9TH PLANT NITRIC OXIDE INTERNATIONAL MEETING

ABSTRACT BOOK

28TH-29TH FEB 2024

NATIONAL INSTITUTE OF PLANT GENOME RESEARCH
(NIPGR), NEW DELHI, INDIA-110067

Organizer's Message

Dear Delegates

Warm Greeting!

On behalf of the International Plant Nitric Oxide Club, I would like to cordially welcome you all to the 9th Plant Nitric Oxide International Meeting (PNO9), Delhi, India

This 9th International Plant Nitric Oxide Club Meeting (PNO9) is a unique platform for leaders and young emerging talents in the field of plant nitric oxide to share their latest research and discuss future directions. I wish this conference will help our Nitric oxide community to exchange the most advanced techniques and approaches to address the different biological puzzles taken by the different leading groups around the globe.

Looking forward to the most exciting and successful conference!!!

Thank You.

Dr. Jagadis Gupta Kapuganti FABAP, FNASc, FNAAS

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Hyderabad, India

Prof. Renu Deswal
New Delhi, India

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Mysore, India

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9th Plant Nitric oxide International Meeting (PNO9), New Delhi, 2024

INAUGURAL SESSION

Day 1 (28th February, Wednesday)		
Inauguration of 9th Plant Nitric oxide International Meeting (PNO9)- 2024		
CET (European time)	**IST (Indian time)**	**Details**
9:30-9:35	14-00-14:05	Welcome Address by **Dr. Jagadis Gupta Kapuganti**, Organizer, PNO9-2024.
9:35-9:45	14:05-14:15	Address by **Dr. Subhra Chakraborty**, Director, NIPGR. New Delhi
9:45-9:50	14:15-14:20	Address by **Prof. Christine H Foyer,** University of Birmingham, UK.
9:50-10:00	14:20-14:30	Address by **Prof. A.S. Raghavendra** & **Prof. Renu Deswal**

TECHNICAL PROGRAM & ABSTRACTS

Session 1			
Chairs: Prof Joerg Durner & Prof A.S Raghavendra			
Key Note Talk			
10:00-10:30	14:30-15:00	**Prof. Gary J Loake**: *S*-nitrosylation of a cytoplasmic receptor-like kinase regulates plant immunity	**1**
Lead talks			
10:30-10:50	15:00-15:20	**John T. Hancock**: Does nitric oxide use protein cavities?	**2**
10:50-11:10	15:20-15:40	**Lorena Aranda-Caño:** Modulation of the nitro-alkylated proteome by nitro-fatty acids during development and abiotic stress in plants.	**3**
11:10-11:30	15:40-16:00	**Ginevra M. E. Peppi:** Uncovering molecular and regulatory mechanisms underlying GSNO degradation by aldo-keto reductase from *Arabidopsis thaliana*.	**4**
11:30-11:35	16:00-16:05	Special issue announcement by **Prof. David Wendehenne.**	-
Session 2			
Chairs: Prof. Francisco J Corpas & Dr. Girigowda Manjunatha			
Lead Talks			
11:35-11:55	16:05-16:25	**Farida Minibayeva:** Role of nitric oxide in autophagy in plants.	**5**
11:55-12:15	16:25-16:45	**Lekshmy Sathee:** Elevated CO_2 exacerbates nitric oxide production and modulates nitrogen metabolism in bread wheat.	**6**
12:15-12:35	16:45-17:05	**Ádám Solti:** Nitric oxide accumulates under iron deficiency in chloroplasts.	**7**
12:35-12:55	17:05-17:25	**Ankita Sehrawat:** S-nitrosoproteome analysis in *Brassica juncea* from apoplast to nucleus during cold stress	**8**
12:55-14:00	17:25-18:30	**Break**	**-**
Session 3			
Chairs: Prof. Juan B. Barroso & Prof. Renu Deswal			
Lead Talks			
14:00-14:20	18:30-18:50	**Dandan Huang:** Nitric Oxide maintained mitochondrial function in peach fruit during cold storage.	**9**
14:20-14:40	18:50-19:10	**Sunita Kataria**: Involvement of nitrate reductase-dependent nitric oxide production in magnetopriming-induced salt tolerance in soybean.	**10**
14:40-15:00	19:10-19:30	**Loriana Demecsová:** Nitric oxide sustains root surface redox activity and growth under hypoxic conditions in barley root tip.	**11**

