Nitric oxide regulation of temperature acclimation: a molecular genetic perspective

Inmaculada Sánchez-Vicente and Oscar Lorenzo*

Departamento de Botánica y Fisiología Vegetal, Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Facultad de Biología, Universidad de Salamanca, C/ Río Duero 12, 37185 Salamanca, Spain

* Correspondence: oslo@usal.es

Editor: Graham Noctor, Université Paris-Sud, France

The current environmental situation is dominated by climate change, including heat and cold waves that trigger adverse conditions for plant growth and development. Identification of key signalling molecules during stress-related events can help in the development of strategies to mitigate detrimental effects and to improve plant tolerance. Among stress regulators, nitric oxide (NO) has emerged as a central gasotransmitter involved in the control of adaptive responses, acting to tailor plant growth and stress responses. Here, we outline the implications of this control by NO and highlight the genetic and molecular evidence for its role in enhancing tolerance and adaptation to heat and cold stress, which in turn has revealed possible new approaches for confronting future environmental challenges.

Involvement of nitric oxide during tolerance to heat stress

As a consequence of climate change, increased temperatures have become an important threat to the maintenance of agriculture worldwide, and regional and local warming is predicted to have an even greater impact on biological systems over the coming years. Increases above optimum temperatures for plant development lead to what is broadly termed heat stress (HS), where serious damage is caused to growth and developmental processes. Several reports have associated high temperatures with molecular and physiological modifications that compromise the correct status of cells (see Box 1).

Disturbed NO homeostasis deeply affects plant growth and development, for example as shown in Arabidopsis mutants either under- or over-producing NO, in S-nitrosoglutathione (GSNO) over-producer mutants, and in transgenic plants overexpressing GSNO reductase (Fig. 1). Although the plants grow faster with increasing temperature independently of the genotype (15–21–25 °C), the final seed production is compromised relative to the wild-type when NO and GSNO production is disturbed in both the NO- and GSNO-altered lines. These phenotypes suggest that maintenance of NO homeostasis is essential for adaptation to increasing temperatures and for a proper heat-stress response (HSR) to avoid severely reduced seed yields. Thus, the hot5-2 and nox1 mutants, which have altered levels of S-nitrosothiols (SNO) and NO, respectively, show thermotolerance defects that can be partially alleviated by the NO-scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) (Lee et al., 2008). In addition, mutants impaired in NO biosynthesis, such as noa1 and nia1 nia2, exhibit lower rates of survival under HS conditions, which is reversible through exogenous treatments with NO donors (Xuan et al., 2010). A large-scale analysis has also shown changes in the nitroproteome, revealing that tyrosine nitration is increased under HS (Chaki et al., 2011).

NO initiates HSRs from multiple molecular features made up of a signalling network that includes activation of oxidative defences, accumulation of osmolytes and HEAT-SHOCK PROTEINS (HSPs), and protection of photosynthesis.

Heat shock promotes the production of reactive oxygen species (ROS) in plants (Wang et al., 2014; Alamri et al., 2019), which leads to cell dysfunction. To cope with this oxidative imbalance, plants readjust antioxidant systems to minimize cellular damage. NO accumulation has also been observed under elevated temperatures (Lee et al., 2008) and counteracts this effect through the regulation of key enzymes that coordinate oxidative defence.

Osmotic adjustment protects cells against abiotic stresses, and plays a key role in the regulation of membrane fluidity, ROS...
Scavenging, and protein stabilization. Among the solutes, proline (Pro) is widely considered to be a universal osmoprotectant that prevents damage to cells. Nevertheless, its role during HSR depends on the species, with Pro accumulation increasing the sensitivity of Arabidopsis and tobacco plants whilst conversely it might be crucial in providing better resistance in broad bean plants (Alamri et al., 2019). Interestingly, the increase of NO during HS in broad bean correlates positively with Pro synthesis, which in turn improves plant thermotolerance.

Photosynthesis is a heat-sensitive process, and is mainly damaged by harmful effects in PSII. Exogenous NO ameliorates cell damage at the reaction centre and throughout the electron transport chain, improving PSII recovery (Chen et al., 2013).

