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Nitric oxide regulation of plant metabolism

Kapuganti Jagadis Gupta[1]*, Vemula Chandra Kaladhar [1], Teresa B. Fitzpatrick[2], Alisdair R. Fernie[3], Ian Max Møller[4], Gary J. Loake[5*]

[1]National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, Delhi, 110067 India

[2] Vitamins and Environmental Stress Responses in Plants, Department of Botany and Plant Biology, University of Geneva, Geneva, 1211 Switzerland

[3]Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, Potsdam-Golm, D-14476 Germany

[4]Department of Molecular Biology and Genetics, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse, Denmark

[5]Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3BF, United Kingdom

Address for correspondence

*Kapuganti Jagadis Gupta
National Institute of Plant Genome Research
Aruna Asaf Ali Marg
110067
New Delhi
Email: jgk@nipgr.ac.in
Phone +91-11-26735111

*Gary J. Loake
Institute of Molecular Plant Sciences
School of Biological Sciences
University of Edinburgh, Edinburgh, UK
G.Loake@ed.ac.uk

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Abstract

Nitric oxide (NO) has emerged as an important signal molecule in plants, having myriad roles in plant development. In addition, NO also orchestrates both biotic and abiotic stress responses, during which intensive cellular metabolic reprogramming occurs. Integral to these response is the location of NO biosynthetic and scavenging pathways in diverse cellular compartments, enabling plants to effectively organize signal transduction pathways. NO regulates plant metabolism and in turn, metabolic pathways reciprocally regulate NO accumulation and function. Thus, these diverse cellular processes are inextricably linked. This review addresses the numerous redox pathways, located in the various subcellular compartments, which produce NO, in addition to the mechanisms underpinning NO scavenging. We focus on how this molecular dance is integrated into the metabolic state of the cell. Within this context, a reciprocal relationship between NO accumulation and metabolite production is often apparent. We additionally showcase cellular pathways including those associated with nitrate reduction that provide evidence for this integration of NO function and metabolism. Finally, we discuss the potential importance of the biochemical reactions governing NO levels in determining plant responses to a changing environment.

Introduction

Plants are sessile organisms exposed to an ever-changing environment and their metabolism must be sufficiently flexible to allow them to acclimate to changing conditions. Changes in light, temperature, mineral nutrients, or stress conditions such as drought, flooding and salinity are all external factors that require such metabolic adjustments. As an example, flooding leads to hypoxia in submerged organs and the plant responds by changing its energy metabolism in those regions to fermentation as a replacement of oxidative phosphorylation in the mitochondria (Taiz et al., 2015). This requires signalling within and between the affected cells leading in the short term to modifications of key enzymes and in the long term to changes in the network of gene expression that controls the related metabolism. A number of small molecules are involved in this signal transduction, such as the plant hormones auxin and ethylene, in addition to NO. The purpose of this review is to summarize the current state-of-the-art regarding aspects of NO signalling that are integral to plant metabolism.

Metabolic pathways associated with NO production and removal

Plants utilise various oxidative and reductive pathways to generate NO (Gupta et al*.,* 2011; Kolbert et al., 2019). Oxidative NO pathways operate under normoxic conditions and the enzymes responsible for NO production via these pathways have not been thoroughly investigated. These pathways are well studied in mammalian cells, with three central NO synthase (NOS) enzymesbeing described: neuronal NOS, inducible NOS and endothelial NOS (Gupta et al*.,* 2011; Kolbert et al., 2019). However, a previously reported plant NOS in *Arabidopsis* did not display significant sequence homology to any of the mammalian NOS isoforms (Guo et al., 2003). This *Arabidopsis* enzyme was found to lack NOS activity and to act instead as a GTPase and was therefore subsequently renamed Nitric Oxide Associated1 (Zemojtel et al*.,* 2006). Nonetheless, NOS-like activity has been detected in peroxisomes and chloroplasts in plants (reviewed in Astier et al*.,* 2018; Corpas and Barroso, 2017) and inhibitors of mammalian NOS (e.g. L -arginine analogues) are able to diminish peroxisomal NOS-like activity (Corpas and Barroso, 2017). To detect this activity, it was necessary to provide L-arginine and the co-substrates, NADPH and oxygen, together with multiple co-enzymes, including flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), as well as proteins such as calmodulin and tetrahydrobiopterin (BH_4) (Figure 1A) (Corpas and Barroso, 2017; Barroso et al., 1999; Corpas et al., 2004). Despite the conclusions of the aforementioned studies, the identification of proteins contributing to this NOS-like activity requires further analysis.

A recent bioinformatic study of the genomes of 1300 higher plant species failed to identify any NOS homologs (Jeandroz et al., 2016). This finding therefore raised the question of the identity of the enzymatic processes underlying the observed L-arginine dependent NO biosynthesis route in *Arabidopsis* (reviewed in Corpas et al*.,* 2009), suggesting the enzyme responsible is not structurally related to mammalian NOS. Despite this, a mammalian NOS homolog was identified, and subsequently characterized, in the microalgae, *Ostreococcus tauri* (Foresi et al*.,* 2010). This enzyme produces large amounts of NO during exponential growth, suggesting an important role for NO in the biology of microalgae. Lack of evidence of NOS in higher plants suggests that land plants may have lost this enzyme during evolution. The apparent absence of a NOS-like enzyme in higher plants has led to several other sources of NO being proposed, including polyamine and hydroxylamine-based pathways. Polyamines such as putrescine, spermine and spermidine have all been implicated in

NO production (Tun et al., 2006; Zhou et al., 2016). For example, experimental evidence suggests that addition of putrescine, spermine and spermidine significantly induced NO accumulation (Tun et al., 2006) and 2-aminoethyl-2-thiopseudourea, an inhibitor of animal NOS enzymes inhibited polyamine-induced NO production. Copper amine oxidases (CuAOs) are enzymes of the polyamine pathways that regulate oxidation reactions. The loss of the CuAO1 and CuAO8 enzymes leads to reduced NO production in *Arabidopsis*, implying a potential polyamine-mediated NO production route in plants (Wimalasekera et al*.,* 2011; Groß et al., 2017). Moreover, elicitor- or salt-induced NO production is lower in *Arabidopsis cuao1* and *cuao8* mutants compared to wild-type supporting a role of these enzymes in NO metabolism (Groß et al., 2017). On the other hand, tobacco cell suspension cultures produced NO in an oxygen- and reactive oxygen species (ROS) dependent reaction upon application of hydroxylamine (Rümer et al*.,* 2009), but this NO biosynthetic pathway has not yet been fully characterized.

Cytosolic nitrate reductase (NR) is a well-known enzyme important in nitrogen metabolism and is widely implicated in NO production (Kolbert et al., 2019). NR mediates reduction of nitrate to nitrite using NADH as an electron donor, also requiring molybdopterin, heme and FAD as coenzymes (Campbell, 2001). NR exhibits nitrite:NO reductase activity under certain conditions, such as hypoxia, anoxia, reduced pH or pathogen infection (Yamasaki and Sakihama, 2000; Rockel et al., 2002) and when excess nitrite accumulation occurs due to inhibition of the nitrite reductase activity of NR (Figure 1C). It has been recently reported that an *Arabidopsis* nitrite reductase mutant obtained by CRISPR-Cas9 genome editing as well as transgenic plants overexpressing NR1 and NR2 proteins contained elevated endogenous NO levels under normoxic conditions (Costa-Broseta et al., 2020; 2021), thus supporting the relevance of reductive NR-dependent activity in the production of NO under normoxia. More specifically, under hypoxic or anoxic conditions, mitochondria can reduce nitrite to NO at the sites of complex III and IV and possibly also at alternative oxidase (AOX) (Figure 1 D). In addition, a plasma membrane-bound specific nitrite-NO reductase (NiNOR) is capable of reducing nitrite to NO (Stöhr et al*.,* 2001) (Figure 1E).

Recently, it was demonstrated that a dual system formed by the Amidoxime Reducing Component or Nitric Oxide-Forming Nitrite Reductase (ARC or NOFNiR) and NR molybdo-enzymes mediates nitrite-dependent NO production in the green algae, *Chlamydomonas reinhardtii* (Chamizo-Ampudia et al*.,* 2016). NR and ARC are regulated both transcriptionally and at the protein activity level. This enzyme system is able to produce NO in the presence of nitrate. However, the role of ARC or NOFNiR in NO production is indirect, and it is not known whether a similar system operates in higher plants. Nitrite reduction to NO can also be catalysed by the peroxisomal enzyme xanthine oxidoreductase (XOR) (Figure 1B). This enzyme can generate uric acid and superoxide under aerobic conditions. Under anaerobic conditions, purified XOR has been shown to reduce nitrite to NO, with either xanthine as reducing substrate or NADH (Godber et al., 2000; Del Río et al., 2004).

In addition to NO biosynthesis, plants have evolved enzymes that can scavenge NO and convert this key signalling molecule into nitrate or *S*-nitrosoglutathione (GSNO). Consequently, net NO production is a function of biosynthesis minus scavenging. Two key proteins with known NO metabolism properties in plants are phytoglobins and *S*-

189 nitrosoglutatione reductase (GSNOR) (Figure 2A and B) (Gupta et al., 2020b). The
190 phytoglobin (PGB)-NO cycle (links cytosolic NR and mitochondria for enhanced
191 energy production, as well as the protection of plants from excess NO due to the ability
192 of this cycle to scavenge NO (Igamberdiev et al*.,* 2005). NR converts nitrate to nitrite,
193 which moves into the mitochondrion via an unknown transporter. Here it replaces
194 oxygen as the terminal electron acceptor, leading to the reduction of nitrite to NO
195 (Planchet et al*.,* 2005). Since NO radicals are diffusible molecules they can readily
196 move to the cytosol where PGB1 can oxidize NO to nitrate, which can subsequently
197 be utilized by NR (Gupta et al., 2018). The full turn of the cycle though is not very
198 efficient at producing ATP (Hebelstrup and Møller, 2015), so under most conditions,
199 the cycle is likely to be more important for regulating the cellular NO level (Becana et
200 al., 2020).
201
202 The PGB-NO cycle also plays a key role in anaerobic germination of rice (Kumari et
203 al 2021). Addition of nitrite results in enhanced expression of components of Pgb-NO
204 cycle accompanied by enhanced ATP generation. In addition, the PGB-NO cycle
205 functions in the establishment of nodulation via increased ATP production (Berger et
206 al*.,* 2020) and further, regulates NO generation during mycorrhizal associations
207 (Martínez-Medina et al*.,* 2019), underscoring the functional importance of this cycle.
208
209 The activity of mitochondrial AOX also indirectly reduces NO production (Cvetkovska
210 and Vanlerberghe, 2012). Plants knocked down in AOX generated more superoxide
211 and NO, whereas AOX overexpressing lines produced less of these molecules. AOX
212 prevents over-reduction of the electron transport chain (ETC) thereby lowering the
213 electron leakage to oxygen or nitrite at complex III and IV (Møller, 2001; Hebelstrup
214 and Møller, 2015). Thus, the production of NO under biotic stress conditions, triggered
215 by treatment of roots with the immune elicitor flg22 can be effectively prevented by
216 AOX activity (Vishwakarma et al*.,* 2018).
217
218 **Metabolic pathways are regulated by *S*-nitrosylation**
219 *S*-nitrosylation, the addition of an NO moiety to a reactive cysteine (Cys) thiol to form
220 an *S*-nitrosothiol (SNO) (Figure 3A) (Yun et al*.,* 2016; Lindermayr et al*.,* 2005; Begara-
221 Morales et al., 2014), is thought to be the dominant route for the mediation of plant NO
222 bioactivity. The unique properties of the sulphur atom embedded within the amino acid,
223 Cys, is key to enable the signalling outcomes associated with this modification
224 (Umbreen et al., 2018). Thus, *S*-nitrosylation, as a prototypic redox-based post-
225 translational modification (PTM), is conceptually similar to more established PTMs
226 such as phosphorylation (Zhou et al., 2018; Gupta et al., 2020b). In this context, *S*-
227 nitrosylation can modulate protein function by regulating enzyme activity, protein
228 localization, protein-protein interactions, protein degradation and protein DNA binding
229 (Yu et al*.,* 2014; Cui et al*.,* 2018; Albertos et al., 2015).
230
231 The accumulating data suggests that *S*-nitrosylation is a key switch to control
232 important components of plant metabolism. Wang et al., (2009) demonstrated that a
233 pathogen-triggered nitrosative burst mediates *S*-nitrosylation of Cys280 of Salicylic
234 Acid-Binding Protein 3 (SABP3), suppressing binding to the key immune-related
235 metabolite, salicylic acid (SA), and reducing the cognate carbonic anhydrase (CA)
236 activity of this enzyme (Slaymaker et al., 2002). The CA function of SABP3 is essential
237 for plant defence (Slaymaker et al*.,* 2002). Hence, the inhibition of SABP3 CA function
238 by *S*-nitrosylation may act as part of a negative feedback loop. On the other hand, NO