Session 4: Flash Talks				
Chairs: Prof. Christian Lindermayr & Dr. Zsuzsanna Kolbert				
Time		**Presenter**	**Title**	
CET (European)	**IST (Indian)**			
15:00-15:05	19:30-19:35	Neidiquele M. Silveira	Revealing the influence of nitric oxide signaling on drought tolerance mechanisms in 'Rangpur' lime.	12
15:05-15:10	19:35-19:40	Fernández MB	Role of NO in modulating UV-B stress responses in the cyanobacterium *Synechococcus* PCC 7335.	13
15:10-15:15	19:40-19:45	Dóra Kondak	Nanotechnological Interventions: Exploring the Impact of Nano-Nitric Oxide Donors in *Brassica napus*	14
15:15-15:20	19:45-19:50	Priyanka Jain	Identification of Nitric Oxide Responsive Genes in Rice Upon Fungal Infection	15
15:20-15:25	19:50-19:55	Patrícia Juliana Lopes Oliveira	Impacts of GSNOR manipulation on tomato heat stress responses.	16
15:25-15:30	19:55-20:00	Josepheena Joseph	Mitochondrial alternative oxidase pathway helps in nitro oxidative stress tolerance in germinating chickpea.	17
15:30-15:35	20:00-20:05	Joanna Gajewska	Host-derived nitrosative stress activates *Phytophthora infestans* HDAC3.	18
15:35-15:40	20:05-20:10	P. Bharath	Cytosolic alkalinization of guard cell pH facilitates the increase in nitric oxide levels and stomatal closure in *Arabidopsis thaliana*	19
15:40-15:45	20:10-20:15	Ruirui Wang	Effects of red light, far red light and NO on embryo germination of *Sorbus pohuashanensis*	20
15:45-15:50	20:15-20:20	Anastasia Mazina	S-nitrosylation of proteins during stress-induced autophagy in wheat *triticum aestivum*	21
15:50-15:55	20:20-20:25	Eduardo Gómez-Copoví	Role of nitric oxide on trans-resveratrol production in grapevine cells elicited with cyclodextrins and methyl jasmonate	22

15:55-16:00	20:25-20:30	Yaiphabi Sougrakpam	Low abundant myrosinases S-nitrosation suggest myrosinases involvement in nitic oxide signalling in *Brassica juncea*.	23
16:00-16:05	20:30-20:35	Rekha	PHYTOGLOBIN - Boon or Bane during *Botrytis* fungus infection in Arabidopsis and Tomato	24
16:05-16:10	20:35-20:40	María A Muñoz-Vargas	Leucine aminopeptidase (LAP) activity from sweet pepper fruits is modulated during ripening and by NO and reducing events	25
16:10-16:15	20:40-20:45	Vijay Pratap Singh	The function of peroxynitrite in rice roots under waterlogging stress	26
Session 5: Flash Talks				
Chairs: Prof. José Manuel Palma & Prof. Francisco J Corpas				
Time		**Presenter**	**Title**	
CET (European)	**IST (Indian)**			
16:15-16:20	20:45-20:50	Garima Balyan	Mechanisms derived from titanium dioxide nanoparticles and nitric oxide interaction to alleviate salinity stress in wheat seedlings	27
16:20-16:25	20:50-20:55	Nidhi Kandhol	The Essential Role of Nitric Oxide in Zinc-induced cadmium stress resistance in rice roots	28
16:25-16:30	20:55-21:00	Kaiser Iqbal Wani	Nitric Oxide Regulates Antioxidant Machinery, Enhances PSII Efficiency, Improves Chloroplast Ultrastructure, and Promotes Artemisinin Biosynthesis in Artemisia annua Under Cadmium Stress	29
16:30-16:35	21:00-21:05	Asif Hussain Hajam	Exogenous application of nitric oxide alleviates arsenic-induced toxicity and enhances antioxidant defense mechanisms in Ocimum basilicum	30
16:35-16:40	21:05-21:10	Sheen Khan	Nitric oxide boosts photosynthetic nitrogen and sulfur-use efficiency and the ascorbate-glutathione cycle to mitigate high-temperature stress in rice plants	31

