell closure, induce programmed cell death or autophagy, or hypothetically adjust metabolism for reparation needs [14]. The voltage-independent group is less studied, but it mediates both  $\text{Ca}^{2+}$ -influx and  $\text{K}^+$  efflux, co-existing with other types of conductances in same patches. Selectivity studies are contradicting, showing that ROS-activated channels could be fully nonselective, including permeation to anions, or can specifically conduct only cations. Most reports suggest that ROS-activated channels are cation-selective. However further studies on selectivity are definitely needed. *Arabidopsis*  $\text{Ca}^{2+}$ -permeable ROS-activated channels are blocked by lanthanides, verapamil and nifedipine, while the same channels in pea are also sensitive to some anion channel blockers, 5-nitro-2(3-phenylpropylamino)-benzoate and niflumate. ROS-activated  $\text{K}^+$ -permeable channels are blocked by  $\text{TEA}^+$ . Time-dependence of activation can also vary including rapidly- and slowly-activated channels. Both  $\text{Ca}^{2+}$  influx and  $\text{K}^+$  efflux ROS-activated conductances include rapidly- and slowly-activated current components. Few preparations showed single-channel characteristics revealing 14.5-pS  $\text{Ca}^{2+}$  influx and 16-pS  $\text{K}^+$  efflux ROS-activated unitary conductances. Molecular nature of ROS-activated conductances is defined only for  $\text{K}^+$  efflux channels, which are encoded by SKOR and GORK genes, both having ROS sensing cysteine moieties. Moreover, more genes can be predicted from the analysis of NADPH oxidase-mediated ROS- $\text{Ca}^{2+}$ -hubs. Established physiological functions of ROS-activated conductances include root cell and pollen tube growth, regulation of osmotic balance and stomata aperture, responses to major stresses, amplification of external stress and phytohormonal signals via ROS- $\text{Ca}^{2+}$ -hub, programmed cell death, and autophagy. Undoubtedly, this list will be extended in further studies on ROS-activated ion channels.

**Table 2.** Biophysical and functional characteristics of ROS-activated ion channels in plant plasma membranes.

Preparation	Activating ROS	Selectivity and Kinetics of Activation	Blockers, Modulators	Function	References
<i>Nitella flexilis</i>					
- intermodal cells	$\text{Cu}^{2+}$ , $\text{HO}^\bullet$	NS *, VI *, RA *	Lanthanides, verapamil, nifedipine	Sensing transition metals, copper toxicity	[17,22]
<i>Arabidopsis thaliana</i>					
- root hairs	$\text{HO}^\bullet$	NS, IR *, SA *	Lanthanides	Growth	[6,12]
- root mature epidermis	$\text{HO}^\bullet$ , $\text{H}_2\text{O}_2$	NS, IR, SA	Lanthanides, verapamil, $\text{TEA}^+$	Stress response	[12,18]
- root elongation zone	$\text{HO}^\bullet$	KS *, OR *, SA, NS, VI, RA	Lanthanides, verapamil	Growth, stress response	[12]
- guard cells	$\text{H}_2\text{O}_2$ , $\text{H}_2\text{O}_2$	KS, OR, SA, NS, VI, RA, NS, IR	Lanthanides, verapamil	ABA signaling, stomata closure	[10]
<i>Pyrus pyrifolia</i>					
- pollen tube	$\text{H}_2\text{O}_2$	IR	Activation by polyamines	Pollen tube growth	[57]
<i>Pisum sativum</i>					
- root	$\text{HO}^\bullet$	NS, VI, RA	Stimulation by polyamines		[21]
<i>Lilium longiflorum</i>					
- pollen tube	$\text{H}_2\text{O}_2$	NS, IR, SA, KS, OR, SA	Lanthanides, nifedipine, $\text{TEA}^+$	Pollen tube growth	[88]

\* NS—non-selective, RA—rapidly-activating, SA—slowly-activating, VI—voltage-independent, OR—outwardly-rectifying, IR—inwardly-rectifying, KS— $\text{K}^+$ -selective.

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