239 accumulation promotes transcription of *SRG1* which encodes a zinc finger
240 transcription factor (Cui et al., 2018), that functions as a positive regulator of plant
241 immunity, including the accumulation of the defence metabolite, SA. SRG1 is a
242 transcriptional repressor; thus, to positively regulate immunity, this protein presumably
243 represses an immune repressor. Accordingly, sustained NO accumulation resulted in
244 *S*-nitrosylation of this protein at Cys87, which released SRG1 binding from its cognate
245 *cis*-element and by extension, the associated SRG1 transcriptional repression activity.
246 Subsequently, this may enable the expression of a negative regulator, subsequently
247 curbing the immune response, including a decrease in SA biosynthesis (Cui et al*.,*
248 2018).
249
250 *S*-nitrosylation also modulates ethylene biosynthetic pathways. For instance,
251 methionine adenosyltransferase (MAT), an enzyme that plays a key role in the
252 formation of *S*-adenosylmethionine (SAM), which is required for various methylation
253 reactions and ethylene biosynthesis, has been shown to be *S*-nitrosylated (Pérez-
254 Mato et al., 1999), leading to suppression of its activity (Lindermayr et al*.,* 2006). Key
255 antioxidant metabolic enzymes are also known to be regulated by *S*-nitrosylation.
256 Yang et al. showed that this redox-based PTM modified ascorbate peroxidase 1
257 (APX1) at Cys32 enhancing its hydrogen peroxide-metabolising activity, thereby
258 reducing oxidative stress (Yang et al., 2015). In addition, *S*-nitrosylation of the
259 *Arabidopsis* RBOHD at Cys890 reduced its capacity to generate ROS, curbing the
260 oxidative burst and thereby limiting the extent of cell death associated with the
261 hypersensitive response (Yun et al*.,* 2011). Interestingly, Cys890 is evolutionarily
262 conserved and *S*-nitrosylation of homologs of this RBOHD in flies and humans also
263 reduces enzyme activity, indicating that this mechanism is conserved across kingdoms
264 (Yun et al*.,* 2011).
265
266 Peroxynitrite ($ONOO^-$) metabolism is also thought to play an important role in the
267 development of pathogen-triggered hypersensitive cell death (Delledonne et al*.,*
268 1998). In this context, it has been demonstrated that *S*-nitrosylation inhibits the
269 hydrogen peroxide-metabolism (peroxidase) activity of peroxiredoxin IIE (PrxII E). This
270 protein has a key function in metabolising $ONOO^-$. Thus, inhibition of PrxII E leads to
271 increased $ONOO^-$ content, which can drive hypersensitive cell death development
272 (Romero-Puertas et al., 2007).
273
274 The metabolism of glycine by the glycine decarboxylase complex (GDC) is governed
275 by a series of enzymes that are triggered in response to high concentrations of the
276 amino acid glycine. The same set of enzymes is sometimes referred to as glycine
277 synthase when it runs in the reverse direction to form glycine. The glycine cleavage
278 system is composed of four proteins: the T-protein, P-protein, L-protein, and H-
279 protein. Treatment of *Arabidopsis* cell cultures with the natural NO metabolite,
280 GSNO, leads to *S*-nitrosylation of the GDC enzyme subunits P2 and H1, which
281 contribute to the modulation of glycine and by extension, photorespiration. In this
282 vein, *S*-nitrosylation of the P2 and H1 subunits leads to the inhibition of this system,
283 resulting in an increased ROS production (Palmieri et al*.,* 2010).
284
285 It is also becoming increasingly appreciated that *S*-nitrosylation can regulate a number
286 of more well-characterised PTMs (Gupta et al., 2020), significantly expanding the
287 influence of NO over key cellular processes including metabolism. Recently, it was
288 shown that NO regulates conjugation of proteins with SUMO (small ubiquitin-like

289 modifier), so-called SUMOylation, through *S*-nitrosylation of SUMO-conjugating
290 enzymes (Skelly et al., 2019). SUMOylation has been shown to negatively regulate
291 the deployment of plant immune responses, underpinned by SA accumulation.
292 Pathogen recognition promotes NO accrual, which subsequently results in *S*-
293 nitrosylation of SUMO conjugating enzyme 1 (SCE1) at Cys139, reducing the activity
294 of this enzyme and by extension, decreasing global SUMO 1 and 2 dependent
295 SUMOylation. The global reduction of SUMO 1/2 SUMOylation subsequently enables
296 the attainment of maximal levels of the metabolite, SA, and subsequent SA-dependent
297 immune responses. Significantly, the human homolog of SCE1, UBC9, is similarly
298 regulated by *S*-nitrosylation of this conserved Cys residue, suggesting that this
299 mechanism to control global SUMOylation is also conserved across kingdoms (Skelly
300 et al*.,* 2019).
301
302 **Protein denitrosylation and transnitrosylation**
303 An important feature of the addition of PTMs to their protein targets associated with
304 cellular signalling is their selective reversal to disengage the given signal networks. In
305 this context, it is possible that specificity in redox signalling is accomplished
306 predominantly by reversal rates of Cys modifications, rather than by their formation,
307 as rapidly degraded redox PTMs may have less impact than more persistent ones
308 (Derakhshan et al., 2007). Thus, different protein-SNOs can have widely diverse
309 biological lifetimes (Seth and Stamler, 2015). While a proportion of this can be
310 attributed to the innate chemical stability of a given protein-SNO, this property is also
311 influenced by potential non-enzymatic breakdown, for example, by either ascorbate or
312 glutathione, key cellular antioxidants (Masella et al., 2005; Feechan et al., 2005;
313 Benhar et al., 2008; Kneeshaw et al., 2014).
314
315 The metabolite, GSNO, can function as a natural NO donor and effectively acts as a
316 relatively stable pool of NO bioactivity. Thus, increasing concentrations of GSNO in
317 *Arabidopsis* promotes elevated levels of total protein *S*-nitrosylation. Conversely,
318 decreasing GSNO concentrations result in reduced levels of this redox-based PTM
319 (Feechan et al., 2005). GSNO can be metabolised by the activity of GSNOR, thus this
320 enzyme indirectly controls the global levels of protein *S*-nitrosylation (Feechan *et al.*,
321 2005; Lee *et al.*, 2008; Chen *et al.*, 2009). In the context of metabolism, *Arabidopsis*
322 GSNOR, via its ability to regulate *S*-nitrosylation, has been shown to control the
323 biosynthesis of the immune activator, SA (Feechan et al., 2005). Similar phenotypes
324 to those of *Arabidopsis* have also been described in tomato GSNOR RNAi lines
325 (Hussain *et al.*, 2019), suggesting that the function of this enzyme is conserved across
326 dicotyledonous species.

327 In phosphorylation, for example, a well-established signal transduction process,
328 specificity is achieved via the result of a delicate poise between kinase and
329 phosphatase activities. Recently, the conceptual equivalent of protein phosphatases
330 associated with redox signalling has begun to emerge. Thioredoxins (Trxs) are present
331 in all living organisms and their activity can be recycled by NADPH-dependent
332 thioredoxin reductase (TrxR). Trx/TrxR mediated denitrosylation has been uncovered
333 as a key mechanism to control NO signalling in mammals (Benhar et al., 2008).
334 Subsequently, *Arabidopsis* Trxh5 has emerged as a plant denitrosylase, which
335 selectively denitrosylates the transcriptional co-activator, NPR1, which promotes SA
336 biosynthesis and SA signalling during plant immunity (Kneeshaw et al., 2014) (Figure
337 3A). It is noteworthy, however, that NPR1 activity is also influenced by GSNOR

338 function (Feechan et al., 2005; Tada et al., 2008), implying that the *S*-nitrosylation
339 status of this co-activator maybe controlled directly via Trxh5 and also indirectly via
340 GSNOR. Moreover, Trxh5 and TrxR may denitrosylate a sub-set of the *Arabidopsi*s
341 SNO proteome directly and selectively *in vivo* (Kneeshaw et al., 2014). Consequently,
342 the regulation of denitrosylation at additional Cys thiol residues embedded in
343 regulatory proteins might also be mediated by Trxh5 and TrxR. Trx enzymes are
344 encoded by a sizeable gene family in *Arabidopsis*, thus additional Trx family proteins
345 may operate in conjunction with TrxR as direct and selective denitrosylases for a
346 variety of other substrates.
347
348 In addition to Trxh5 and possibly other Trx proteins, *Arabidopsis* possesses two
349 nucleoredoxins, NRX1 and NRX2. To date, NRX1 has unexpectedly been shown to
350 be required for the protection of enzymes associated with ROS metabolism from
351 oxidation within ROS-rich environments, including plant cells undergoing immune
352 responses (Kneeshaw et al., 2017). Perhaps these two enzymes might also function
353 as specific denitrosylases of key regulatory proteins within the plant nucleus.
354
355 Counterpoint to denitrosylation, the emerging evidence across life kingdoms suggests
356 protein *S*-nitrosylation may occur within multiprotein macro-complexes, where an *S*-
357 nitrosylated protein transfers an NO group directly to a target protein, a process termed
358 transnitrosylation (Figure 3B) (Seth et al., 2018; Chen et al., 2020). Here, the protein
359 transferring the NO moiety, termed a nitrosylase, is increasing both the efficiency and
360 specificity of this redox-based PTM in an enzyme-like fashion. It is likely that examples
361 of transnitrosylation relevant to plant metabolism will also be uncovered in the near
362 future.
363
364 Collectively, the current state-of-the-art suggests that Trx and TrxR enzymes and
365 possibly also NRX1 and NRX2, can function as direct and selective denitrosylases to
366 regulate a subset of plant *S*-nitrosylated proteins associated with metabolic processes.
367 In addition, our appreciation of how NO maybe transferred to target Cys residues
368 relevant to metabolism by transnitrosylation, resulting from the activity of nitrosylases,
369 is also set to increase.
370

371 **Protein Tyr-nitration modulates metabolic pathways**

372 An additional NO based redox modification is tyrosine nitration, where protein tyrosine
373 side-chains are nitrated to give 3-nitrotyrosine (NO_2-Tyr) by peroxynitrous acid
374 (HOONO), which is formed by the reaction of superoxide and NO, followed by a
375 protonation (Chaki et al*.,* 2014; Holzmeister et al., 2011) (Figure 3C). It is becoming
376 clear that this is also a major regulatory PTM given that it appears to be involved in
377 the control of a wide range of metabolic pathways. Many nitrated proteins and
378 enzymes have been identified (Chaki et al*.,* 2014; Holzmeister et al., 2011), but we will
379 here only highlight examples relevant to metabolism, where the site of tyrosine
380 nitration has been identified and the cognate effect established. Note to date this effect
381 has always been inhibitory, which may point at a fundamental property of this type of
382 metabolic regulation.
383
384 During senescence of pea roots, a total of 16 NO_2-Tyr proteins were identified. One of
385 these, cytosolic NADP-isocitrate dehydrogenase, which is involved in amino acid
386 interconversions and NADPH production, was shown to be inhibited by tyrosine
387 nitration (Begara-Morales et al., 2013). Further, the ascorbate-glutathione cycle