16:40-16:45	21:10-21:15	Giovanna Camargo do Carmo	Effect of nitric oxide-releasing nanoparticles on the growth and physiology of Cecropia pachystachya Trécul seedlings subjected to moderate water deficit	32
16:45-16:50	21:15-21:20	Abhaypratap Vishwakarma	A discrete strategy of AOX during hypoxia to increase nitric oxide and ATP synthesis	33
16:50-16:55	21:20-21:25	Hugo R. Guariz	S-nitrosoglutathione-loaded chitosan nanoparticles improve the physiological performance and reduce the mortality of neotropical tree seedlings after transplanting to the field	34
16:55-17:00	21:25-21:30	Anjali Pande	Chitosan-GSNO nanoparticles: a positive modulator of drought stress tolerance in soybean	35
17:00-17:05	21:30-21:35	Rosa M. Rivero	Unraveling the interplay between Melatonin and Nitric Oxide during the PSII/PSI gene dynamics under single and combined abiotic stresses	36

DAY-2

Day 2 (29th February, Thursday)			
Session 1			
Chairs: Prof Gary Loake & Dr Jagadis Gupta Kapuganti			
CET (European time)	**IST (Indian time)**	**Details**	
Key Note Talk			
9:00-9:30	13:30-14:00	**Jose F. Moran:** A new oxidative pathway of nitric oxide production from oximes in plants.	**37**
Lead talks			
9:30-9:50	14:00-14:20	**Kapuganti Jagadis Gupta:** Nitric oxide role in mitochondrial biogenesis in plants.	**39**
9:50-10:10	14:20-14:40	**Zoé Chaudron:** Identification and functional characterization of S-nitrosated proteins during salt stress in *Klebsormidium nitens.*	**40**
10:10-10:30	14:40-15:00	**Ling Yang:** Role of nitric oxide in stress-induced somatic embryogenesis in trees.	**41**
10:30-10:50	15:00-15:20	**Urszula Krasuska:** An update to the model of "nitrosative door" in seed biology.	**42**
10:50-11:00	15:20-15:30	Ecophysics	-
Session 2			
Chairs: Prof. José Manuel Palma & Prof. David Wendehenne			
Lead talks			
11:00-11:20	15:30-15:50	**Prof Raghavendra:** Nitric oxide is complemented by reactive oxygen species and calcium during stomatal closure and plant defence against abiotic and biotic stress	**43**
11:20-11:40	15:50-16:10	**María José Iglesias:** NO and ROS promote hypocotyl cell growth under shade in plants.	**44**
11:40-1200	16:10-16:30	**Amedea B. Seabra:** Nitric oxide-releasing polymeric nanoparticles in plants recent progress, perspective and challenges.	**45**
12:00-12:20	16:30-16:50	**Riddhi Datta:** Unmasking the Hero: How the GSH-GSNO Module Defeats Iron Deficiency in Plants.	**46**
12:20-12:40	16:50-17:10	**Staszek P:** Canavanine-induced decrease in NO synthesis alters cell cycle regulation.	**47**
12:40-13:00	17:10-17:30	**Inmaculada Sánchez-Vicente:** Molecular regulation of bZIP67 by S-nitrosylation impacts on fatty acid storage during seed development	**48**
13:00-14:00	17:30-18:30	**Break**	**-**

