REVIEW PAPER

ROS and RNS in plant physiology: an overview

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Abstract

The production of reactive oxygen species (ROS) is the unavoidable consequence of aerobic life. ROS is a collective term that includes both oxygen radicals, like superoxide ($O_2^{\cdot-}$) and hydroxyl ($^{\cdot}OH$) radicals, and other non-radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$ or $^1\Delta g$), etc. In plants, ROS are produced in different cell compartments and are oxidizing species, particularly hydroxyl radicals and singlet oxygen, that can produce serious damage in biological systems (oxidative stress). However, plant cells also have an array of antioxidants which, normally, can scavenge the excess oxidants produced and so avoid deleterious effects on the plant cell bio-molecules. The concept of 'oxidative stress' was re-evaluated in recent years and the term 'oxidative signalling' was created. This means that ROS production, apart from being a potentially harmful process, is also an important component of the signalling network that plants use for their development and for responding to environmental challenges. It is known that ROS play an important role regulating numerous biological processes such as growth, development, response to biotic and environmental stresses, and programmed cell death. The term reactive nitrogen species (RNS) includes radicals like nitric oxide ($NO^{\cdot}$) and nitric dioxide ($NO_2^{\cdot}$), as well as non-radicals such as nitrous acid (HNO_2) and dinitrogen tetroxide (N_2O_4), among others. RNS are also produced in plants although the generating systems have still not been fully characterized. Nitric oxide ($NO^{\cdot}$) has an important function as a key signalling molecule in plant growth, development, and senescence, and RNS, like ROS, also play an important role as signalling molecules in the response to environmental (abiotic) stress. Similarly, $NO^{\cdot}$ is a key mediator, in co-operation with ROS, in the defence response to pathogen attacks in plants. ROS and RNS have been demonstrated to have an increasingly important role in biology and medicine.

Key words: Antioxidants, hydrogen peroxide (H_2O_2), nitric oxide (NO), oxidative signalling, oxidative stress, superoxide ($O_2^{\cdot-}$), reactive oxygen species (ROS), reactive nitrogen species (RNS), review, ROS signalling, RNS signalling, environmental stress.

Introduction

'All substances are poisons: there is none which is not a poison.
The right dose differentiates a poison and a remedy'

Paracelsus (1493–1541)

The production of reactive oxygen species (ROS) is the unavoidable consequence of aerobic life. ROS is a collective term that includes both oxygen radicals, like superoxide ($O_2^{\cdot-}$), hydroxyl ($^{\cdot}OH$), peroxyl ($ROO^{\cdot}$), etc, and other non-radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$ or $^1\Delta g$), ozone (O_3), etc (Halliwell, 2006; Halliwell and Gutteridge, 2007) (Table 1). Reactive nitrogen species (RNS) is a similar collective term that includes radicals like nitric oxide ($NO^{\cdot}$) and nitric dioxide ($NO_2^{\cdot}$), as well as non-radicals such as nitrous acid (HNO_2) and dinitrogen tetroxide (N_2O_4) among others (Halliwell and Gutteridge, 2007; Nasir Khan *et al.*, 2014) (Table 2). ROS and RNS have been demonstrated to have an important role in biology and medicine.

Table 1. *Main reactive oxygen species (ROS) (Halliwell and Gutteridge, 2007)*

Free radicals	Non-radicals
Superoxide, $O_2^{\cdot-}$	Hydrogen peroxide, H_2O_2
Hydroperoxyl, $HO_2^{\cdot}$	Singlet oxygen, $^1O_2$ or $^1\Delta g$
Hydroxyl, $^{\cdot}OH$	Ozone, O_3
Peroxyl, $ROO^{\cdot}$	Hypochlorous acid, $HOCl$
Alkoxyl, $RO^{\cdot}$	Peroxynitrite, $ONOO^-$

Table 2. *Main reactive nitrogen species (RNS) (Halliwell and Gutteridge, 2007)*

Free radicals	Non-radicals
Nitric oxide, $NO^{\cdot}$	Nitrous acid, HNO_2
	Nitrosonium cation, NO^+
Nitric dioxide, $NO_2^{\cdot}$	
	Nitroxyl anion, NO^-
Nitrate radical, $NO_3^{\cdot}$	
	Peroxynitrite, $ONOO^-$
	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3

Some pioneer studies in ROS research

There are key milestones in modern ROS and RNS research and the author would like to mention some of the major pioneers in these fields. Their important initial and seminal work, together with that from many other colleagues, has led to our current knowledge on ROS and RNS in plant physiology.

In 1954, an Argentinian human physiologist, Rebeca Gerschman (Fig. 1A) and co-workers at the University of Rochester, New York, in a study on oxygen poisoning and ionizing radiation, postulated that most of the damaging effects of O_2 were due to oxygen radicals. An increase in oxygen partial pressure or a decrease in the antioxidant defence would equally lead to cell and tissue damage, with the conclusion that oxygen toxicity was a continuous phenomenon (Gerschman *et al.*, 1954). This theory was rather advanced and 'revolutionary' at that time and implied the involvement of oxygen radicals in the origin of certain diseases and ageing processes. It shocked the scientific community since it postulated new and audacious ideas on oxygen poisoning but, unfortunately, not much attention was given to it and this proposal fell into oblivion for many years. On the other hand, Rebeca Gerschman was also very active in the fight for women's rights in science.