388 detoxifies hydrogen peroxide in all the major subcellular compartments.
389 Monodehydroascorbate reductase (MDAR) and APX are both additionally integral to
390 this cycle and they are both inhibited by tyrosine nitration (Begara-Morales et al., 2014;
391 Begara-Morales et al., 2015). Interestingly, mitochondrial manganese superoxide
392 dismutase (Mn-SOD), which converts superoxide into hydrogen peroxide, is inhibited
393 by peroxinitrate mediated tyrosine nitration (Holzmeister et al., 2015). Thus, the
394 superoxide formed by the mitochondrial ETC is not metabolised and therefore is free
395 to react with any available NO to generate more peroxinitrate. In addition, peroxisomal
396 hydroxypyruvate reductase (HPR1), part of the photorespiratory pathway, is prone to
397 tyrosine nitration, which inhibits its activity (Corpas et al*.,* 2013a). The last step in the
398 assimilation of sulphur (from sulphate) is catalysed by the enzyme *O*-
399 acetylserine(thiol)lyase. This enzyme is also inhibited by tyrosine nitration (Alvarez et
400 al., 2010). Finally, in root nodules, glutamine synthetase (Melo et al., 2011) is another
401 enzyme inhibited by tyrosine nitration, while leghemoglobin nitration is thought to act
402 as a sink for potentially damaging nitrogen radicals thus protecting other proteins
403 (Sainz et al., 2015).
404
405 A considerable limitation associated with a potential signalling role for NO_2-Tyr in
406 metabolic regulation, is that while Cys *S*-nitrosylation, is readily reversible, there is no
407 known pathway for the reversal of tyrosine nitration. Indeed, this PTM has been linked
408 with protein degradation. For example, the turnover of ABA receptors following
409 tyrosine nitration (Castillo et al., 2015). However, the degradation of tyrosine nitrated
410 proteins is an energetically expensive solution and may only be appropriate when the
411 plant cell is closing down a metabolic process or is undergoing senescence.
412
413 **Influence of NO on peroxisomal enzymes linked to oxidative metabolism**
414 Peroxisomes are one of the major sites for ROS generation (Del Rio, and Lopez-
415 Huertas, 2016), and these organelles are also involved in NO production and
416 constitute targets for NO (Corpas and Barroso, 2014; Begara-Morales et al., 2015).
417 Peroxisomes contain antioxidant metabolic enzymes, including catalase (CAT),
418 MDAR and Mn-SOD, to control the generation of ROS, mainly superoxide radicals
419 and hydrogen peroxide (Corpas and Barroso, 2018; Rodríguez-Ruiz et al*.,* 2019).
420 Several lines of evidence suggest that CAT is inhibited by NO and $ONOO^-$ (Clark et
421 al., 2000). Both *S*-nitrosylation and tyrosine nitration retard CAT activity in pea
422 leaves and pepper fruits (Chaki et al., 2015; Ortega-Galisteo et al., 2012). However,
423 the role of *S*-nitrosylation and tyrosine nitration-mediated inhibition of CAT requires
424 further investigation to uncover the detailed molecular mechanisms associated with
425 this PTM.
426
427 ROS and reactive nitrogen species (RNS) are essential for modulating peroxisomal
428 function during pepper fruit (*Capsicum annuum L.*) ripening especially under nitro-
429 oxidative stress when CAT and other potential enzyme candidates are *S*-nitrosylated
430 (Rodríguez-Ruiz et al*.,* 2019). Purified recombinant MDAR from pea leaf peroxisomes
431 is inhibited by both $ONOO^-$ and GSNO. Furthermore, MDAR undergoes nitration at
432 Tyr345, in addition to *S*-nitrosylation at Cys68 (Begara-Morales et al., 2015). SOD is
433 also prone to nitration at Tyr115 (Holzmeister et al., 2015), which leads to irreversible
434 inhibition and increased superoxide accumulation (Corpas et al*.,* 2019). HPR1, a
435 peroxysomal enzyme, is also inhibited by Tyr-nitration (Corpas et al., 2013a), while
436 APX can be both activated by reversible *S*-nitrosylation and inactivated by irreversible

nitration (Begara-Morales et al., 2014). Thus, numerous studies have now established NO as a ubiquitous regulator of antioxidant metabolic enzymes in the peroxisome.

NO influences mitochondrial metabolism under stress

Mitochondria are the energy powerhouses of the cell, but they also generate NO and contain protein targets for NO regulation (Møller, 2001; Hebelstrup and Møller, 2015; Gupta et al., 2018; Møller et al*.,* 2020). All of the main complexes of the mitochondrial ETC interact, either directly or indirectly, with NO (Figure 4) (Gupta et al., 2018). For example, mutations in NADH dehydrogenase subunit 7 in *Nicotiana sylvestris* impaired NO production and resulted in cytoplasmic male-sterility (Shah et al., 2013). This mutant showed an increased expression of Phytoglobin 1 (PGB1) under differential oxygen concentrations (Shah et al., 2013). It was recently demonstrated that nitrite protects mitochondrial structure and function under hypoxia (Gupta et al*.,* 2017). Several complexes and super complexes are affected in this process. Interestingly, the specific activity of complex I was higher in the presence of NO. In addition, the supercomplex $I+III_2$ showed enhanced activity suggesting a specific role of this supercomplex in mitochondrial protection. NO might also have a specific role in enhancing electron channelling via mitochondrial supercomplexes under hypoxia.

Complex II which participates in both the tricarboxylic acid cycle and the ETC, also interacts with NO. Simonin and Galina (2013) found that application of NO donors such as *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) or diethylenetriamine nonoate led to a dramatic increase in the K_m (succinate), up to 45-fold under anoxic conditions.

Complex III is also both a site for the production of and a target for NO (Planchet et al., 2005; Alber et al*.,* 2017). The site of NO production is the Q cycle, analogous to superoxide generation, where electron pressure in the Q cycle during stress leads to increased electron leakage and concomitant superoxide or NO production (Alber et al*.,* 2017; Sun and Trumpower, 2003; Møller,2001). The production of NO under anoxia by tobacco cell suspensions or tobacco roots is sensitive to myxothiazol, which inhibits the Q cycle of the bc1 complex of the mitochondrial ETC (Sun and Trumpower, 2003).

The cytochrome (COX) pathway is also sensitive to NO at Complex IV (Millar and Day, 1996) and its inhibition leads to increased AOX abundance and engagement. In barley roots overexpressing PGB1 decreased the NO concentration and inhibited respiration, thereby increasing internal oxygen, reducing ROS production and subsequently enhancing metabolic flux via the oxidative pentose phosphate pathway (Gupta et al*.,* 2014). Bulky tissues such as germinating seeds contain low internal oxygen levels, that can slow down germination. In this context, a recent discovery demonstrated that exogenously supplied NO stimulated germination of the slow germinating kabuli chickpea variety, which produces reduced levels of NO (Pandey et al*.,* 2019). Application of NO lead to increased internal oxygen concentrations and lowered ROS levels, which prompted germination. Chickpeas are a key crop on the Indian subcontinent where they provide an important source of protein and fibre. Thus, breeding programmes to enhance NO production in chickpea might help in increasing the rate of seed germination of this important crop species (Pandey et al*.,* 2019).

AOX catalyzes ubiquinol oxidation with a four-electron reduction of oxygen to water (Møller, 2001; Moore et al., 2013). Electron transfer via AOX does not lead to proton

487 translocation via complex III and IV but plays a role in preventing over-reduction of the
488 ubiquinone pool and concomitant ROS and NO production (Møller, 2001; Cvetkovska
489 and Vanlerberghe, 2012). Both an AOX knockout mutant of tobacco and an AOX
490 antisense line displayed higher NO accumulation than wild-type (Cvetkovska and
491 Vanlerberghe, 2012). Increased levels of NO inhibit COX and induce AOX activity,
492 which may help to compensate for COX inhibition, as AOX is insensitive to NO (Millar
493 and Day, 1996). This feature can convey an additional advantage to mitochondrial
494 energy production in conditions such as hypoxia. Several lines of evidence suggest
495 that *AOX* transcripts and protein are induced by NO. For example, the bacterial elicitor
496 harpin leads to the accumulation of NO and the transcriptional activation of *AOX*
497 (Huang et al., 2002). In addition, the pathogen associated molecular pattern flg22,
498 consisting of a 22 amino acid peptide from bacterial flagellin or hypoxia also resulted
499 in the activation of *AOX* expression (Vishwakarma et al*.,* 2018). As both of these cues
500 elicit NO production, this molecule might be a key signal for regulation of AOX.
501
502 NO produced by the ETC participates in the regulation of other aspects of
503 mitochondrial metabolism (Møller et al*.,* 2020). The TCA-cycle enzyme, aconitase, is
504 regulated by NO and ROS (Gupta et al*.,* 2012). This enzyme contains an iron-sulphur
505 (Fe-S) cluster, presumably targeted by NO and is involved in the interconversion of
506 three tricarboxylic acids (citrate, *cis*-aconitate, and isocitrate) (Navarre et al*.,* 2000).
507 Inhibition of aconitase by hypoxia-induced NO, results in increased citrate levels and
508 enhanced AOX activity (Gupta et al*.,* 2012).
509
510 **NO regulation of chloroplast enzymes**
511 In addition to mitochondria, another organelle linked with NO production and function
512 is the chloroplast. Several lines of evidence suggests that chloroplasts are also a
513 source of NO production (Galatro et al., 2013; Tewari et al., 2013). Using electron
514 paramagnetic resonance (EPR) spectroscopy together with the spin trap, iron (II)-N-
515 methyl-D-glucamine dithiocarbamate (Fe(MGD)$_{2)}$, it has been demonstrated that
516 purified chloroplasts from soybean leaves can generate NO (Puntarulo et al., 2007**)**. It
517 was also found that both L-arginine- and nitrite-dependent pathways operate to
518 generate NO in chloroplasts (Jasid et al., 2006). In addition, NO influences
519 photophosphorylation, electron transport and PSII activity (Misra et al., 2014). In this
520 context, ETC components of PSII are targets of NO (Diner and Petrouleas, 1990): NO-
521 binding to the PSII component $Q_AFe^{2+}Q_B$ leads to a significant (10-fold) decrease of
522 the electron transfer rate between Q_A and Q_B. Employing pulse amplitude modulation
523 (PAM) fluorescence coupled with flash oxygen evolution approaches on isolated pea
524 thylakoid membranes, it was demonstrated that the electron donor site of PSII is the
525 probable target of NO action (Vladkova et al., 2011). It was also found that several
526 chloroplast proteins of *Arabidopsis* are *S*-nitrosylated in response to NO treatment
527 including the Rubisco small chain 1a precursor, Rubisco activase, Rubisco large
528 subunit, several PSII components and the Rieske Fe-S protein (Lindermayr et al.,
529 2005). In addition, a range of other proteins were found to undergo tyrosine nitration
530 (Lozano-Juste et al., 2011). Investigation into the specific roles of tyrosine nitrated and
531 *S*-nitrosylated proteins in chloroplasts will provide information on NO function
532 associated with metabolism linked to this organelle.
533
534 **NO regulates amino acid metabolism and polyamine production**
535 NO accumulation correlates with increases in the levels of amino acids of the
536 glutamate family (León et al*.,* 2016). Furthermore, an increased level of γ-