Session 3: Flash Talks				
Chairs: Dr. Alexandre Boscari, Sanjib Bal Samant & Jagannath Swain				
Time		**Presenter**	**Title**	
CET (European)	**IST (Indian)**			
14:00-14:05	18:30-18:35	Correa-Aragunde, Natalia	Exploring new insights into the role of nitric oxide synthase in nitrogen metabolism among photosynthetic organisms	49
14:05-14:10	18:35-18:40	Ana Cristina Preisler	Study of the interaction of a free and nanoencapsulated nitric oxide donor with soybean roots	50
14:10-14:15	18:40-18:45	Gábor Fejes	The effect of plasma-activated water on wheat seedling development and the levels of *in planta* reactive oxygen and nitrogen species	51
14:15-14:20	18:45-18:50	Selahattin Kondak	Ecotype-Dependent Responses to Nickel Oxide Nanoparticles in Ni Hyperaccumulator *Odontarrhena lesbiaca*: Unraveling the Impact Across Cellular, Tissue, Organ, and Molecular Level.	52
14:20-14:25	18:50-18:55	Maciej Piekarniak	Can HCN be converted to NO under physiological conditions?	53
14:25-14:30	18:55-19:00	Yue Zhang	Mechanism of nitric oxide alleviating alkali-induced inhibition of embryo germination in *Sorbus pohuashanensis*	54
14:30-14:35	19:00-19:05	Aakanksha Wany	Trichoderma supplementation enhances defense responses under low nitrate nutrition in Arabidopsis	55
14:35-14:40	19:05-19:10	Priyanka Babuta	*Brassica juncea* has multiple forms of S-nitrosoglutathione reductase (GSNOR) suggesting multiple roles	56
14:40-14:45	19:10-19:15	Tamás Bodor	The effect of seed priming with plasma activated water on germination and RONS content of *Arabidopsis thaliana* L.	57
14:45-14:50	19:15-19:20	Caihong Zhao	Concentration-based regulation of dormancy-emergence mechanisms in *Sorbus pohuashanensis* embryos by nitric oxide.	58
14:50-14:55	19:20-19:25	Jorge Taboada	NO modulates in pepper fruits the gene expression of tryptamine 5-hydroxylase (*T5H*), a gene involved in the serotonin generation	59

14:55-15:00	19:25-19:30	S. Gómez-Jiménez	Nitric oxide sensing by TGA transcription factors is essential for the root stem cell niche preservation.	60
15:00-15:05	19:30-19:35	Salvador González-Gordo	Unraveling the dynamics of the ascorbate-glutathione cycle enzymes in pepper fruits during ripening and under a NO-enriched atmosphere.	61
15:05-15:10	19:35-19:40	Pooja Singh	Phytoglobin-NO cycle and AOX pathway play a role in anaerobic germination and growth of deepwater rice.	62
15:10-15:15	19:40-19:45	Laura Lopes Maldonado	Emergence and Initial Development of Seedlings of Atlantic Forest Tree Species in Response to Seed Priming with Nanoencapsulated Nitric Oxide Donor	63
15:15-15:20	19:45-19:50	Anik Sarkar	Status of nitric oxide during fungal disease development in plants.	64
15:20-15:25	19:50-19:55	Shruti Sharma	Understanding the putative role of Nitric Oxide in growth and development of *Dioscorea alata* tubers.	65
15:25-15:30	19:55-20:00	Neha Gupta	Unraveling the involvement of Nitric oxide synthase in mitigating oxidative burst in cyanobacterium *Aphanizomenon flos-aquae* 2012/KM1/D3	66
15:30-15:35	20:00-20:05	Sonika Pandey	Nitric oxide stimulates seed germination via regulating the respiration in chickpea.	67
15:35-15:40	20:05-20:10	Agnieszka Wal	RNS and ROS alterations in *Nepenthes* x V*entrata*	68
Session 4				
Chairs: Prof. A. S. Raghavendra				
Lead talks				
15:40-16:00	20:10-20:30	**Patrick Treffon:** Organomercury-based capture of S-nitrosated proteins links protein quality control to fertility defects.		69
16:00-16:10	20:30-20:40	Concluding remarks by **Prof. Gary Loake** and **Prof. Jorg Durner**		
16:10-16:20	20:40-20:50	Announcement of winners of Flash talks (Will be announced by **Prof. Gary Loake** and **Prof. Francisco J Corpas**		
16:20-16:30	20:50-21:00	Vote of Thanks by **Dr. Jagadis Gupta Kapuganti**.		

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9TH PLANT NITRIC OXIDE INTERNATIONAL MEETING

NIPGR, NEW DELHI, INDIA

DAY- 1

WEDNESDAY,28 TH FEB 2024

KEY NOTE TALK

S-nitrosylation of a cytoplasmic receptor like kinase regulates plant immunity.

Prof. Gary J. Loake

University of Edinburgh, UK.