It was in 1969 when the theory of Rebeca Gerschman *et al.* was reconsidered. Joe McCord and Irwin Fridovich (Fig. 1B), at the Duke University Medical Center, Durham, USA, discovered the enzyme superoxide dismutase (SOD) that uses an oxygen free radical (superoxide) as substrate (McCord and Fridovich, 1969). In the course of very interesting and intriguing research work (whose history is worth reading), they demonstrated that this enzyme catalyses the disproportionation or dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide. The enzyme was found to be identical to erythrocuprein or haemocuprein,

a protein whose existence had been known for many years but where no enzymatic activity had been detected before (McCord and Fridovich, 1969). This was a very important and seminal discovery and, later on, the existence of different SOD isoenzymes in most prokaryotic and eukaryotic organisms was demonstrated, as well as the fact that superoxide and other radicals derived thereof were produced in biological systems (Halliwell and Gutteridge, 2007; Imlay, 2011; Schnell and St Clair, 2014). Certainly, it can be said that, as a result of that important finding of Joe McCord and Irwin Fridovich in 1969, the biology of oxygen free radicals was born. In this respect, it may be interesting to keep in mind Irwin Fridovich's advice on research work: 'When, by chance, you make an observation that cannot be explained in terms of current knowledge, do not hesitate to pursue it, even though it may seem esoteric or unimportant. It may lead you to discoveries of considerable importance' (Imlay, 2011). No less interesting is Joe McCord's advice to students: 'Science requires the same creativity, inventiveness, and passion that we expect from artists, composers, and writers. When you feel it, you know it. If you don't feel it, then science probably isn't the best career choice for you. This has little to do with whether science seems easy or difficult. It always seemed difficult to me as a student, as it probably should' (Schnell and St Clair, 2014).

Another important milestone in ROS research was the discovery, by Bernard M Babior and co-workers (Fig. 1C) at Harvard University Medical School, Boston, that white cells (leukocytes) produced superoxide radicals by a 'respiratory burst' and used these to kill invading bacteria in the fagocytosis process, using ROS as potent bactericidal agents (Babior *et al.*, 1973). Further research showed that the generating system of superoxide radicals in phagocytes was an NADPH oxidase and this enzyme was characterized (Babior, 2004). This was a very important finding that had a deep impact in biomedicine (Curnutte, 2004; Halliwell and Gutteridge, 2007), and further research demonstrated the existence in plants of homologues of animal NADPH oxidases (Sagi and Fluhr, 2006).

The founder of the concept of 'oxidative stress' was Helmut Sies (Fig. 1D), Heinrich Heine University, Düsseldorf, Germany (Sies, 1985; Jones and Radi, 2014). Helmut Sies and Britton Chance, in a collaborative work, were the first to identify hydrogen peroxide as a normal aerobic metabolite and designed a method to quantify H_2O_2 concentration and turnover in cells (Sies and Chance, 1970). Helmut Sies made fundamental contributions to the physiology of GSH, selenium nutrition, singlet oxygen biochemistry, and the health benefits of dietary lycopene and cocoa flavonoids which supplied nutritional strategies against cancer, cardiovascular disease, and ageing. His formulation of the concept of oxidative stress stimulated research on oxidants and antioxidants, and his quantitative approach to redox biochemistry provided a foundation for modern redox systems biology (Sies, 2014; Jones and Radi, 2014). As Helmut Sies has said: 'The joy of exploring the unknown and finding something novel and noteworthy: what a privilege!' (Jones and Radi, 2014).

Some important landmarks in ROS research were also made in the plant field, such as those that came from the

Fig. 1. Some fundamental pioneers in basic ROS research. (A) The Argentinian physiologist Rebeca Gerschman (1903–1986) (reprinted from Boveris AA. Rebeca Gerschman: a brilliant woman scientist in the fifties. *Free Radical Biology and Medicine* 21(1): 5-6. Copyright 1996, with permission from Elsevier). (B) Biochemists Irwin Fridovich and Joe McCord, from left to right (courtesy of McCord). At present, they are both Emeritus Professors at Duke University, NC and the University of Colorado, Denver, respectively. (C) Biochemist Bernard M Babior M.D. (1935–2004) (from Curnutte, JT. *J Clin Invest.* 2004;114(8):1054–1057. Copyright © 2004, The American Society for Clinical Investigation). (D) Biochemist Helmut Sies M.D., Heinrich Heine University Düsseldorf, Germany (from Jones DP and Radi R. Redox pioneer: Professor Helmut Sies. *Antioxidants & Redox Signaling*. 2014, 21(18): 2459–2468).

laboratory of Erich F Elstner, Technische Universität, München, Freising-Weihenstephan, Germany (Fig. 2A). He systematically studied the biochemistry of activated oxygen during plant stress and also showed that ROS were formed in photosynthetic electron transport (Elstner and Osswald, 1994). The biosynthesis of the phytohormone ethylene and its relationship with ROS was also investigated in his laboratory in Munich. Erich Elstner was one of the first who recognized the central significance of ROS and ethylene in signal transduction pathways in both plant and animal systems. His contributions to ROS biochemistry cover a wide scientific field from biochemistry and plant physiology to plant and human pathology (Elstner, 1990; Denke *et al.*, 1999). Elstner's global interest in oxygen biochemistry and oxygen toxicity, and in establishing connections with colleagues from different but overlapping scientific fields, led him to the foundation of the famous 'Münchner Sauerstoffclub' (Munich Oxygen Club) in 1977, where members with interests in ROS from different areas, including chemistry, biology, food chemistry, botany, radiation biology, phytopathology, and medicine, met periodically and had informal, high-level, scientific seminars. Those

who worked with Erich Elstner will never forget his deep biochemical and botanical knowledge, and his excellent scientific criterion and warm personality (Denke *et al.*, 1999).