537 aminobutyric acid (GABA) was observed coinciding with increased levels of γ-
538 hydroxybutyrate and alanine, which play a role in conditions such as hypoxia (Rocha
539 et al*.,* 2010). NO accumulation also correlated with increased levels of proline, which
540 can act as an osmolyte, antioxidant and metal chelator (Hayat et al*.,* 2012).
541
542 NO-enhanced metabolic flux via the oxidative pentose phosphate pathway and
543 glycolysis plays a role in providing pyruvate for the Tricarboxylic acid (TCA) cycle to
544 enhance energy production (Pandey et al*.,* 2019). NO also enhanced the content of
545 polyamines such as putrescine and spermidine as well as agmatine (León et al*.,*
546 2016). These polyamines are thought to play an important role in stress tolerance
547 (Khajuria et al*.,* 2018). Increased polyamine levels were observed under nitrate
548 nutrition, which resulted in enhanced NO production and associated increased plant
549 disease resistance (Mur et al*.,* 2019). NO-induced polyamine accumulation
550 additionally plays a role in the delay of fruit ripening (Lokesh et al*.,* 2019). Collectively,
551 these findings suggest that NO is emerging as a key regulator for amino acid
552 metabolism and associated polyamine biosynthesis.
553
554 **A potential role for NO in vitamin B_6 metabolism**
555 Recently, NO has been implicated in vitamin B_6 metabolism (Xia et al*.,* 2014). Vitamin
556 B_6 is a family of molecules most notable among which is pyridoxal 5'-phosphate (PLP),
557 known for its essential role as a coenzyme for numerous metabolic enzymes, with
558 those involved in amino acid metabolism being among the best characterized. Other
559 non-coenzyme forms of the vitamin B_6 family (pyridoxamine 5'-phosphate (PMP),
560 pyridoxine 5'-phosphate (PNP) and non-phosphorylated derivatives pyridoxal (PL),
561 pyridoxamine (PM) and pyridoxine (PN) are also emerging as potential key players in
562 cellular metabolism and some are even touted as potent antioxidants (Mooney, S. and
563 Hellmann, 2010). A recent screen for NO hypersensitive mutants led to the isolation
564 of a PL kinase (which phosphorylates PL to PLP) mutant termed *sno1* (*sensitive to*
565 *nitric oxide 1*) (Xia et al*.,* 2014). The *sno1* mutant is allelic to *sos4* (*salt overly sensitive*
566 *4*) isolated in a screen for sensitivity to sodium chloride (Shi and Zhu, 2002). NO is
567 thought to play a signalling role in salt stress tolerance, through modulation of the Na^+
568 to K^+ ratio via the action of the Na^+/H^+ antiporter and the K^+ channel, (AKT1) (Campos
569 et al*.,* 2019). AKT1 activity was repressed in *sno1* plants as well as in the NO *nox1*
570 mutant, which accumulates NO (Xia et al*.,* 2014). However, the NO content was
571 reportedly not increased in *sno1* plants (Xia et al*.,* 2014). Instead, it was proposed that
572 increased PLP levels measured in *sos4*/*sno1* lines inhibit AKT1 activity (Xia et al*.,*
573 2014).
574
575 Conversely, it has recently been shown that PLP levels are decreased in the
576 *sos4*/*sno1* mutants (Gorelova et al., 2021), which while supportive of the role of SOS4
577 as a PL kinase does not support the explanation for decreased AKT1 in the mutant
578 lines. Therefore, the mechanism linking NO hypersensitivity, salt sensitivity and
579 misregulation of vitamin B_6 biosynthesis in *sno1*/*sos4* plants remains to be elucidated.
580 Notably, this recent study demonstrated severe developmental defects in *sos4*/*sno1*
581 mutants under standard growth conditions due to loss of vitamin B_6 homeostasis,
582 which is suggested to render them hypersensitive to stress (Gorelova et al., 2021).
583 Interestingly, loss of Pyridox(am)ine oxidase 3 (PDX3) function, another enzyme
584 involved in vitamin B_6 metabolism, which oxidizes PMP/PNP to PLP, leads to a
585 reduction in NR activity (Colinas et al*.,* 2016). Therefore, NO levels may also be
586 modulated in these plants and could be implicated in the constitutive upregulation of

587 defence-related genes observed in *pdx3* mutant lines (Colinas et al*.,* 2016), but this
588 remains to be deciphered. Nonetheless, given that members of the vitamin B_6 family
589 of molecules are claimed to function as antioxidants and have been implicated in
590 numerous abiotic stress responses (Colinas et al*.,* 2016; Gorelova et al., 2021), it is
591 possible that the imbalance derived from impairing enzymes of vitamin B_6 biosynthesis
592 impact the level of ROS, as well as reactive nitrogen species (RNS). Indeed, an
593 emerging theme is crosstalk between these reactive species (Lindermayr, 2017). For
594 example, the GSNO pool, and thus level of SNO proteins, can be regulated by the
595 direct effect of ROS on GSNOR (Kovacs et al., 2016). Thus, ROS/RNS homeostasis
596 may be disrupted when there is also misregulation of vitamin B_6 homeostasis. A more
597 rigorous study of vitamin B_6 metabolism and the role of specific vitamers (i.e. bioactive
598 forms), particularly the non-coenzyme forms, will provide a clearer picture of the
599 connection between the regulation of N metabolism, vitamin B_6 homeostasis and ROS.
600
601 Interestingly, Tyr-nitration has been reported for the PLP synthase proteins PDX1.1
602 and PDX1.3 in *Arabidopsis* (Lozano-Juste et al., 2011) and needs to be investigated
603 further to unravel biological context. NO signalling is also intimately connected with
604 ethylene, as mentioned above, which in turn requires vitamin B_6 (i.e. PLP) as a
605 coenzyme for its biosynthesis via 1-aminocyclopropane-1-carboxylic acid synthase
606 activity (Boycheva et al., 2015). While the interplay between NO and ethylene may be
607 synergistic, it is generally reported to be antagonistic. For example, ethylene mediates
608 NO depletion during acclimation to flooding stress (Hartman et al*.,* 2019). In addition,
609 NO affects the levels of other hormones, e.g. auxin (Campos et al., 2019), which also
610 requires PLP-dependent enzymes for its biosynthesis (Boycheva et al*.,* 2015).
611 Therefore, unravelling the interplay of vitamin B_6 with N metabolism and NO bioactivity
612 may also require consideration of plant hormone function associated with these
613 processes.
614

615 **NO association with fatty acid metabolism**

616 NO is involved in fatty acid metabolism, which is an important pathway for
617 maintenance of structural integrity and energy provision for various metabolic
618 processes (Lim et al., 2017). Nitro fatty acids (NO_2-FAs) are formed in a reaction
619 between either NO or $ONOO^-$ with unsaturated fatty acids (Aranda-Caño et al., 2019).
620 In animal systems NO_2-FAs play important roles as signal molecules in protection
621 against cardiac ischemic injury and are integral to inflammation cascades (Cui et al.,
622 2006). Recent evidence also suggests that NO_2-FAs play a role in plant metabolism
623 (Aranda-Caño et al., 2019; Mata-Pérez et al., 2020). These molecules can react with
624 biological nucleophiles such as glutathione and can therefore indirectly modulate ROS
625 homeostasis (Aranda-Caño et al., 2019; Fazzari et al., 2014). Further, in *Arabidopsis*
626 endogenous nitro-linolenic acid (NO_2-Ln) was found at picomolar concentrations and
627 was shown to be working as a signal molecule (Mata-Pérez et al., 2016b).
628 Transcriptomic analyses showed that NO_2-Ln was involved in plant defence via
629 induction of heat shock proteins by an unknown mechanism. NO_2-Ln can also
630 modulate GSNO biosynthesis suggesting that this metabolite plays a role in NO
631 homeostasis. The NO-FA content is elevated under various stress conditions such as
632 osmotic stress, low temperature, wounding and cadmium (Cd^{2+}) treatment (Mata-
633 Pérez et al., 2016a). In *Arabidopsis* NO_2-FAs are involved in ROS production and
634 stomatal moments via modulating the activity of the superoxide-producing enzyme,
635 NADPH oxidase activity (Di Palma et al., 2020). Therefore, generating further insights

636 related to the role of NO in the control of fatty acid metabolism will be of significant
637 value.
638
639 **NO control of ethylene biosynthesis and polyamine function in fruit ripening**
640 Ethylene is a plant hormone extensively involved in several stages of plant
641 development, including fruit ripening. NO modulates the ethylene biosynthetic
642 pathway by regulating transcriptionally, post translationally and enzymatically and
643 therefore influences ethylene production and fruit ripening. In the final step in ethylene
644 biosynthesis, catalysed by the aminocyclopropane-1-carboxylic acid (ACC) oxidase
645 (ACCO), ACC is oxidized to give ethylene (Pattyn et al., 2021). NO-mediated signal
646 transduction can transcriptionally antagonize ethylene biosynthesis with impacts
647 linked to fruit ripening (Manjunatha et al., 2010). It has also been reported that NO
648 reacts with ACCO by binding to the active site of this enzyme (Tierney et al., 2005).
649 Further, the ethylene biosynthetic enzyme MAT is subjected to *S*-nitrosylation, leading
650 to its inhibition (Lindermayr et al., 2006). NO and ACCO also form a complex, which
651 is further chelated by ACC to produce a stable ternary ACC–ACCO–NO complex
652 leading to ACCO inhibition, which negatively impacts ethylene biosynthesis (Tierney
653 et al., 2005). In peach fruit, NO and/or $ONOO^-$ generated in a reaction between NO
654 and ROS can retard ACCO activities via oxidative inactivation of their co-factors,
655 leading to a decrease in ethylene levels (Zhu et al., 2006). Also related to fruit ripening,
656 NO alters expression of enzymes responsible for cell wall metabolism, associated with
657 both the lignification and pigmentation of fruits, thereby extending fruit shelf life
658 (Manjunatha et al., 2010).
659
660 Polyamines (PAs), inducers of NO production, are involved in the delay of fruit ripening
661 (Malik & Singh, 2004). The application of spermidine, the smallest polyamine with
662 three amine groups, to peach fruit slowed down ripening by impairing ripening-related
663 gene expression of aminocyclopropane-1-carboxylate synthase ACS1 (Torrigiani et
664 al. 2021). Since spermidine application can lead to NO production (Tun et al., 2006)
665 the observed delay of ripening mediated by spermidine most likely occurs via NO.
666 Application of PAs to banana fruits caused a significant delay of the ripening processes
667 including: softening, slowing of peel colour transition, suppression of ethylene
668 production, decreased mitochondrial respiration and reduced ACCO activity (Purwoko
669 et al., 2002). In *Arabidopsis*, NO inactivates *S*-adenosyl-L-methionine synthase 1
670 (SAMS1) by *S*-nitrosylation (Lindermayr et al., 2006). It is therefore likely that *S*-
671 nitrosylation affects ethylene biosynthesis in plants by both targeting multiple steps in
672 this pathway.
673
674 *S*-Adenosyl-L-methionine (SAM) is a common precursor for both ethylene and PA
675 biosynthesis. Recently, it has been shown that in banana fruit the biosynthesis of
676 polyamines occurs via L-arginine-dependent pathways, but not via competitive
677 diversion of SAM (Lokesh et al., 2019). Interestingly, NO fumigation of tomato fruits
678 with NO gas reduced hydrogen peroxide scavenging capacity, elevated the levels of
679 antioxidants such as ascorbate and enhanced NO-mediated PTMs such as protein *S*-
680 nitrosylation (Zuccarelli et al., 2021). In addition, NO differentially affected a multitude
681 of metabolic processes including carotenoid, tocopherol and flavonoid production.
682 Thus, a 60% higher flavonoid accumulation was found in NO-treated fruits relative to
683 control untreated fruits. The content of several secondary metabolites such as
684 naringenin chalcone, naringenin glucoside, kaempferol rutinoside, quercetin

685 diglycoside and apigenin derivatives were also elevated in NO-treated fruits (Zuccarelli
686 et al., 2021).
687
688 NO treatment also regulates biochemical pathways related to tomato flavour such as
689 glutamate and aspartate production. Additionally, GSNOR activity was down-
690 regulated several-fold during ripening of pepper (*Capsicum annuum* L.) fruits
691 accompanied by enhanced abundance of *S*-nitrosylated proteins (Rodríguez-Ruiz et
692 al., 2019). Several enzymes involved in ROS production were differentially impacted
693 by NO during the ripening process. Peroxisomal catalase activity was down-regulated
694 by both tyrosine nitration and *S*-nitrosylation in sweet pepper (*Capsicum annuum* L.)
695 fruits during ripening **(**Rodríguez-Ruiz et al., 2019). The respiratory burst oxidase
696 homolog (RBOH), responsible for the production of extracellular ROS, was
697 upregulated during ripening of pepper fruits **(**Chu-Puga et al., 2019), while NADP-malic
698 enzyme activity was suppressed (Muñoz-Vargas et al., 2020). NO also regulates
699 phenylpropanoid metabolism during ripening. In this context, NO treatment promoted
700 enhanced activities of phenylalanine ammonia-lyase, cinnamate-4-hydroxylase and 4-
701 coumaroyl-CoA ligase enzymes in peach fruit (Li et al., 2017). In addition, it has been
702 shown that that the lipid metabolite, inositol 1,4,5-triphosphate, plays a major role in
703 NO-induced chilling tolerance via enhanced activity of enzymes associated with
704 antioxidant metabolism including SOD, peroxidase (POD), CAT, APX, Glutathione S-
705 transferase (GST) and glutathione reductase (GR), leading to increased postharvest
706 shelf-life and enhanced disease resistance (Jiao et al., 2019).
707
708 NO therefore regulates ethylene biosynthesis in plants by targeting multiple steps in
709 the biosynthetic pathway of this key gaseous hormone and by extension controls
710 associated processes linked to ethylene function, including fruit ripening. NO gas
711 treatment of fruits such as tomato may thus provide novel future strategies for
712 increasing fruit quality (Corpas et al., 2018; Zuccarelli et al., 2021).
713
714 **Conclusions and future perspectives**
715 The accumulating evidence indicates that NO plays a key role in regulating numerous
716 metabolic enzymes principally via *S*-nitrosylation. NO also orchestrates, either directly
717 or indirectly, an array of responses to both biotic and abiotic stresses and central to
718 this ability is wide-ranging metabolic reprogramming, involving a plethora of
719 metabolites from numerous pathways. The biosynthesis of several important nutrients
720 including amino acids, fatty acids and perhaps vitamins, in addition to the key immune-
721 related metabolite, SA, all appear to be regulated by NO. In addition, organelles such
722 as peroxisomes, chloroplasts and mitochondria are all thought to be sites of NO
723 production and function associated with plant metabolism. These organelles not only
724 generate NO, but also ROS, and the interplay between NO and ROS in these
725 organelles is important in regulating numerous metabolic processes.
726
727 However, a number of key questions remain to be addressed. Given the existing
728 absence of clarity regarding the different possible enzymatic sources of NO in plants,
729 these sources should be more rigorously characterised and their potential contribution
730 to NO production in relation to plant metabolism carefully established. Also, it would
731 be helpful to have a greater understanding of how NO production pathways might be
732 manipulated both temporally and spatially in order to enable metabolic
733 reprogramming. In addition, can redox switches, that enable key regulatory proteins
734 to be controlled by cellular NO levels, be redesigned resulting in enhanced metabolic