Among the crosstalk that occurs between NO and biomolecules, nitro-fatty acids (NO2-FA) are considered as signalling molecules involved in HSR in animals, and nitro-linolenic acid (NO2-Ln) has been identified to play such a role in Arabidopsis, since exogenous treatments induce the expression of genes

---

**Box 1. Schematic representation of plant molecular and physiological modifications in response to heat stress**

Heat stress (HS) disturbs the redox balance and affects the coordination of organelles and the structure and functionality of macromolecules (i.e. lipid oxidation, DNA damage, protein destabilization and aggregation, and disruption of enzyme activity; reviewed by Ohama et al., 2017). Plants have evolved various mechanisms that control the signalling pathways involved in the acquisition of tolerance, including antioxidant defences and the accumulation of osmoprotectants, HEAT SHOCK FACTORS (HSFs), and HEAT SHOCK PROTEINS (HSPs). The figure compiles the effects of NO together with the specific molecular processes that act to improve plant tolerance to HS (created with BioRender.com).

Exogenous NO confers thermotolerance by increasing the activity of enzymes that scavenge reactive oxygen species (ROS), including SUPEROXIDE DISMUTASE (SOD), CATALASE (CAT), and ASCORBATE PEROXIDASE (APX), as observed in reed calluses and wheat plants (Hasanuzzaman et al., 2012). In addition, NO has been reported to act upstream of CALMODULIN 3 (CaM3) in Arabidopsis, inducing thermotolerance by enhancing the binding of HSFs to DNA and increasing the accumulation of HSPs (Xuan et al., 2010; Wang et al., 2014), whilst NO metabolism is modulated by CaM3, which binds to GSNO REDUCTASE (GSNOR) under HS (Zhang et al., 2020). There is an influx of calcium (Ca2+) into the cell in response to HS, and intracellular Ca2+ and H2O2 are known to act upstream of NO in the response to HS (Peng et al., 2019). Indeed, both molecules increase the survival rate of rice seedlings by promoting the expression of HSP26 (Uchida et al., 2002).
involved in HSR and the acquisition of thermotolerance, mainly HSPs and HEAT-SHOCK FACTORS (HSFs) (Mata-Pérez et al., 2016).

Although a pivotal role for NO during HSR is clear, some studies have identified a reduction in the content of NO after exposure to HS (Chaki et al., 2011). These apparently contradictory results might depend on the plant species, the tissue, and the specific heat treatment, as well as the highly dynamic nature of NO.

**Association of nitric oxide with cold stress**

Similar to HS, low temperatures represent one of the most harmful abiotic stresses, resulting in significant damage to plants, and consequently to potentially extreme yield losses. Under cold stress, plants suffer changes in both biochemical and physiological processes, and as a result they have evolved several strategies to minimize the effects (see Box 2). Cold acclimation constitutes a highly complex process that can confer great resistance to exposure to low, non-freezing temperatures.

Several NO genetic tools (e.g. Fig. 1) and NO-mediated mechanisms for coping with cold stress and improving tolerance have been described. Large-scale analyses have shed light on the functions of NO during plant responses to low temperature, which involve changes in hormonal pathways, accumulation of osmoprotectants, and oxidative responses. NO acts to attenuate the freezing response, modulating the switch between constitutive feedback and normal growth under optimal conditions. This explains the cold-related phenotype observed in the nia1 nia2 noa1-2 triple-mutant, which is characterized by increased survival under cold stress (Costa-Broseta et al., 2018) but with a lower capacity to tolerate freezing conditions when compared to cold-acclimated wild-type plants (Costa-Broseta et al., 2019). Nevertheless, earlier work reported a lower survival rate for the double-deficient mutant nia1 nia2 (Zhao et al., 2009). In a similar way to HS, these apparently contradictory observations might be explained by the different conditions used to carry out the experiments.

NO is able to modify the nitrosoproteome in *Brassica juncea* and Arabidopsis during the first hours of exposure to cold
stress, highlighting its involvement in post-translational regulation. The most common mechanisms that are susceptible to modification by NO are those related to photosynthesis, redox homeostasis, metabolism, and signalling pathways. Although studies have shown that nitrosated proteins are clearly accumulated, only about 30% of those identified are consistent between the different analyses reported, which emphasizes the highly dynamic nature of NO regulation. Other recent research has also drawn attention to the subcellular changes that occur when NO is attached to cysteine residues, suggesting the existence of nitrosation-mediated nuclear trafficking that has an impact on cellular metabolism and redox status (Sehrawat et al., 2019). These findings are also supported by results obtained in bermudagrass that show that NO has a
crucial role in maintaining cell membrane stability, in antioxidant responses, and in PSII recovery, with temperature changes involving interconnections between H₂O₂ and NO during the plant responses (Fan et al., 2013).