Abstract

Perception of pathogen/microbial-associated molecular patterns (P/MAMPs) by plant cell-surface receptors leads to a sustained burst of reactive oxygen species (ROS), a key feature of P/MAMP-triggered immunity (PTI). Here we report that P/MAMP recognition leads to a rapid nitrosative burst, initiating the accumulation of nitric oxide (NO), subsequently leading to the *S*-nitrosylation of the cytoplasmic receptor-like kinase, BIK1, at Cys^{80}. This redox-based, post-translational modification, promotes the phosphorylation of BIK1, subsequently resulting in BIK1 activation and stabilisation. Further, BIK1 *S*-nitrosylation increases its physical interaction with RBOHD, the source of the apoplastic oxidative burst, promoting ROS formation. Our data identifies mechanistic links between rapid NO accumulation and the expression of PTI, providing novel insights to help guide the development of disease resistant crops.

LEAD TALKS

Does nitric oxide use protein cavities?

John T. Hancock

University of the West of England, Bristol, UK

Abstract

It has been known for a long time that Xenon (Xe) frequents cavities in proteins, with such hydrophobic regions often being referred to as "xenon pockets". The interaction of this inert gas has two consequences. Firstly, it can alter the structure of the proteins, and secondly, it can be used for the study of the protein's topology. The model proteins used for such studies are usually the globins, i.e., haemoglobin and myoglobin. Other inert gases, such as Argon (Ar), Krypton (Kr), and Helium (He) have also been studied for their biological effects, and such atoms may well be interacting with proteins in a manner analogous to Xe. Recently, there has been a flurry of research activity to investigate the biological activity of molecular hydrogen (H_2). This gas is also relatively inert and there has been some debate about how it enacts its biological effects. Although it is thought to be a scavenger of the hydroxyl radical ($^{\cdot}OH$), such activity would not account for all the biological effects seen when H_2 is used, and therefore other mechanisms of action have been suggested. One of these is that H_2 acts in a similar way to Xe, i.e., it enters Xe pockets and so alters the structure or dynamics of proteins, such as the globins. The question which could be asked is do other small biomolecules have the ability to act in the same way? Could nitric oxide (NO) also enter Xe pockets in proteins and have some of its effects in this manner? Clearly there has been a lot of work on how NO interacts with haem groups (such as in soluble guanylyl cyclase (sGC)) and on how NO partakes in post-translational modification of proteins through *S*-nitrosylation, but is there scope for another mechanism? Is NO too reactive? Is it too large? Or does it have the ability to compete with other gases such as Xe, H_2 etc. to enter protein cavities, and finally, is this worthy of some future research?

Modulation of the Nitro-Alkylated Proteome By Nitro-Fatty Acids During Development and Abiotic stress In Plants

Lorena Aranda-Caño[1], Raquel Valderrama[1], Juan Carlos Begara-Morales[1], Mounira Chaki[1], José Rafael Pedrajas[1], Manuel Melguizo[2], Juan B. Barroso[1]

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Abstract

Nitro-fatty acids (NO_2-FA) are generated from the interaction of unsaturated fatty acids and nitric oxide (NO)-derived molecules. These molecules are involved in plant defence processes against abiotic stress conditions via induction of the chaperone network and antioxidant systems (1, 2). These electrophilic lipids also interfere with intracellular signalling pathways through the reversible post-translational modification of nucleophilic protein residues by nitroalkylation (3) and their ability to release NO (4). Moreover, an *in vitro* reversible esterification of NO_2-FAs in complex lipids has been recently stablished (5). In this work, the previously unknown storage biomolecules of NO_2-FAs in *Arabidopsis thaliana* were identified during development and different abiotic stress conditions. In this sense, phytosterol esters (SE) and triacylglycerides (TAGs) acted as the main reservoir biomolecules in seeds, which were replaced by phospholipids and proteins in the vegetative, generative, and senescence stages (6). On the other hand, the accumulation of NO_2-FAs in phospholipids and their depletion in protein storages was highlighted under stress situations. Esterification of NO_2-FAs in phospholipids and proteins remarks their involvement in both biomembrane dynamics and signalling processes.Additionally, the global proteome susceptible to nitroalkylation has been characterized for the first time in Arabidopsis development and under abiotic stress situations. In the plant development, nitroalkylated proteins presented a noteworthy participation in stress response, biosynthetic and developmental processes, reproduction, and autophagy. On the other hand, the nitro-oxidative stress observed during the different abiotic stress caused a decrease in the levels of biosynthetic and stress-responsive nitroalkylated proteins, as a consequence of the oxidation of Michael adducts and a parallel increase in the esterification of NO_2-FAs with complex lipids. It is noteworthy that the decrease in the levels of nitroalkylation of the pool of proteins studied, restored their functionality in a generalized way by suppressing the negative modulation exerted by nitroalkylation. In summary, these results evidence the regulatory role of NO_2-FA-mediated nitroalkylation in protein functionality.