735 outputs? Might it be possible to modify plant NO levels and associated signalling
736 through differential nitrogen-based feeding or modification of the enzymatic pathways
737 involved in nitrogen assimilation? Further, the interconversion of NO and its related N-
738 oxides, occurring via different metabolic routes in plants, requires greater granularity.
739 The emerging evidence also suggests that NO interacts with the deployment of other
740 PTMs and signalling systems linked with plant metabolism. Detailed insights into the
741 associated molecular mechanisms my help shape agriculturally relevant plant traits.
742
743 It is now well established that massive metabolite exchange occurs in plants during
744 stress responses. It would be important to establish how NO orchestrates these
745 exchanges together with deeper insights into the associated metabolic fluxes. A clear
746 understanding of how NO influences highly complex plant metabolism is a crucial area,
747 where progress might lead to novel strategies for plant breeding or crop design.
748
749
750 **Figure Legends**
751
752 **Figure 1. Nitric oxide biosynthetic pathways in plants**.
753 A) NO is produced by oxidative pathways and reductive pathways. The former include
754 a NOS-like enzyme, a polyamine-mediated pathway and a hydroxylamine pathway,
755 while the latter include NR and XOR. A NOS-like enzyme may use L-arginine as
756 substrate and produce L-citrulline and NO. This activity requires several cofactors
757 such as BH_4, CaM (calmodulin), FAD, FMN, Ca^{2+} and oxygen. B) XOR catalyses the
758 reduction of nitrite to NO using NADH or xanthine as reducing substrate. C) NR
759 catalyses reduction of nitrite (NO_2^-) to NO. Under aerobic conditions, the cytoplasmic
760 nitrate (NO_3^-) regulates NR activity, because nitrate competitively inhibits nitrite
761 reduction. Thus, a lower nitrite concentration does not favour its reduction due to an
762 increased K_m requirement. Under conditions such as hypoxia NR is inhibited, leading
763 to an increased nitrite concentration and its concomitant removal. D) The nitrite
764 generated under hypoxia is transported to mitochondria via a putative nitrite
765 transporter. Under hypoxic conditions, nitrite reduction to NO takes place at complex
766 III, IV and possibly AOX. COX (cytochrome c oxidase), IMM (inner mitochondrial
767 membrane). E) NO is also generated by the combined action of a plasma membrane-
768 bound nitrite-NO reductase (PM-NINOR) and a plasma membrane-bound NR (PM-
769 NR).
770
771 **Figure 2. NO scavenging by Pgb and GSNOR**. A) PGB - NO cycle operates via
772 interconversion of nitrite, NO and nitrate. Under certain conditions such as hypoxia
773 nitrate (NO_3^-) is reduced to nitrite (NO_2^-). The nitrite is reduced to NO at different sites
774 (complex III complex IV and possibly AOX). The produced NO diffuses to the cytosol
775 where it is converted to nitrate (NO_3^-) by phytoglobin ($PgbO_2$) which then yields
776 metphytoglobin (MetPgb), which is reduced by metphytoglobin reductase (MetPgbR).
777 The produced NO_3^- will again become a substrate for NR. Operation of this cycle leads
778 to the biosynthesis of a limited amount ATP. B) The role of GSNOR in the regulation
779 of GSNO homeostasis in plants. NO and reduced glutathione (GSH) react with each
780 other to form GSNO. This product can be converted to oxidized glutathione (GSSG)
781 and ammonia (NH_3) by GSNOR. In the process of transnitrosylation GSNO can also
782 transfer NO to a reduced Cys residue of a given protein leading to protein *S*-
783 nitrosylation.
784

785 **Figure 3. Chemistry of protein *S*-nitrosylation, denitrosylation and**
786 **transnitrosylation**. A) Schematic drawing showing the process of *S*-nitrosylation and
787 denitrosylation. *S*-nitrosylation is a prominent PTM in which the covalent addition of
788 an NO group to a Cys thiol leads to formation of a *S*-nitrosothiol (SNO). The thioredoxin
789 (Trx) system denitrosylates *S*-nitrosylated proteins via a dithiol moiety leading to
790 formation of a reduced protein thiol (-SH) and oxidized Trx, which is subsequently
791 reduced by NADPH-dependent Trx reductase (NTR). B) Transnitrosylation is
792 catalysed by a transnitrosylase carrying an SNO group that transfers the NO moiety
793 to a target protein. C) A superoxide radical reacts with NO leading to formation of
794 peroxynitrite which can drive tyrosine nitration.
795
796 **Figure 4**. **Under hypoxia or anoxia, nitrite can serve as an alternative electron**
797 **acceptor in mitochondrial ETC leading to the generation of NO**. Mitochondrial
798 electron transport components complex III, IV and possibly AOX are involved in nitrite
799 reduction to NO. The NO produced can inhibit aconitase leading to enhanced
800 accumulation of citrate which can activate AOX. The activated AOX lowers the
801 reduction level of complexes I, III and IV and therefore ROS production. NO also
802 induces SOD, ascorbate oxidase, catalase and APX which all help remove ROS. NO
803 produced by the mitochondria affects mitochondrial function, integrity, formation of
804 supercomplex formation, redox regulation, induction of programmed cell death,
805 regulation of respiration and oxygen homeostasis by inhibition of COX, regulation of
806 oxidative pentose phosphate pathway and nitrite-driven ATP production.
807
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816
817 **References**
818 **Alber, N.A., Sivanesan, H., Vanlerberghe, G.C.** (2017). The occurrence and control
819 of nitric oxide generation by the plant mitochondrial electron transport chain. Plant Cell
820 Environ. **40**:1074-1085.
821
822 **Albertos, P., Romero-Puertas, M.C., Tatematsu, K., Mateos, I., Sanchez-Vicente,**
823 **I., Nambara, E., and Lorenzo, O.** (2015). S-nitrosylation triggers ABI5 degradation to
824 promote seed germination and seedling growth. Nat. Commun. **6**:8669.
825
826 **Alvarez, C., Calo, L., Romero, L.C., García, I., Gotor, C.** (2010). An O-
827 acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates
828 cysteine homeostasis in *Arabidopsis*. Plant Physiol. **152**:656-69.
829
830 **Aranda-Caño, L., Sánchez-Calvo, B., Begara-Morales, J.C., Chaki, M., Mata-**
831 **Pérez, C., Padilla, M.N., Valderrama, R., Barroso, J.B.** (2019). Post-Translational
832 Modification of Proteins Mediated by Nitro-Fatty Acids in Plants: Nitroalkylation. Plants
833 **8**:82.
834

835 **Astier, J., Gross, I., Durner, J.** (2018). Nitric oxide production in plants: an update. J.
836 Exp. Bot. **69**:3401-3411.
837
838 **Barroso, J.B., Corpas, F.J., Carreras, A., Sandalio, L.M., Valderrama, R., Palma,**
839 **J.M., Lupianez, J.A., and Del Río, L.A.** (1999). Localization of nitric oxide in plant
840 peroxisomes. J. Biol. Chem. **274**:36729-36733.
841
842 **Becana, M., Yruela, I., Sarath, G., Catalán, P., Hargrove, M.S.** (2020). Plant
843 hemoglobins: a journey from unicellular green algae to vascular plants. New Phytol.
844 **227**:1618-1635.
845
846 **Benhar, M., Forrester, M.T., Hess, D.T., Stamler, J.S.** (2008). Regulated protein
847 denitrosylation by cytosolic and mitochondrial thioredoxins. Science **320**:1050-1054.
848
849 **Begara-Morales, J. C., Chaki, M., Sánchez-Calvo, B., Mata-Pérez, C., Leterrier,**
850 **M., Palma, J. M., et al.** (2013). Protein tyrosine nitration in pea roots during
851 development and senescence. J. Exp. Bot. **64**:1121-1134.
852
853 **Begara-Morales, J.C., Sánchez-Calvo, B., Chaki, M., Mata-Pérez, C., Valderrama,**
854 **R., Padilla, M.N., López-Jaramillo, J., Luque, F., Corpas, F.J., Barroso,**
855 **J.B.** (2015). Differential molecular response of monodehydroascorbate reductase and
856 glutathione reductase by nitration and *S*-nitrosylation. J. Exp. Bot. **66**:5983-5996.
857
858 **Begara-Morales, J.C., Sánchez-Calvo, B., Chaki, M., Valderrama, R., Mata-Pérez,**
859 **C., López-Jaramillo, J., Padilla, M.N., Carreras, A., Corpas, F.J., Barroso, J.B.**
860 (2014). Dual regulation of cytosolic ascorbate peroxidase (APX) by Tyr nitration and
861 S-nitrosylation. J. Exp. Bot. **65**:527-538.
862 **Berger, A., Guinand, S., Boscari, A., Puppo, A., Brouquisse, R.** (2020). Medicago
863 truncatula Phytoglobin 1.1 controls symbiotic nodulation and nitrogen fixation via the
864 regulation of nitric oxide level. New Phytol. **227**:84-98.
865
866 **Boycheva, S., Dominguez, A., Rolcik, J., Boller, T., Fitzpatrick, T.B.** (2015).
867 Consequences of a deficit in vitamin B6 biosynthesis de novo for hormone
868 homeostasis and root development in *Arabidopsis*. Plant Physiol. **167**:102-117.
869
870 **Campbell, W.H.** (2001). Structure and function of eukaryotic NAD(P)H: nitrate
871 reductase. CMLS. Cell Mol. Plant Sci. **58**:194-204.
872
873 **Campos, F.V., Oliveira, J.A., Pereira, M.G., Farnese, F.S**. (2019). Nitric oxide and
874 phytohormone interactions in the response of *Lactuca sativa* to salinity stress. Planta
875 **250**:1475-1489.
876
877 **Castillo, M.C., Lozano-Juste, J., González-Guzmán, M., Rodriguez, L.,**
878 **Rodriguez, P.L., León, J.** (2015). "Inactivation of PYR/PYL/RCAR ABA receptors by
879 tyrosine nitration may enable rapid inhibition of ABA signaling by nitric oxide in
880 plants." Sci. Signal **8**:392.
881

882 **Chaki, M., Álvarez de Morales, P., Ruiz, C., Begara-Morales, J.C., Barroso, J.B.,**
883 **Corpas, F.J., Palma, J.M.** (2015). Ripening of pepper (*Capsicum annuum*) fruit is
884 characterized by an enhancement of protein tyrosine nitration. Ann. Bot. **116**:637-647.
885
886 **Chaki, M., Kovacs, I., Spannagl, M., Lindermayr, C.** (2014). Computational
887 prediction of candidate proteins for S-nitrosylation in *Arabidopsis thaliana*. PLoS
888 One **9**:e110232.
889
890 **Chamizo-Ampudia, A., Sanz-Luque, E., Llamas, Á., Ocaña-Calahorro, F.,**
891 **Mariscal, V., Carreras, A., Barroso, J.B., Galván, A., Fernández, E.** (2016). A dual
892 system formed by the ARC and NR molybdoenzymes mediates nitrite-dependent NO
893 production in *Chlamydomonas*. Plant Cell Environ. **39**:2097-2107.
894
895 **Chen, R., Sun, S., Wang, C., Li, Y., Liang, Y., An, F., Li, C., Dong, H., Yang, X.,**
896 **Zhang, J., & Zuo, J.** (2009). The Arabidopsis PARAQUAT RESISTANT2 gene
897 encodes an S-nitrosoglutathione reductase that is a key regulator of cell death. Cell
898 research **19**:1377-1387.