Further relevant milestones in ROS research in plants were established by Professor Kozi Asada, in Kyoto University, Japan, related to photosynthesis and photoinhibition, as well as to the environmental responses of photosynthesis (Fig. 2B). He demonstrated the univalent reduction of molecular oxygen to superoxide by chloroplasts on illumination (Asada *et al.*, 1974). The production and scavenging of ROS in chloroplasts and their functions were thoroughly studied in his laboratory (Asada, 2006) and he proposed the existence of a water–water cycle in chloroplasts as a system of scavenging 'active oxygens' and dissipating excess photons (Asada, 1999). In his laboratory, the presence of superoxide dismutase in chloroplasts was demonstrated for the first time and carried out the purification to homogeneity, characterization, and recrystallization of this Cu,Zn-SOD from spinach leaves (Asada *et al.*, 1973). This was excellent pioneering work since, at that time, very few superoxide dismutases had been isolated and purified particularly from higher plants.

Fig. 2. Some important pioneers in ROS research in plants. (A) From second left to right, Christine H Foyer, Erich F Elstner, and Alain Puppo. (B) The three pioneers in the study of H_2O_2 metabolism in chloroplasts, and discoverers of the ascorbate–glutathione cycle, also known as the Foyer–Halliwell–Asada pathway. From left to right, Barry Halliwell, Kozi Asada (sadly deceased in December 2013), and Christine H Foyer.

An important finding was the discovery of the ascorbate-glutathione cycle in chloroplasts. This cycle is also called Foyer–Halliwell–Asada cycle, after the names of the three major contributors, and is a crucial mechanism for H_2O_2 metabolism in both animals and plants (Halliwell and Gutteridge, 2007; del Río, 2011a). The main contributors to this discovery were Christine H Foyer and Barry Halliwell (Foyer and Halliwell, 1976), Kings College, University of London, UK: Christine Foyer has recently been recognized as Redox Pioneer by the USA journal *Antioxidants & Redox Signaling* (del Río, 2011a). The three pioneers in the study of H_2O_2 metabolism in chloroplasts, Christine H Foyer, Barry Halliwell, and Kozi Asada (regrettably passed away in 2013) are shown in Fig. 2B on the occasion of the 30th anniversary of the discovery of the ascorbate–glutathione cycle, in a meeting celebrated in Ghent, Belgium, in 2007. Christine Foyer has made a very significant contribution to

our current understanding of the crucial roles of ascorbate and glutathione in redox biology, particularly in relation to photosynthesis, respiration, and chloroplast and mitochondrial redox signalling networks (Foyer and Noctor, 2005, 2011; del Río, 2011a).
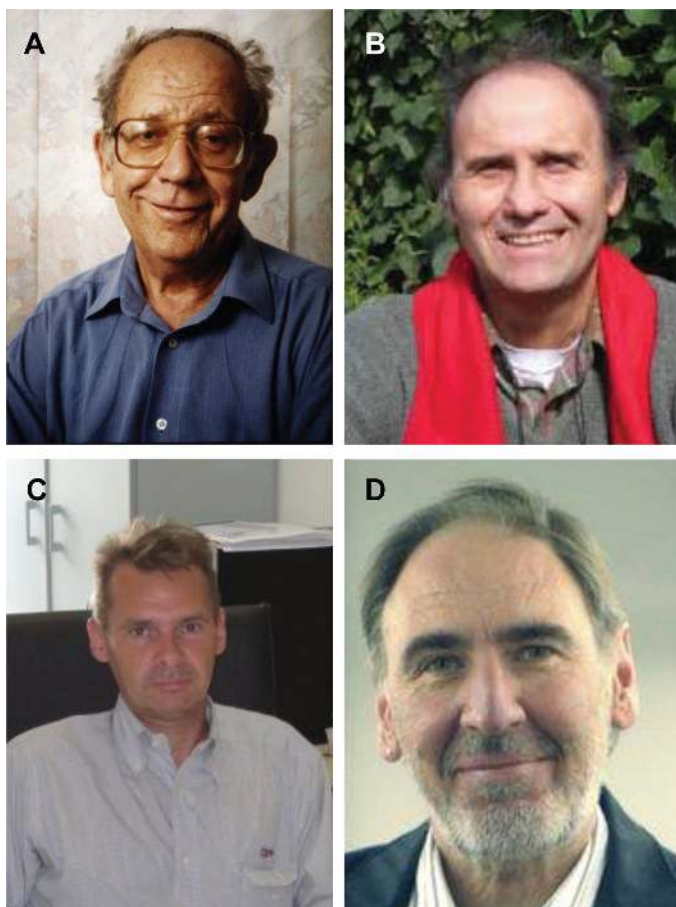
The group of Alain Puppo, University of Nice, France, was a pioneer in the study of the role of ROS and redox-related processes in the symbiosis between N_2-fixing bacteria (rhizobia) and legumes (Puppo *et al.*, 1982) (Fig. 2A). They studied the signalling function of H_2O_2, NO, and GSH in the establishment and functioning of this interaction between *Rhizobium* and the legume, and the role of those signalling molecules in the regulation of the cellular redox state involved in root nodule functioning and senescence (Puppo *et al.*, 2005, 2013). Alain Puppo's group has put special emphasis on the role of NADPH oxidases, nitrate reductases, and GSH in the legume symbiotic interaction (Marino *et al.*, 2012; Puppo *et al.*, 2013).