Similar to HS, Pro is linked to cold tolerance, and its levels are modulated by NO production via NITRATE REDUCTASE (NR), which stimulates its synthesis and reduces its degradation through the control of 5PC SYNTHASE1 (5PCS1) and Pro DEHYDROGENASE (ProDH), respectively (Zhao et al., 2009).

Among the transduction molecules related to cold perception, lipid-derived signals represent one of the earliest responses. NO is involved in the formation of these signals, and it specifically regulates the production of phytosphingosine phosphate (PHS-P) and ceramide phosphate (Cer-P) (Cantrel et al., 2011). Overall, studies consistently indicate that NO is crucial for providing increased resistance to the harmful effects of cold stress.

**Perspective**

Exposure to extreme conditions outside of optimal temperatures reduces both crop yield and quality. NO has been shown to be a crucial gasotransmitter during temperature acclimation and as such it holds great promise for contributing to our assessments of how plants respond to climate change. Advances in research on temperature sensing have identified NO as a key temperature-sensitive mediator that helps to ensure that plants thrive under temperature-related stress. In order to improve and accurately focus future research, further knowledge is required regarding the molecular players that participate within the complex NO network, enabling us to differentiate the free NO and SNO contents, since their dynamics are not yet well understood. To this end, the plant material used, the developmental stages, and the specific treatments must all be carefully scrutinized as many contradictory results have arisen. To aid in this task, NO-controlled post-translational modifications (PTMs), such as S-nitrosation of cysteine residues, S-nitrosylation of metals, and nitration of tyrosine residues, constitute versatile signals that alter protein functionality through directly modifying stability, the ability to form multicomplexes, protein activity, and localization. The huge capacity for protein regulation influences adaptation to abiotic stresses. Protein 3D structural features should be analysed more in depth, since the proteins identified as putative NO targets through wide-scale analyses do not all present conserved primary sequences of adjacent amino acid residues for PTMs. The effects of NO₂ – FAs to directly drive S-nitrosation should also be considered. Furthermore, special attention should be paid to the potential use of the bZIP and NAC families of transcription factors as molecular players that respond to heat and cold stress, either as targets of PTMs or as regulators of NO homeostasis.

**Acknowledgements**

We would like to thank the Spanish network BIO2015-68957-R-REDT and RED2018-102397-T for stimulating discussions, and Emma Keck for English revision. This work was financially by grants from the ‘EcoSeed Impacts of Environmental Conditions on Seed Quality’ ‘EcoSeed-311840’ ERC.KBBE.2012.1.1-01, BIO2017-85758-R from the Ministry of Science, Innovation and Universities (Spain), grants SA313P18 and SA137P20 from the Regional Government of Castile and Leon and “Escalera de Excelencia” CLIU-2018-04 co-funded by the PO. FEDER, of Castile and Leon 2014–2020 Spain (to OL) and “Fundación Solórzano” FS/16 2019 (to IS-V).

**Keywords:** Cold stress, gasotransmitter, GSNO reductase (GSNOR), heat stress, nitrate reductase (NR), reactive oxygen species (ROS), transcription factors (TFs).

**References**

Alamri SA, Siddiqui MH, Al-khaishany MY, Khan MN, Mohamed H, Alakeel KA. 2019. Nitric oxide-mediated cross-talk of proline and heat shock proteins induce thermotolerance in Vicia faba L. Environmental and Experimental Botany 161, 290–302.

Arfan M, Zhang D-W, Zou L-J, Luo S-S, Tan W-R, Zhu T, Lin H-H. 2019. Hydrogen peroxide and nitric oxide crosstalk mediates brassinosteroids induced cold stress tolerance in Medicago truncatula. International Journal of Molecular Sciences 20, 144.

Cantrel C, Vazzquez T, Puyaubert J, Lesch M, Kaiser WM, Dutilleul C, Guillas I, Zachowski A, Baudouin E. 2011. Nitric oxide participates in cold-responsive phosphosphingolipid formation and gene expression in Arabidopsis thaliana. New Phytologist 189, 415–427.