899 **Chu-Puga, Á., González-Gordo, S., Rodríguez-Ruiz, M., Palma, J.M., Corpas, F.J.**
900 (2019). NADPH Oxidase (Rboh) activity is up regulated during sweet pepper
901 (*Capsicum annuum* L.) fruit ripening**.** Antioxidants (Basel) **8**:9.
902
903 **Clark, D., Durner, J., Navarre, D.A., Klessig, D.F.** (2000). Nitric oxide inhibition of
904 tobacco catalase and ascorbate peroxidase. Mol. Plant Microbe Interact. **13**:1380-
905 1384.
906
907 **Colinas, M., Eisenhut, M., Tohge, T., Pesquera, M., Fernie, A.R., Weber, A.P.,**
908 **Fitzpatrick, T.B.** (2016). Balancing of B6 vitamers is essential for plant development
909 and metabolism in Arabidopsis. Plant Cell **28**:439-453.
910
911 **Corpas, F.J., Barroso, J.B.** (2014). Functional implications of peroxisomal nitric oxide
912 (NO) in plants. Front. Plant Sci. **5**:97.

913 **Corpas, F.J. and Barroso, J.B.** (2017). Nitric oxide synthase-like activity in higher
914 plants. Nitric Oxide **68**:5-6.
915
916 **Corpas, F.J., and Barroso, J.B.** (2018). Peroxisomal plant metabolism - an update
917 on nitric oxide, Ca(2+) and the NADPH recycling network. J. Cell Sci. **131**:202978.

918 **Corpas, F.J., Barroso, J.B., Carreras, A., Quirós, M., León, A.M., Romero-**
919 **Puertas, M.C., Esteban, F.J., Valderrama, R., Palma, J.M., Sandalio, L.M., Gómez,**
920 **M., Del Río, L.A.** (2004). Cellular and subcellular localization of endogenous nitric
921 oxide in young and senescent pea plants. Plant Physiol. **136**:2722-2733.
922
923 **Corpas, F.J., Leterrier, M., Begara-Morales, J.C., Valderrama, R., Chaki, M.,**
924 **López-Jaramillo, J., Luque, F., Palma, J.M., Padilla, M.N., Sánchez-Calvo, B.,**
925 **Mata-Pérez, C., Barroso, J.B.** (2013a). Inhibition of peroxisomal hydroxypyruvate
926 reductase (HPR1) by Tyr nitration. Biochim. Biophys. Acta. **1830**:4981-4989**.**
927

928 **Corpas, F.J., Palma, J.M., Del Río, L.A., Barroso, J.B.** (2009). Evidence supporting
929 the existence of L-arginine dependent nitric oxide synthase activity in plants. New
930 Phytol. **184**:9-14.
931
932 **Corpas, F.J., Río, L.A.D., Palma, J.M.** (2019). Impact of nitric oxide (NO) on the ROS
933 Metabolism of peroxisomes. Plants (Basel) **8**:37.
934
935 **Costa-Broseta, Á., Castillo, M., León, J.** (2020). Nitrite Reductase 1 Is a Target of
936 Nitric Oxide-Mediated Post-Translational Modifications and Controls Nitrogen Flux
937 and Growth in Arabidopsis. Int. J. Mol. Sci. **21**:7270.

938 **Costa-Broseta, Á., Castillo, M., León, J**. (2021). Post-Translational Modifications of
939 Nitrate Reductases Autoregulates Nitric Oxide Biosynthesis in Arabidopsis. Int. J. Mol.
940 Sci. **22**:549.

941 **Cui, B., Pan, Q., Clarke, D., Villarreal, M.O., Umbreen, S., Yuan, B., Shan, W.,**
942 **Jiang, J., Loake, G.J.** (2018). *S*-nitrosylation of the zinc finger protein SRG1 regulates
943 plant immunity. Nat. Commun. **9**:4226.
944
945 **Cui, T., Schopfer, F.J., Zhang, J., Chen, K., Ichikawa. T., Baker, P.R., Batthyany,**
946 **C., Chacko, B.K., Feng, X., Patel, R.P., Agarwal, A., Freeman, B.A., Chen, Y.E.**
947 (2006). Nitrated fatty acids: endogenous anti-inflammatory signaling mediators. J. Biol.
948 Chem. **281**:35686-35698.
949
950 **Cvetkovska, M., and Vanlerberghe, G.C.** (2012). Alternative oxidase modulates leaf
951 mitochondrial concentrations of superoxide and nitric oxide. New Phytol. **195**:32-39.
952
953 **Delledonne, M., Xia, Y., Dixon, R.A., Lamb, C**. (1998). Nitric oxide functions as a
954 signal in plant disease resistance. Nature **394**:585-588.

955 **Del Río, L.A., Corpas, J.F., and Barroso, J.B.** (2004). Nitric oxide and nitric oxide
956 synthase activity in plants. Phytochemistry **65**:783-792.

957 **Del Río, L.A., and López-Huertas, E.** (2016). ROS generation in peroxisomes and
958 its role in cell signaling. Plant Cell Physiol. **57**:1364-1376.

959 **Derakhshan, B., Wille, P.C., and Gross, S.S.** (2007). Unbiased identification of
960 cysteine S-nitrosylation sites on proteins. Nat. Protoc. **2**:1685-1691.

961 **Diner, B.A., and Petrouleas, V.** (1990). Formation by NO of nitrosyl adducts of redox
962 components of the photosystem II reaction center. II: evidence that $HCO3^{-}/CO2$ binds
963 to the acceptor-side non-heme iron. Biochim. Biophys. Acta. **1015**:141-149.
964
965 **Di Palma, A.A., Di Fino, L.M., Salvatore, S.R., D'Ambrosio, J.M., García-Mata, C.,**
966 **Schopfer, F.J., Laxalt, A.M.** (2020). Nitro-oleic acid triggers ROS production via
967 NADPH oxidase activation in plants: A pharmacological approach. J. Plant Physiol.
968 **246-247**:153128.

969 **Fazzari, M., Trostchansky, A., Schopfer, F.J., Salvatore, S.R., Sánchez-Calvo, B.,**
970 **Vitturi, D., Valderrama, R., Barroso, J.B., Radi, R., Freeman, B.A., Rubbo, H.**

(2014). Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. PLoS One **9**:e84884.

Feechan, A., Kwon, E., Yun, B.W., Wang, Y., Pallas, J.A., Loake, G.J. (2005). A central role for *S*-nitrosothiols in plant disease resistance. Proc. Natl. Acad. Sci. U S A **102**:8054-8059.

Foresi, N., Correa-Aragunde, N., Parisi, G., Caló, G., Salerno, G., Lamattina, L. (2010). Characterization of a nitric oxide synthase from the plant kingdom: no generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell **22**:3816-3830.

Galatro, A., Puntarulo, S., Guiamet, J.J., Simontacchi, M. (2013). Chloroplast functionality has a positive effect on nitric oxide level in soybean cotyledons. Plant Physiol Biochem. **66**:26-33.

Godber, B.L., Doel, J.J., Sapkota, G.P., Blake, D.R., Stevens, C.R., Eisenthal, R., Harrison, R. (2000). Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. J. Biol. Chem. **275**:7757-7763.

Gorelova, V., Colinas, M., Dell Aglio, E ., Flis, P., Salt, D.E., and Fitzpatrick, T.B. (2021). A deficit in phosphorylated B_6 vitamers in SALT OVERLY SENSITIVE 4 mutants compromises shoot and root development. Plant Physiol. (In Press)

Groß, F., Rudolf, E.E., Thiele, B., Durner, J., Astier, J. (2017). Copper amine oxidase 8 regulates arginine-dependent nitric oxide production in *Arabidopsis thaliana*. J. Exp. Bot. **68**:2149-2162.

Guo, F.Q., Okamoto, M., and Crawford, N.M. (2003). Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science. **302**:100-103.

Gupta, K.J., Fernie, A.R., Kaiser, W.M., van Dongen, J.T. (2011). On the origins of nitric oxide. Trends Plant Sci. **16**:160-168.

Gupta, K.J., Hebelstrup, K.H., Kruger, N.J., George Ratcliffe, R. (2014). Nitric oxide is required for homeostasis of oxygen and reactive oxygen species in barley roots under aerobic conditions. Mol. Plant **7**:747-750.

Gupta, K.J., Kolbert, Z., Durner, J., Lindermayr, C., Corpas, F.J., Brouquisse, R., Barroso, J.B., Umbreen, S., Palma, J.M., Hancock, J.T., Petrivalsky, M., Wendehenne, D., & Loake, G.J. (2020b). Regulating the regulator: nitric oxide control of post-translational modifications. New Phytol. **227**:1319–1325.

Gupta, K.J., Kumari, A., Florez-Sarasa, I., Fernie, A.R., Igamberdiev, A.U. (2018). Interaction of nitric oxide with the components of plant mitochondrial electron transport chain. J. Exp. Bot. **69**:3413-3424.

Gupta, K.J., Lee, C.P., Ratcliffe, R.G. (2017). Nitrite protects mitochondrial structure and function under hypoxia. Plant Cell Physiol. **58**:175-183.

1103
1104 **Gupta, K.J., Shah, J.K., Brotman, Y., Jahnke, K., Willmitzer, L., Kaiser, W.M.,**
1105 **Bauwe, H., Igamberdiev, A.U.** (2012). Inhibition of aconitase by nitric oxide leads to
1106 induction of the alternative oxidase and to a shift of metabolism towards biosynthesis
1107 of amino acids. J. Exp. Bot. **63:**1773-1784.
1108
1109 **Hartman, S., Liu, Z., Veen, V.H., Vicente, J., Reinen, E., Martopawiro, S., Zhang,**
1110 **H., van Dongen, N., Bosman, F., Bassel, G.W., Visser, E.J.W., Bailey-Serres, J.,**
1111 **Theodoulou, F.L., Hebelstrup, K.H., Gibbs, D.J., Holdsworth, M.J., Sasidharan,**
1112 **R., Voesenek, L.A.C.J.** (2019). Ethylene-mediated nitric oxide depletion pre-adapts
1113 plants to hypoxia stress. Nat. Comm. **10**:4020.
1114
1115 **Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A.**
1116 (2012). Role of proline under changing environments. Plant Signal. Behav. **7**:1456-
1117 1466.
1118
1119 **Hebelstrup, K.H., and Møller, I.M.** (2015). Mitochondrial signaling in plants under
1120 hypoxia: Use of reactive oxygen species (ROS) and reactive nitrogen species (RNS).
1121 In Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants, J.
1122 Gupta, and and A.U. Igamberdiev, eds (Heidelberg, Germany: Springer), 63-77.
1123
1124 **Holzmeister, C., Fröhlich, A., Sarioglu, H., Bauer, N., Durner, J., Lindermayr, C.**
1125 (2011). Proteomic analysis of defense response of wildtype Arabidopsis thaliana and
1126 plants with impaired NO- homeostasis. Proteomics **11:**1664-1683.
1127
1128 **Holzmeister, C., Gaupels, F., Geerlof, A., Sarioglu, H., Sattler, M., Durner, J.,**
1129 **Lindermayr, C.** (2015). Differential inhibition of *Arabidopsis* superoxide dismutases
1130 by peroxynitrite-mediated Tyr nitration. J. Exp. Bot. **66**:989-999.
1131
1132 **Huang, X., von Rad, U., Durner, J.** (2002). Nitric oxide induces transcriptional
1133 activation of the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension
1134 cells. Planta **215**:914-923.
1135
1136 **Hussain, A., Yun, B.W., Kim, J.H., Gupta, K.J., Hyung, N.I., & Loake, G.J.** (2019).
1137 Novel and conserved functions of S-nitrosoglutathione reductase in tomato. J. Exp.
1138 Bot. **70**:4877-4886.