Some pioneer studies in RNS research

In animal systems, the gaseous radical nitric oxide (NO·) is known to be an important inter- and intracellular messenger molecule involved in important regulatory functions in the nervous, vascular, and immune systems (Martínez-Ruiz *et al.*, 2011). NO was the subject of the Nobel prize in 1998 and was named 'molecule of the year' in 1992 by the journal *Science*. The use of NO by plants had already been observed by Fewson and Nicholas (1960), and the emission of NO by plants had been described by Klepper (1979), in soybean plants treated with herbicides.

In the field of plant physiology, relevant research on the NO function has also been carried out. It was in the last 17–18 years when some plant physiologists became interested in the function of NO in plants. Ya'acov Leshem (Fig. 3A), Bar-Ilan University, Israel, and Tomoya Noritake, Nagoya University, Japan, were pioneers in the study of the function of NO in plants. Their papers on the role of NO in senescence (Leshem and Haramaty, 1996) and plant immunity (Noritake *et al.*, 1996) were two important key points in the study of NO in plant physiology, and Professor Leshem published the first book on nitric oxide in plants (Leshem, 2000). One year after those two earlier publications, an Argentinian scientist, Dr Lorenzo Lamattina (Fig. 3B), published an article on the effect of NO in the preservation of chlorophyll in plants infected by a fungus (Laxalt *et al.*, 1997). Since then, Lamattina's group has made very significant contributions to the field of the physiological function of NO in plants.

One year later, two independent papers appeared by Massimo Delledonne *et al.* (1998) (Fig. 3C) and Jörg Durner *et al.* (1998) (Fig. 3D). These publications pointed to a new function of NO in plants which, until then, had not been considered. They postulated a role for nitric oxide as a signal molecule in plant defence (or resistance) to bacterial infection. This new function of NO in plant pathogenesis represented a very significant turning point in the research on the physiological role of NO in plants.

Fig. 3. Some important pioneers in RNS research in plants. (A) Ya'acov Y Leshem (1931–2011), Bar-Ilan University, Israel. (B) Lorenzo Lamattina, National University of Mar del Plata, Argentina. (C) Massimo Delledonne, University of Verona, Italy. (D) Jörg Durner, Helmholtz Zentrum München, Germany.

Table 3. *Main plant antioxidants*

Enzymatic	Non-enzymatic or low molecular weight
Catalase	Glutathione (GSH)
Superoxide dismutase (SOD)	Ascorbic acid (vitamin C)
Ascorbate-glutathione cycle enzymes	Tocopherols (vitamin E)
Peroxidases	Polyphenols (flavonoids)
NADP-dehydrogenases	
Peroxiredoxin (Prx)	

ROS (Table 1) are oxidizing species, particularly the hydroxyl radicals ($^{\cdot}$OH) and singlet oxygen ($^1O_2$). These species are very powerful oxidants that can react with nearly all the components of living cells producing severe damage to lipids, proteins, and nucleic acids (oxidative stress situations) (Bailey-Serres and Mittler, 2006; Halliwell and Gutteridge, 2007; del Río and Puppo, 2009; Sies, 2014). However, to prevent oxidative stress situations, plant cells have an array of enzymatic and non-enzymatic antioxidants (Table 3) which, under normal conditions, can scavenge the excess oxidants produced and so avoid the deleterious effects on plant cell bio-molecules.

This was the opinion that prevailed on ROS in plant biology until recently. However, genetic evidence has suggested that, *in planta*, purely physicochemical damage may be more limited than previously thought (Foyer and Noctor, 2005). This means that the concept of 'oxidative stress', which strictly implies a state to be avoided, was re-evaluated and the term 'oxidative signalling' or 'redox signaling' was created (Foyer and Noctor, 2005). This means that ROS production, which was originally considered as an exclusively harmful and dangerous process, is also an important component of the signalling network that plants use for their development and for responding to environmental challenges. The evolution of efficient antioxidant systems has, most likely, enabled plant cells to overcome ROS toxicity and to use these reactive species as signal transducers (del Río and Puppo, 2009; Mittler *et al.*, 2011).

It is now widely accepted that ROS play an important signalling role in plants, as key regulators of processes such as growth, development, response to biotic and environmental stimuli, plant metabolism, and programmed cell death (del Río and Puppo, 2009; Mittler *et al.*, 2011; Inzé *et al.*, 2012; Sandalio *et al.*, 2012; Baxter *et al.*, 2014). A representation of 'the double role of ROS' is shown in Fig. 4. Unfavourable environmental conditions result in excessive ROS production that leads to oxidative cell injuries at high concentrations. To prevent ROS-dependent cellular damage, plants have a broad range of antioxidant mechanisms at their disposal to use ROS simultaneously as a signal within various biological processes (Vanderauwera *et al.*, 2009).