Chaki M, Valderrama R, Fernández-Ocaña AM, et al. 2011. High temperature triggers the metabolism of S-nitrosohistidines in sunflower mediating a process of nitrosative stress which provokes the inhibition of ferredoxin-NADP reductase by tyrosine nitration. Plant, Cell & Environment 34, 1803–1818.

Chen K, Chen L, Fan J, Fu J. 2013. Alleviation of heat damage to photosystem II by nitric oxide in tall fescue. Photosynthesis Research 116, 21–31.

Costa-Broseta Á, Perea-Resa C, Castillo MC, Ruiz MF, Salinas J, León J. 2018. Nitric oxide controls constitutive freezing tolerance in Arabidopsis by attenuating the levels of osmoprotectants, stress-related hormones and anthocyanins. Scientific Reports 8, 9268.

Costa-Broseta Á, Perea-Resa C, Castillo MC, Ruiz MF, Salinas J, León J. 2019. Nitric oxide deficiency decreases C-repeat binding factor-dependent and -independent induction of cold acclimation. Journal of Experimental Botany 70, 3283–3296.

Fan J, Chen K, Amombo E, Hu Z, Chen L, Fu J. 2015. Physiological and molecular mechanism of nitric oxide (no) involved in bermudagrass response to cold stress. PLOS ONE 10, e0132991.

Hasanuzzaman M, Nahar K, Alam M, Fujita M. 2012. Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (Triticum aestivum L.) seedlings by modulating the antioxidant defense and glyoxalase system. Australian Journal of Crop Science 6, 1314–1323.

Lee U, Wie C, Fernandez BO, Feelisch M, Vierling E. 2008. Modulation of nitrosative stress by S-nitrosoglutathione reductase is critical for thermotolerance and plant growth in Arabidopsis. The Plant Cell 20, 786–802.

Liu T, Xu J, Li J, Hu X. 2019. NO is involved in JA- and H₂O₂-mediated ALA-induced oxidative stress tolerance at low temperatures in tomato. Environmental and Experimental Botany 161, 334–343.
Lv X, Ge S, Jalal Ahammed G, Xiang X, Guo Z, Yu J, Zhou Y. 2017. Crosstalk between nitric oxide and MPK1/2 mediates cold acclimation-induced chilling tolerance in tomato. Plant & Cell Physiology 58, 1963–1975.

Mata-Pérez C, Sánchez-Calvo B, Padilla MN, et al. 2016. Nitro-fatty acids in plant signaling: nitro-linolenic acid induces the molecular chaperone network in Arabidopsis. Plant Physiology 170, 686–701.

Ohama N, Sato H, Shinozaki K, Yamaguchi-Shinozaki K. 2017. Transcriptional regulatory network of plant heat stress response. Trends in Plant Science 22, 53–65.

Peng X, Zhang X, Li B, Zhao L. 2019. Cyclic nucleotide-gated ion channel 6 mediates thermotolerance in Arabidopsis seedlings by regulating nitric oxide production via cytosolic calcium ions. BMC Plant Biology 19, 368.

Sehrawat A, Sougrakpam Y, Deswal R. 2019. Cold modulated nuclear S-nitrosoproteome analysis indicates redox modulation of novel Brassicaceae specific, myrosinase and napin in Brassica juncea. Environmental and Experimental Botany 161, 312–333.

Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T. 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Science 163, 515–523.

Wang L, Guo Y, Jia L, Chu H, Zhou S, Chen K, Wu D, Zhao L. 2014. Hydrogen peroxide acts upstream of nitric oxide in the heat shock pathway in Arabidopsis seedlings. Plant Physiology 164, 2184–2196.

Xuan Y, Zhou S, Wang L, Cheng Y, Zhao L. 2010. Nitric oxide functions as a signal and acts upstream of AtCaM3 in thermotolerance in Arabidopsis seedlings. Plant Physiology 153, 1895–1906.

Zhang X, Wang W, Kang X, Zhao L. 2020. Arabidopsis CaM3 inhibits nitric oxide accumulation and improves thermotolerance by promoting S-nitrosoglutathione reductase via direct binding. Plant Growth Regulation 90, 41–50.

Zhao MG, Chen L, Zhang LL, Zhang WH. 2009. Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in Arabidopsis. Plant Physiology 151, 755–767.