1139 **Igamberdiev, A.U., Baron, K., Manach-Little, N., Stoimenova, M., Hill, R.D.** (2005).
1140 The haemoglobin/nitric oxide cycle: involvement in flooding stress and effects on
1141 hormone signalling. Ann. Bot. **96**:557-564.
1142
1143 **Jasid, S., Simontacchi, M., Bartoli, C.G., Puntarulo, S.** (2006). Chloroplasts as a
1144 nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids
1145 and proteins. Plant Physiol. **142**:1246-55.

1146 **Jeandroz, S., Wipf, D., Stuehr, D.J., Lamattina, L., Melkonian, M., Tian, Z., Zhu,**
1147 **Y., Carpenter, E.J., Wong, G.K., Wendehenne, D.** (2016). Occurrence, structure,
1148 and evolution of nitric oxide synthase-like proteins in the plant kingdom. Sci. Signal.
1149 **9**:re2.

1150
1151 **Jiao, C., Chai, Y., Duan, Y.** (2019). Inositol 1,4,5-Trisphosphate mediates nitric-oxide-
1152 induced chilling tolerance and defense response in postharvest peach fruit. J. Agric.
1153 Food Chem. **67**:4764-4773.
1154
1155 **Khajuria, A., Sharma, N., Bhardwaj, R., Ohri, P.** (2018). Emerging Role of
1156 Polyamines in Plant Stress Tolerance. Curr. Protein Pept. Sci. **19**:1114-1123.
1157
1158 **Kikuchi, G.** (1973). The glycine cleavage system: composition, reaction mechanism,
1159 and physiological significance. Mol. Cell Biochem. **1**:169-87.
1160
1161 **Kneeshaw, S., Gelineau, S., Tada, Y., Loake, G.J., Spoel, S.H.** (2014). Selective
1162 protein denitrosylation activity of Thioredoxin-h5 modulates plant Immunity. Mol. Cell
1163 **56**:153-62.
1164
1165 **Kneeshaw, S., Keyani, R., Delorme-Hinoux, V., Imrie, L., Loake, G.J., Le Bihan,**
1166 **T., Reichheld, J.P., Spoel, S.H.** (2017). Nucleoredoxin guards against oxidative
1167 stress by protecting antioxidant enzymes. Proc Natl Acad Sci USA **114**:8414-8419.
1168
1169 **Kolbert, Z., Barroso, J.B., Brouquisse, R., Corpas, F.J., Gupta, K.J., Lindermayr,**
1170 **C., Loake, G.J., Palma, J.M., Petřivalský, M., Wendehenne, D., Hancock,**
1171 **J.T.** (2019). A forty year journey: The generation and roles of NO in plants**.** Nitric Oxide
1172 **93**:53-70.
1173
1174 **Kovacs, I., Holzmeister, C., Wirtz, M., Geerlof, A., Fröhlich, T., Römling, G.,**
1175 **Kuruthukulangarakoola, G.T., Linster, E., Hell, R., Arnold, G.J., Durner. J.,**
1176 **Lindermayr, C**. (2016). ROS mediated inhibition of S-nitrosoglutathione reductase
1177 contributes to the activation of anti-oxidative mechanisms. Front. Plant Sci. **7**:1669.
1178
1179 **Kumari, A., Singh, P., Kaladhar, V.C., Manbir, Paul, D., Pathak, P.K., Gupta, K.J.**
1180 (2021). Phytoglobin-NO cycle and AOX pathway play a role in anaerobic germination
1181 and growth of deepwater rice. Plant Cell Environ. (In Press).
1182
1183 **Lee, U., Wie, C., Fernandez, B.O., Feelisch, M., Vierling, E.** (2008). Modulation of
1184 nitrosative stress by S-nitrosoglutathione reductase is critical for thermotolerance and
1185 plant growth in *Arabidopsis*. Plant Cell **20**:786-802.
1186
1187 **León, J., Costa, Á., Castillo, M.C.** (2016). Nitric oxide triggers a transient metabolic
1188 reprogramming in Arabidopsis. Sci. Rep. **6**:37945.
1189
1190 **Li, G., Zhu, S., Wu, W., Zhang, C., Peng, Y., Wang, Q., Shi, J**. (2017). Exogenous
1191 nitric oxide induces disease resistance against *Monilinia fructicola* through activating
1192 the phenylpropanoid pathway in peach fruit. J. Sci. Food Agric. **97**:3030-3038.
1193
1194 **Lindermayr, C.** (2017). Crosstalk between reactive oxygen species and nitric oxide in
1195 plants: key role of S-nitrosoglutathione reductase. Free Radic. Biol. Med. **122**:110 -
1196 115.
1197

1198 **Lindermayr, C., Saalbach, G., Bahnweg, G., Durner, J.** (2006). Differential inhibition
1199 of *Arabidopsis* methionine adenosyltransferases by protein *S*-nitrosylation*.* J. Biol.
1200 Chem. **281**:4285-4291.
1201
1202 **Lindermayr, C., Saalbach, G., Durner, J.** (2005). Proteomic identification of S-
1203 nitrosylated proteins in Arabidopsis. Plant Physiol. **137**:921-930.

1204 **Lim, G.H., Singhal, R., Kachroo, A., Kachroo, P.** (2017). Fatty Acid- and Lipid-
1205 Mediated Signaling in Plant Defense. Annu. Rev. Phytopathol. **55**:505-536.

1206 **Lokesh, V., Manjunatha, G., Hegde, N.S., Bulle, M., Puthusseri, B., Gupta, K.J.,**
1207 **Neelwarne, B.** (2019). Polyamine induction in postharvest banana fruits in response
1208 to NO donor SNP occurs via L-arginine mediated pathway and not via competitive
1209 diversion of S-adenosyl-l-methionine. Antioxidants (Basel) **8**:358.

1210 **Lozano-Juste, J., Colom-Moreno, R., León, J.** (2011). In vivo protein tyrosine
1211 nitration in *Arabidopsis thaliana*. J. Exp. Bot. **62**:3501-3517.
1212
1213 **Malik, A. U., and Singh, Z.** (2004). Endogenous free polyamines of mangos in relation
1214 to development and ripening. J. Am. Soc. Hortic. Sci. **129**:280-286.
1215
1216 **Manjunatha, G., Lokesh, V., Neelwarne, B.** (2010). Nitric oxide in fruit ripening:
1217 trends and opportunities. Biotechnol Adv. **28:**489-499.

1218 **Martínez-Medina, A., Pescador, L., Fernández, I., Rodríguez-Serrano, M., García,**
1219 **J.M., Romero-Puertas, M.C., Pozo, M.J.** (2019). Nitric oxide and phytoglobin
1220 PHYTOGB1 are regulatory elements in the *Solanum lycopersicum* - *Rhizophagus*
1221 *irregularis* mycorrhizal symbiosis. New Phytol. **223**:1560-1574.
1222
1223 **Masella, R., Di Benedetto, R., Vari, R., Filesi, C., Giovannini, C.** (2005). Novel
1224 mechanisms of natural antioxidant compounds in biological systems: involvement of
1225 glutathione and glutathionerelated enzymes. J. Nutr. Biochem. **16**:577-586.
1226
1227 **Mata-Pérez, C., Sánchez-Calvo, B., Padilla, M.N., Begara-Morales, J.C., Luque,**
1228 **F., Melguizo, M., Jiménez-Ruiz, J., Fierro-Risco, J., Peñas-Sanjuán, A.,**
1229 **Valderrama, R., Corpas, F.J., Barroso, J.B.** (2016a). Nitro-Fatty Acids in Plant
1230 Signaling: Nitro-Linolenic Acid Induces the Molecular Chaperone Network in
1231 Arabidopsis. Plant Physiol. **170**:686-701.
1232
1233 **Mata-Pérez, C., Begara-Morales, J.C., Chaki, M., Sánchez-Calvo, B., Valderrama,**
1234 **R., Padilla, M.N., Corpas, F.J., Barroso, J.B.** (2016b). Protein Tyr nitration during
1235 development and abiotic stress response in plants. Front. Plant Sci. **7**:1699.
1236
1237 **Mata-Pérez, C., Padilla, M.N., Sánchez-Calvo, B., Begara-Morales, J.C.,**
1238 **Valderrama, R., Chaki, M., Aranda-Caño, L., Moreno-González, D., Molina-Díaz,**
1239 **A., Barroso, J.B.** (2020). Endogenous Biosynthesis of S-Nitrosoglutathione From
1240 Nitro-Fatty Acids in Plants. Front. Plant Sci. **11**:962.

1241 **Melo, P.M., Silva, L.S., Ribeiro, I., Seabra, A.R., and Carvalho, H.G.** (2011).
1242 Glutamine synthetase is a molecular target of nitric oxide in root nodules of *Medicago*
1243 *truncatula* and is regulated by tyrosine nitration. Plant Physiol. **157**:1505-1517.
1244
1245 **Millar, A.H., and Day, D.A.** (1996). Nitric oxide inhibits the cytochrome oxidase but
1246 not the alternative oxidase of plant mitochondria. FEBS Lett. **398**:155-158.
1247
1248 **Misra, A.N., Vladkova, R., Singh, R., Misra, M., Dobrikova, A.G., Apostolova, E.L.**
1249 (2014). Action and target sites of nitric oxide in chloroplasts. Nitric Oxide **39**:35-45.
1250
1251 **Møller, I.M.** (2001). Plant mitochondria and oxidative stress: electron transport,
1252 NADPH turnover, and metabolism of reactive oxygen species. Annu. Rev. Plant
1253 Physiol. Plant Mol. Biol. **52**:561-591.
1254
1255 **Møller, I.M., Igamberdiev, A.U., Bykova, N.V., Finkemeier, I., Rasmusson, A.G.,**
1256 **Schwarzländer, M.** (2020). Matrix Redox Physiology Governs the Regulation of Plant
1257 Mitochondrial Metabolism through Posttranslational Protein Modifications. Plant
1258 Cell **32**: 573-594.
1259
1260 **Moore, A.L., Shiba, T., Young, L., Harada, S., Kita, K., Ito, K.** (2013). Unraveling
1261 the heater: new insights into the structure of the alternative oxidase. Annu. Rev. Plant
1262 Biol. **64**: 637-663.
1263
1264 **Mooney, S., and Hellmann, H.** (2010). Vitamin B6: Killing two birds with one stone?
1265 Phytochemistry **71**:495-501.
1266
1267 **Muñoz-Vargas, M.A., González-Gordo, S., Palma, J.M., Corpas, F.J.**
1268 (2020).Inhibition of NADP-malic enzyme activity by H2S and NO in sweet pepper
1269 (*Capsicum annuum* L.) fruits. Physiol. Plant. **168**:278-288.
1270
1271 **Mur, L.A.J., Kumari, A., Brotman, Y., Zeier, J., Mandon, J., Cristescu, S.M.,**
1272 **Harren, F., Kaiser, W.M., Fernie, A.R., Gupta, K.J.** (2019). Nitrite and nitric oxide are
1273 important in the adjustment of primary metabolism during the hypersensitive response
1274 in tobacco. J. Exp. Bot. **70**:4571-4582.
1275
1276 **Navarre, D.A., Wendehenne, D., Durner, J., Noad, R., Klessig, D.F.** (2000). Nitric
1277 oxide modulates the activity of tobacco aconitase. Plant Physiol. **122**:573-582.
1278
1279 **Ortega-Galisteo, A.P., Rodríguez-Serrano, M., Pazmiño, D.M., Gupta, D.K,**
1280 **Sandalio, L.M., Romero-Puertas, M.C.** (2012). *S*-Nitrosylated proteins in pea (*Pisum*
1281 *sativum* L.) leaf peroxisomes: Changes under abiotic stress. J. Exp. Bot. **63**: 2089-
1282 2103.
1283
1284 **Palmieri, M.C., Lindermayr, C., Bauwe, H., Steinhauser, C., Durner, J.** (2010).
1285 Regulation of plant glycine decarboxylase by S-nitrosylation and glutathionylation.
1286 Plant Physiol. **152**:1514-1528.
1287