In plants, the homologue of the respiratory-burst NADPH oxidase of leukocytes has been identified (Sagi and Fluhr, 2006), and this has led to the demonstration that plant cells, like mammalian cells, can initiate and most likely amplify ROS production for the purpose of signalling (Suzuki *et al.*,

Production and function of ROS in plants

In plants, H_2O_2 is produced by different enzymes as a product of their catalytic reactions, and important generators of H_2O_2 are the peroxisomal flavin-containing enzymes glycolate oxidase and acyl-CoA oxidase, which are involved in the photorespiratory and fatty acid β-oxidation pathways, respectively. It is now well established that a major source of $O_2^{\cdot-}$ is the plasma membrane-localized NADPH oxidase (NOX) (Suzuki *et al.*, 2011; Marino *et al.*, 2012; Baxter *et al.*, 2014), but other contributing sources of superoxide and hydrogen peroxide are the chloroplasts, mitochondria, and peroxisomes as a result of different oxidative and electron transport reactions (Asada, 2006; del Río *et al.*, 2006; Rhoads *et al.*, 2006; Halliwell and Gutteridge, 2007; del Río and Puppo, 2009; del Río, 2013). Another important source of ROS are cell wall-bounded peroxidases which can generate H_2O_2 (Bolwell and Daudi, 2009; O'Brien *et al.*, 2012). In addition, chloroplasts are also important producers of the non-radical ROS singlet oxygen ($^1O_2$) through different photodynamic reactions (Asada, 2006; Triantaphylidés and Havaux, 2009).

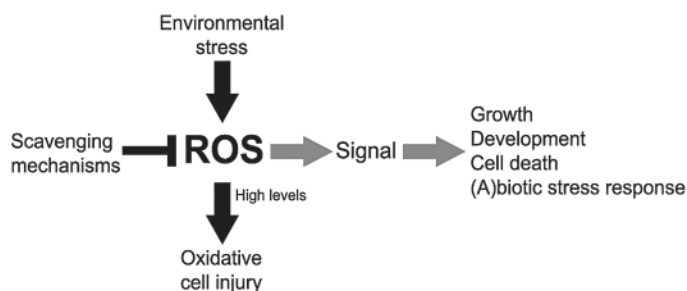**Fig. 4.** The double role of ROS. Environmental stress conditions induce over-production of ROS which, at high concentrations, can produce oxidative cell injuries. However, plants have a broad array of antioxidant systems that allow ROS to be used as a signal in distinct biological processes, such as growth and development, responses to biotic and abiotic stresses, and programmed cell death (PCD). Reproduced from Vanderauwera et al. (2009) in *Reactive oxygen species in plant signaling*, p. 150. Springer-Verlag.

2011; Marino *et al.*, 2012). The localized ROS production in organelles such as chloroplasts, peroxisomes, and mitochondria is very important from the viewpoint of initiating signalling cascades. A simple view of ROS signalling in plants is shown in Fig. 5. ROS formed in plant cells by many stimuli, trigger signal transduction events, eliciting specific cellular responses. The influence of these molecules on cellular processes is regulated by an equilibrium between the continuation of their production and their scavenging by the different antioxidant systems (Bailey-Serres and Mittler, 2006). Therefore, apart from single increases in ROS production, controlled down-regulation of antioxidant enzymes can also be involved, in the signalling mechanisms during plant stress.

Generation and function of nitric oxide in plants

In animal systems, most of the NO produced is due to the enzyme nitric oxide synthase (NOS; EC 1.14.13.39) (Alderton *et al.*, 2001). This enzyme catalyses the oxygen- and NADPH-dependent oxidation of L-arginine to NO and citrulline, in a complex reaction requiring FAD, FMN, tetrahydrobiopterin (BH$_4$), Ca^{2+}, and calmodulin (Knowles and Moncada, 1994; Alderton *et al.*, 2001). However, a gene or a protein with homology to mammalian NOS enzymes has not been found in *Arabidopsis thaliana* (The *Arabidopsis* genome initiative, 2000). The different molecular approaches developed so far to clone a higher plant NOS based on the sequence of animal NOS, have always given negative results (del Río, 2011b). The only case reported to date in the plant kingdom of a NOS that has been characterized is that of a unicellular species of marine green alga, *Ostreoccus tauri* (Foresi *et al.*, 2010). The length sequence of *O. tauri* NOS showed a similarity of 42, 43, and 34% with respect to eNOS, iNOS, and nNOS, respectively.

In plants there are several potential sources of NO including enzymatic and non-enzymatic systems (Wilson *et al.*, 2008; del Río, 2011b; Mur *et al.*, 2012; Hancock, 2012; del Río *et al.*, 2014). A summarized list of some established sources of NO in plant cells, with an indication of the different substrates used, is presented in Table 4. In addition, there are numerous reports
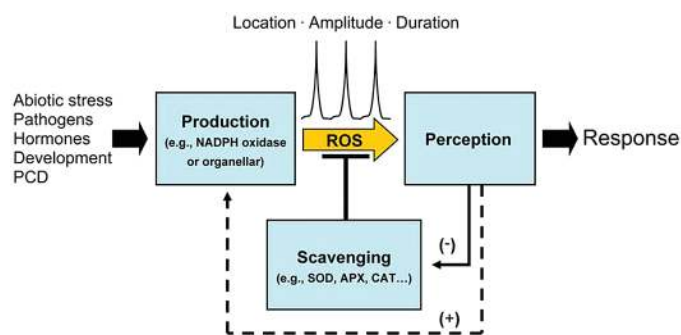
Fig. 5. ROS signalling in plant cells. A simplified model showing how ROS, formed in plant cells by many stimuli, can trigger signal transduction events, provoking specific cellular responses. The control of these molecules on cellular processes is mediated by a balance between the maintenance of their generation and their scavenging by different antioxidant systems, like SOD, catalase, ascorbate peroxidase, etc. Reproduced from Bailey-Serres J, Mittler R. 2006, The roles of reactive oxygen species in plant cells. *Plant Physiology* **141**, p. 311. www.plantphysiol.org. Copyright American Society of Plant Biologists.

Table 4. *Main sources of NO in plants (Corpas et al., 2009; del Río, 2011b)*

Source	Substrates
Non-enzymatic	
NO$_2$	Acid pH (ASC)
Enzymatic	
Nitrate reductase	NO$_2$ + NADH
NOS-like activity	L-Arg+NOS cofactors
Peroxidase	Hydroxyurea+H$_2$O$_2$
Plasma membrane	NO$_2$+reduced Cyt *c*
Cell organelles	
Mitochondria	NO$_2$
Peroxisomes	L-Arg+NOS cofactors
Chloroplasts	NO$_2$ and L-Arg

of L-arginine-dependent NOS activity in extracts from different plant species (Cueto *et al.*, 1996; Jasid *et al.*, 2006; Zhao *et al.*, 2007; reviewed by Corpas *et al.*, 2009, and by del Río, 2011b).

In plant systems, there is little information on the sub-cellular sites where NO is produced. The presence of NOS activity in peroxisomes was first demonstrated in plant tissues (Barroso *et al.*, 1999; Corpas *et al.*, 2004; del Río *et al.*, 2006, 2014; del Río, 2011b). Besides peroxisomes, other cell organelles where the generation of NO has been clearly demonstrated are mitochondria (Gupta and Kaiser, 2010) and chloroplasts (Jasid *et al.*, 2006).

Nitric oxide has an important function, as an inter- and intracellular signalling molecule, in plant growth and development. Apparently, NO regulates different processes by inducing gene transcription or activating secondary messengers (Besson-Bard *et al.*, 2008; Gaupels *et al.*, 2011). NO has multiple functions in different plant physiological and pathological processes and mainly include seed germination, pollen tube growth, cell wall lignification, root organogenesis, establishment and functioning of the legume–*Rhizobium* symbiosis, flowering, fruit ripening and senescence, and biotic and

abiotic stress (Lamattina and Polacco, 2007; Wendehenne and Hancock, 2011; Puppo et al., 2013; Nasir Khan et al., 2014; Yu et al., 2014).

Signalling function of ROS and RNS in plants under physiological and environmental stress conditions

RNS, like ROS, also play an important role as signalling molecules in the response to environmental (abiotic) stress in plants (Wilson et al., 2008; del Río, 2013; Corpas et al., 2013a; Nasir Khan et al., 2014; Yu et al., 2014). Similarly, NO is a key mediator, in co-operation with ROS, in the defence response to pathogen attacks in plants (Bellin et al., 2013; Trapet et al., 2014; Yu et al., 2014).

In general, it has been observed that, when plants are subjected to biotic and environmental stresses, a rapid overproduction of ROS and RNS takes place (del Río and Puppo, 2009; Mittler et al, 2011; Airaki et al., 2012; Sandalio et al., 2012; del Río, 2013; Baxter et al., 2014; Nasir Khan et al., 2014; Yu et al., 2014). A list of different abiotic and biotic stresses, where the induction of ROS and RNS has been reported, is shown in Table 5. Frequently, the responses of the rapid production of NO and ROS unchain a designated programmed cell death (PCD) process. In this process, both NO and ROS play key functions. PCD is an important mechanism to regulate different aspects of growth and development, as well as to eliminate damaged or infected cells during responses to environmental stresses and pathogen attacks (Wang et al., 2013).

It is known that NO in the presence of O_2 can react with reduced glutathione (GSH), by an S-nitrosylation reaction, to form S-nitrosoglutathione (GSNO) which is an important mobile reservoir of NO bioactivity whose presence in different plant species has been demonstrated (Ortega-Galisteo et al., 2012; Barroso et al., 2013; Corpas et al., 2013c; Xu et al., 2013; Kubienová et al., 2014; Yu et al., 2014). On the other hand, the RNS peroxynitrite ($ONOO^-$) is a powerful oxidant/nitrating species which is formed by the rapid reaction between $O_2^{.-}$ and NO (Radi, 2013), and its occurrence in plant organelles, like peroxisomes, has been reported (Corpas and Barroso, 2014).