1288 **Pandey, S., Kumari, A., Shree, M., Kumar, V., Singh, P., Bharadwaj, C., Loake,**
1289 **G.J., Parida, S.K., Masakapalli, S.K., Gupta, K.J.** (2019). Nitric oxide accelerates
1290 germination via the regulation of respiration in chickpea. J. Exp. Bot. **70**:4539–4555.
1291
1292 **Pattyn, J., Vaughan-Hirsch, J., Van de Poel, B.** (2021). The regulation of ethylene
1293 biosynthesis: A complex multilevel control circuitry. New Phytol. 229:770-782.
1294
1295 **Pérez-Mato, I., Castro, C., Ruiz, F.A., Corrales, F.J., Mato, J.M.** (1999). Methionine
1296 adenosyltransferase *S*-nitrosylation is regulated by the basic and acidic amino acids
1297 surrounding the target thiol. J. Biol. Chem. **274**:17075-17079.
1298
1299 **Planchet, E., Jagadis Gupta, K., Sonoda, M., Kaiser, W.M.** (2005). Nitric oxide
1300 emission from tobacco leaves and cell suspensions: rate limiting factors and evidence
1301 for the involvement of mitochondrial electron transport. Plant J. **41**:732-743.
1302
1303 **Purwoko, B.S., Susanto, S., Kodir, K.A., Novita, T., & Harran, S.** (2002). Studies
1304 on the physiology of polyamines and ethylene during ripening of banana and papaya
1305 fruits. Acta Horticulturae **575**:651-657.
1306
1307 **Puntarulo, S., Jasid, S., Simontacchi, M.** (2007). Reactive nitrogen species-
1308 dependent effects on soybean chloroplasts. Plant Signal Behav. **2**:96-8.
1309
1310 **Rocha, M., Licausi, F., Araújo, W.L., Nunes-Nesi, A., Sodek, L., Fernie, A.R., van**
1311 **Dongen, J.T.** (2010). Glycolysis and the tricarboxylic acid cycle are linked by alanine
1312 aminotransferase during hypoxia induced by waterlogging of *Lotus japonicus*. Plant
1313 Physiol. **152**:1501-1513.
1314
1315 **Rockel, P., Strube, F., Rockel, A., Wildt, J., Kaiser, W.M.** (2002). Regulation of nitric
1316 oxide (NO) production by plant nitrate reductase *in vivo* and *in vitro*. J. Exp. Bot.
1317 **53**:103-110.
1318
1319 **Rodríguez-Ruiz, M., González-Gordo, S., Cañas, A., Campos, M. J., Paradela, A.,**
1320 **Corpas, F. J., et al**. (2019). Sweet pepper (*Capsicum annuum* L.) fruits contain an
1321 atypical peroxisomal catalase that is modulated by reactive oxygen and nitrogen
1322 species. Antioxidants (Basel) **8**:E374.
1323
1324 **Romero-Puertas, M.C., Laxa, M., Mattè, A., Zaninotto, F., Finkemeier, I., Jones,**
1325 **A.M., Perazzolli, M., Vandelle, E., Dietz, K.J., Delledonne, M.** (2007). *S*-Nitrosylation
1326 of peroxiredoxin II E promotes peroxynitrite-mediated Tyr nitration. Plant Cell **19**:4120-
1327 4130.
1328
1329 **Rümer, S., Gupta, K.J., Kaiser, W.M.** (2009). Plant cells oxidize hydroxylamines to
1330 NO. J. Exp. Bot. **60**:2065-2072.
1331
1332 **Sainz, M., Calvo-Begueria, L., Pérez-Rontomé, C., Wienkoop, S., Abián, J.,**
1333 **Staudinger, C., et al.** (2015). Leghemoglobin is nitrated in functional legume nodules
1334 in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent
1335 mechanism. Plant J. **81**:723-735.
1336

1337 **Seth, D., and Stamler, J.S.** (2015). SNOs Differ: Methodological and Biological
1338 Implications. Circ. Res. **117:**826-829.
1339
1340 **Shah, J.K., Cochrane, D.W., De Paepe, R., Igamberdiev, A.U.** (2013). Respiratory
1341 complex I deficiency results in low nitric oxide levels, induction of hemoglobin and
1342 upregulation of fermentation pathways. Plant Physiol. Biochem. **63**:185-190.
1343
1344 **Shi, H., and Zhu, J.K.** (2002). SOS4, a pyridoxal kinase gene, is required for root hair
1345 development in *Arabidopsis*. Plant Physiol. **129**:585-593.
1346
1347 **Skelly, M.J., Malik, S.I., Le Bihan, T., Bo, Y., Jiang, J., Spoel, S.H., Loake,
1348 G.J.** (2019). A role for S-nitrosylation of the SUMO-conjugating enzyme SCE1 in plant
1349 immunity. Proc. Natl. Acad. Sci. U S A **116:**17090-17095.
1350
1351 **Simonin, V., and Galina, A.** (2013). Nitric oxide inhibits succinate dehydrogenase-
1352 driven oxygen consumption in potato tuber mitochondria in an oxygen tension-
1353 independent manner. Biochem. J. **449**:263-273.
1354
1355 **Slaymaker, D.H., Navarre, D.A., Clark, D., del Pozo, O., Martin, G.B., Klessig, D.F.**
1356 (2002). The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast
1357 carbonic anhydrase, which exhibits antioxidant activity and plays a role in the
1358 hypersensitive defense response. Proc. Natl. Acad. Sci. USA **99**:11640-11645.
1359
1360 **Stöhr, C., Strube, F., Marx, G., Ullrich, W.R., Rockel, P.** (2001). A plasma
1361 membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from
1362 nitrite. Planta **212**:835-841.
1363
1364 **Sun, J., and Trumpower, B.L.** (2003). Superoxide anion generation by the
1365 cytochrome bc1 complex. Arch. Biochem. Biophys. **419**:198-206.
1366
1367 **Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., and Dong, X.**
1368 (2008). S-nitrosylation and thioredoxins regulate conformational changes of NPR1 in
1369 plant innate immunity. Science **321**:952-956.
1370
1371 **Taiz, L., Zeiger, E., Møller, I.M., and Murphy, A.** (2015). Plant Physiology and
1372 Development. 6th Edition, Sinauer Associates Inc., Sunderland, CT
1373
1374 **Tewari, R.K., Prommer, J., Watanabe, M.** (2013). Endogenous nitric oxide
1375 generation in protoplast chloroplasts. Plant Cell Rep. **32**:31-44.
1376
1377 **Tierney, D.L., Rocklin, A.M., Lipscomb, J.D., Que, L. Jr., Hoffman, B.M.** (2005).
1378 ENDOR studies of the ligation and structure of the non-heme iron site in ACC oxidase.
1379 J. Am. Chem. Soc. **127**:7005-13.
1380
1381 **Torrigiani, P., Bressanin, D., Ruiz, K.B., Tadiello, A., Trainotti, L., Bonghi, C.,
1382 Ziosi, V., Costa, G.** (2012). Spermidine application to young developing peach fruits
1383 leads to a slowing down of ripening by impairing ripening-related ethylene and auxin
1384 metabolism and signaling. Physiol Plant. **146**:86-98.
1385

1386 **Tun, N.N., Santa-Catarina, C., Begum, T., Silveira, V., Handro, W., Floh, E.I.,**
1387 **Scherer, G.F.** (2006). Polyamines induce rapid biosynthesis of nitric oxide (NO) in
1388 *Arabidopsis thaliana* seedlings. Plant Cell Physiol. **47**:346-354.
1389
1390 **Umbreen, S., Lubega, J., Cui, B., Pan, Q., Jiang, J., Loake, G.J.** (2018). Specificity
1391 in nitric oxide signalling. J. Exp. Bot. **69**:3439-3448.
1392
1393 **Vishwakarma, A., Kumari, A., Mur, L.A.J., Gupta, K.J.** (2018). A discrete role for
1394 alternative oxidase under hypoxia to increase nitric oxide and drive energy
1395 production. Free Radic. Biol. Med. **22**:40-51.
1396
1397 **Vladkova, R., Dobrikova, A.G., Singh, R., Misra, A.N., Apostolova, E.** (2011).
1398 Photoelectron transport ability of chloroplast thylakoid membranes treated with NO
1399 donor SNP: changes in flash oxygen evolution and chlorophyll fluorescence. Nitric
1400 Oxide **24**:84-90.
1401
1402 **Wang, Y.Q., Feechan, A., Yun, B.W., Shafiei, R., Hofmann, A., Taylor, P., Xue, P.,**
1403 **Yang, F.Q., Xie, Z.S., Pallas, J.A., Chu, C.C., Loake, G.J.** (2009). *S*-Nitrosylation of
1404 AtSABP3 antagonizes the expression of plant immunity. J. Biol. Chem. **284**:2131-
1405 2137.
1406
1407 **Wimalasekera, R., Villar, C., Begum, T., and Scherer, G.F.** (2011). *COPPER*
1408 *AMINE OXIDASE1 (CuAO1)* of *Arabidopsis thaliana* contributes to abscisic acid- and
1409 polyamine-induced nitric oxide biosynthesis and abscisic acid signal transduction. Mol.
1410 Plant. **4**:663-678.

1411 **Xia, J., Kong, D., Xue, S., Tian, W., Li, N., Bao, F., Hu, Y., Du, J., Wang, Y., Pan,**
1412 **X., Wang, L., Zhang, X., Niu, G., Feng, X., Li, L., He, Y.** (2014). Nitric oxide
1413 negatively regulates AKT1- mediated potassium uptake through modulating vitamin
1414 B6 homeostasis in *Arabidopsis.* Proc. Natl. Acad. Sci. USA **111**:16196-16201.
1415
1416 **Yamasaki, H., and Sakihama, Y.** (2000). Simultaneous production of nitric oxide and
1417 peroxynitrite by plant nitrate reductase: *in vitro* evidence for the NR-dependent
1418 formation of active nitrogen species. FEBS Lett. **468**:89-92.
1419
1420 **Yang, H., Mu, J., Chen, L., Feng, J., Hu, J., Li, L., Zhou, J.M., Zuo, J.** (2015). *S*-
1421 nitrosylation positively regulates ascorbate peroxidase activity during plant stress
1422 responses. Plant Physiol. **167**:1604-1615.
1423
1424 **Yu, M., Lamattina, L., Spoel, S.H., Loake, G.J.** (2014). Nitric oxide function in plant
1425 biology: a redox cue in deconvolution. New Phytol. **202**: 1142-1156.
1426
1427 **Yun, B.W., Feechan, A., Yin, M., Saidi, N.B., Le Bihan, T., Yu, M., Moore, J.W.,**
1428 **Kang, J.G., Kwon, E., Spoel, S.H., Pallas, J.A., Loake, G.J.** (2011). *S*-nitrosylation
1429 of NADPH oxidase regulates cell death in plant immunity. Nature **478**:264-268.
1430
1431 **Yun, B.W., Skelly, M.J., Yin, M., Yu, M., Mun, B.G., Lee, S.U., Hussain, A., Spoel,**
1432 **S.H., Loake, G.J.** (2016). Nitric oxide and S-nitrosoglutathione function additively
1433 during plant immunity. New Phytol. **211:**516-526.
1434

Zemojtel, T., Fröhlich, A., Palmieri, M.C., Kolanczyk, M., Mikula, I., Wyrwicz, L.S., Wanker, E.E., Mundlos, S., Vingron, M., Martasek, P., Durner, J. (2006). Plant nitric oxide synthase: a never-ending story? Trends Plant Sci. **11**:524-525.

Zhou, C., Liu, Z., Zhu, L., Ma, Z., Wang, J., Zhu, J. (2016). Exogenous melatonin improves plant iron deficiency tolerance via increased accumulation of polyamine-mediated nitric oxide. Int. J. Mol. Sci. **17**:1777.

Zhou, H.L., Stomberski, C.T., Stamler, J.S. (2018). Cross talk between *S*-nitrosylation and phosphorylation involving kinases and nitrosylases. Circ. Res. **122**: 1485-1487.

Zhu, S., Liu, M., Zhou, J. (2006). Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage. Postharvest Biology and Technology **42**:41-48.

Zuccarelli, R., Rodríguez-Ruiz, M., Patrícia, J., Lopes-Oliveira, P.J., Grazieli, B., Pascoal, G.R., Sónia, C.S., Andrade, S.C.S., Furlan, C.M., Purgatto, E., P alma, J.M., Corpas, F.J., Rossi, M., Freschi, L. (2021). Multifaceted roles of nitric oxide (NO) in tomato fruit ripening: NO-induced metabolic rewiring and consequences in fruit nutritional quality and flavour. J. Exp. Bot. **72**:941-958.