Table 5. *Induction of ROS and RNS overproduction in plants by stress situations (del Río and Puppo, 2009; Baxter et al., 2014; Nasir Khan et al., 2014; Yu et al., 2014)*

| Stress situations that induce ROS and RNS overproduction |
| --- |
| Infection by pathogens |
| High light intensities |
| UV radiation |
| High and low temperatures |
| Drought and salt stress |
| Heavy metals |
| Atmospheric pollutants |
| Physical and mechanical wounding |

As a result of the presence in plant tissues of NO and GSNO, and the generation of $ONOO^-$, important covalent post-translational modifications (PTMs) can take place in plants under natural and stress conditions, such as S-nitrosylation and the nitration of proteins (Romero-Puertas et al., 2013; Corpas et al., 2013a). In peroxisomes, catalase and glycolate oxidase activity are inhibited by S-nitrosylation and this could regulate the cellular level of key signalling molecules like H_2O_2 (Ortega-Galisteo et al., 2012). On the other hand, the generation of $ONOO^-$ can produce tyrosine nitration of plant proteins and originate nitrosative damage in plant cells, although a basal endogenous nitration could also have a regulatory function. Recent results obtained in pea plants by EM immunogold-labelling have shown the presence of nitrated proteins in different sub-cellular compartments of leaf cells, including peroxisomes, chloroplasts, mitochondria, and the cytosol (Barroso et al., 2013). Moreover, proteomic analysis of isolated pea leaf peroxisomes has shown that peroxisomal NADH-dependent hydroxypyruvate reductase is a target of nitration, and this reaction by peroxynitrite produced a loss of function in the enzyme (Corpas et al., 2013b). A schematic model of different post-translational modifications mediated by NO in plant cells is shown in Fig. 6.

The Society for Free Radical Research (SFRR)

Those colleagues working in the field of ROS and RNS biology, and especially the newcomers in this area, should know about the existence of a very active Society named the 'Society for Free Radical Research' (SFRR), which could be very helpful to them in the course of their research careers. This society was created at Brunel University, Uxbridge, UK, near London, in 1982, 'to promote the interest in all aspects of research related with free radicals in any scientific enterprise'. The Society was founded by an eclectic group of scientists mainly integrated by synthesis organic chemists, electron spin resonance (ESR) spectroscopists, radiation chemists, and biochemists interested in electron transfer processes. Its foundation was an example of the creative and practical spirit of scientific researchers working in very different fields but with a common interest, deciding to join forces and work together and creating a new society of interest for all of them. The Society, very small in the beginning, started to grow quickly in parallel to the exponential number of publications appearing which showed the important role played by oxygen free radicals in many fields, including biomedicine, pharmacology, inorganic and organic chemistry, physical chemistry, biology, etc. The SFRR is distributed over the five continents and there is an international section (SFRR International; http://www.sfrr.org/) that organizes and co-ordinates the different continental branches. The European branch is the SFRR-Europe (http://www.sfrr-europe.org/). This society has played and is playing a key role in keeping its original pioneering spirit, supplying a forum for multiple scientific congresses, symposia, workshops, etc, and is also associated with several international journals that specialize in ROS and RNS where
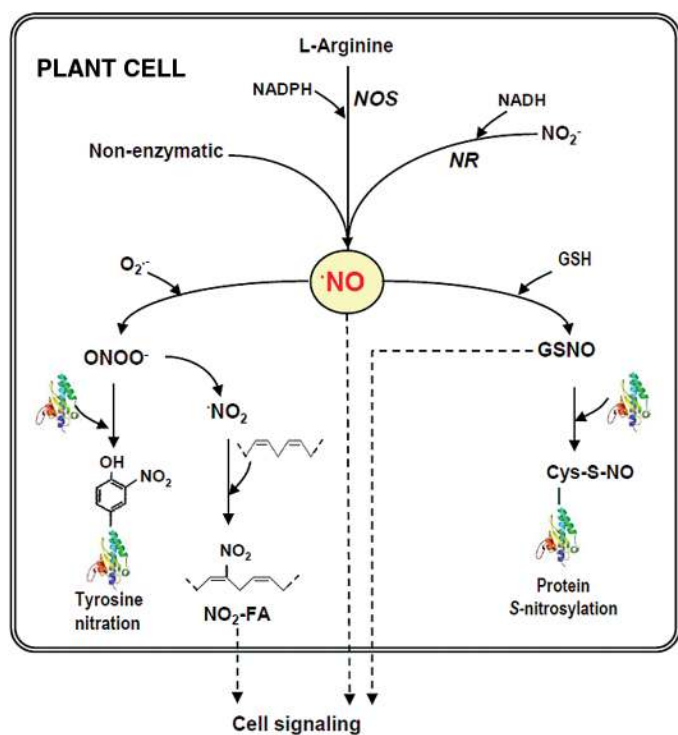
Fig. 6. Model of post-translational modifications mediated by nitric oxide in plant cells. The scheme represents a plant cell. NO generated either by L-arginine-dependent nitric oxide synthase (NOS), nitrate reductase (NR) or other non-enzymatic processes, can react with reduced glutathione (GSH) to form S-nitrosoglutathione (GSNO) that can produce protein S-nitrosylation reactions. But nitric oxide can also react with superoxide radicals ($O_2^{\cdot-}$) to generate peroxynitrite (ONOO$^-$), a powerful oxidant that can mediate the tyrosine nitration of proteins, and fatty acids (FA) where the nitrogen dioxide radical ($\cdot NO_2$) seems to be an intermediate. Nitric oxide, GSNO, and NO_2-FA could participate in cell signalling processes, as well as S-nitrosylated proteins. Taken from Corpas *et al.* (2015) in *Reactive oxygen and nitrogen species signaling and communication in plants*. Series Vol. 23. Springer, 267–281.

papers in these fields can be published. The SFRR organizes many meetings (see the corresponding web pages) where scientists of different disciplines can present and discuss their latest results contributing to the improvement of knowledge. In Spain, there is a group called the 'Spanish Group for Free Radical Research' (GEIRLI; http://www.geirli.net/), associated with the SFRR-Europe, which organizes national or international meetings every year.

The Plant Oxygen Group (POG)

In the SFRR-Europe there is a group dedicated exclusively to research on ROS and RNS in plants, which is named The Plant Oxygen Group (POG). This group was created in 1993 and was the idea of Dr George AF Hendry who was then working at the Department of Animal and Plant Sciences, University of Sheffield. George Hendry, together with Professor RMM Crawford, University of St Andrews, and Dr BA Goodman of the Scottish Crop Research Institute, Invergowrie, Dundee, organized what was considered to be the first and constitutive meeting of this group in September 1993 at the University of St Andrews, Scotland. The meeting

was called 'The First International Conference on the Role of Oxygen in Environmental Stress in Plants'. During this meeting, George Hendry proposed the formation of a European or international group devoted to the study of the function of oxygen in environmental stress in plants, and he proposed for this group the name 'Plant Oxygen Group (POG)'. This proposal was approved and it was decided that the group should be associated with the SFRR-Europe.

That was the very beginning of the POG and I remember the nice and highly stimulating scientific atmosphere of the St Andrews meeting. A book was published with papers selected from the 150 world-wide participants attending this first conference (Crawford *et al.*, 1994). Among the many colleagues attending, apart from the organizers, were AR Wellburn (UK), Ya'acov Leshem (Israel), Martina Schraudner (Germany), Heinrich Sandermann, Jr (Germany), C Triantaphylidès (France), Christian Langebartels (Germany), Erich F Elstner (Germany), Mark N Merzlyak (Russia), Ivan Couée (France), Gary P Creissen (UK), Phil M Mullineaux (UK), Nick Smirnoff (UK), Flavia Navari-Izzo (Italy), Juan Segura-Aguilar (Sweden), Roy H Burdon (UK), Gunnar Wingsle (Sweden), Francisca Sevilla (Spain), Ricardo Izzo (Italy), Mike F Quartacci (Italy), Susana Puntarullo (Argentina),and Luis A del Río (Spain). George Hendry was elected to be the first President of the POG (1993–1995) and I succeeded him as the second President (1995–1998). The following Presidents were: Flavia Navari-Izzo, Christine H Foyer, Alain Puppo, Laura de Gara, Jaakko Kangasjärvi, Stanislaw Karpinski, and the current President is Frank Van Breusegem (Ghent University, Belgium). After the first meeting in St Andrews, periodical meetings were organized, usually every two years, in Aberdeen (UK), Vienna (Austria), Pisa (Italy), Granada (Spain), Nice (France), Freising-Weihenstephan (Germany), Bristol (UK), Ghent (Belgium), Helsinki (Finland), Budapest (Hungary), Warsaw (Poland), and the next meeting will be in Verona (Italy) in 2015.

In conclusión, the POG is a very active group which provides a major interdisciplinary platform for cutting edge research in all aspects of free radical research (ROS and RNS) and redox signalling in plants and organizes conferences every two years to facilitate information transfer and other essential interactions. More information about the Plant Oxygen Group can be found in the web page http://www.plantoxygengroup.org/

Conclusions and perspectives

Although it is now well established that ROS and RNS have a key role in intercellular and intracellular molecular communication, there is still much to learn about the initiation of ROS signalling, the sensing and response mechanisms, and how the delicate balance between production and scavenging is controlled. It is also very important to know more about the interactions between the pathways mediated by RNS, ROS, cellular redox changes, calcium signalling, hormones (auxin, cytokinin, salicylic acid, jasmonic acid, and ethylene), and other messenger molecules. The detailed knowledge of the mechanism and regulation of the cross-talk by ROS,

RNS, and those mentioned signalling molecules in plant cells under physiological and stress conditions will substantially expand our knowledge of ROS and RNS in plant physiology.

While there has been important progress in our understanding of the physiological role of NO in plants there is still scarce information on NO biosynthesis. For its significant physiological repercussions, it is very important to characterize the protein(s) or gene(s) responsible for the L-arginine-dependent nitric oxide synthase (NOS) activity detected in many higher plant species under different physiological conditions. The identification of this important plant NOS-like activity is waiting to be accomplished.

Although the first genes involved in ROS and RNS perception and signal transduction have been identified, it remains a challenge to identify the other players of the gene regulatory network and decipher their mode of action in ROS and RNS signal perception and transduction, as well as their possible role in epigenetic processes. Equally, the development of new and innovative methodologies of imaging analysis to study ROS and RNS at the tissue and cellular level is essential to advance our knowledge. All this will shed new light on ROS and RNS action in plants. No doubt, the forthcoming years will bring new and exciting insights into the mechanism of action of ROS and RNS in plant physiology and their interconnection with other important signalling networks, particularly under environmental stress conditions, an iceberg whose tip we are only starting to see now.

Acknowledgements

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