Uncovering molecular and regulatory mechanisms underlying GSNO degradation by aldo-keto reductase from *Arabidopsis thaliana*

Ginevra M. E. Peppi[1], Patrick Treffon[2], Lorenza Guidotti[1], Giuseppe Gabellini[3], Lauren Velie[2], Simona Fermani[3], Elizabeth Vierling[2], and Mirko Zaffagnini[1]

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[2]Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA, USA
[3]Department of Chemistry "G. Ciamician", University of Bologna, Italy.

Abstract

S-Nitrosoglutathione (GSNO) is considered to be an intracellular reservoir of nitric oxide (NO) and responsible for the NO transport that allows the biological activity of NO to expand. Although GSNO homeostasis is known to be controlled by the ubiquitous NADH-dependent S-nitrosoglutathione reductase (GSNOR), specific aldo-keto reductases (AKRs) have been recently identified as a new class of NADPH-dependent enzymes involved in GSNO catabolism in plant and animal systems. We have obtained extensive data on the kinetic properties of Arabidopsis AKRs belonging to the 4C family (AtAKR4Cs) and correlated their catalytic properties with specific structural elements of the AKR family. Recombinant proteins were purified to homogeneity and the kinetic parameters (K_m and *kcat*) determined for both cofactor and substrate. Among four AtAKR4Cs (C8, 9, 10 and 11 isoforms), the C8 isoform appears to be the most catalytically efficient with an affinity toward GSNO similar to that measured for plant GSNOR (~30 µM). However, catalysis of GSNOR by AtAKR4Cs is ~7-fold lower than that of the nitrosylated coenzyme A (SNO-CoA), indicating that the latter substrate is favoured in the degradation process. Redox sensitivity of AtAKR4Cs was first explored by analyzing available X-ray or modelled structures to establish the solvent accessibility of cysteine residues. Next, we evaluated the effect on enzyme activities after treatment with oxidizing agents including hydrogen peroxide (H_2O_2), oxidized glutathione (GSSG) and GSNO. Intriguingly, only the C8 isoform was significantly inhibited, suggesting that this isoform contains one or more redox sensitive cysteines. Based on solvent accessibility, we identified Cys287 as the most likely candidate for oxidation. To assess the redox sensitivity of Cys287, we replaced this residue with alanine or serine and purified the mutant forms to analyze the impact of oxidants on catalysis. Overall, our findings reveal kinetic and regulatory properties of AtAKR4Cs highlighting the ability of these enzymes to support GSNOR in GSNO catabolism, with the C8 isoform being the most catalytically efficient but also the only isoform showing redox sensitivity. The generation of single and higher order AKR4C mutants in Arabidopsis is ongoing, and the analysis of these mutant plants will be crucial for a deeper understanding of NO homeostasis in plants.

Role of nitric oxide in autophagy in plants

Minibayeva F.V., Mazina A.B., Gazizova N.I., Rakhmatullina R.F.

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Abstract

Autophagy is a highly conserved process that degrades damaged macromolecules and organelles. Although it is widely recognized that autophagy plays significant roles in the response of plants to stresses and is associated with survival and/or programmed cell death, little is known about nitric oxide (NO)-dependent mechanisms that regulate this catabolic process. Contradictory data exist on the role of NO in the regulation of autophagy. Initially, NO was thought to slow down or prevent autophagy in animal cells, but recently, more evidence emerged that NO can induce autophagy. Unlike animals, only limited information is available regarding NO-mediated autophagy in plants. The aim of present work was to unravel the link between NO, redox status, energy metabolism, and autophagy in plant cells. We showed that in four-day old wheat seedlings, treatment with NO donors such as nitrate, nitrite, sodium nitroprusside, and a physiological NO donor spermine, induces autophagy in the roots. This was confirmed by visualization of autophagosomes using LysoTracker and mRFP-ATG8 staining, upregulation of autophagic (*ATG*) genes, and the accumulation of ATG4 and ATG8, the key ATG proteins. Among NO donors, nitrite proved to be the most effective inducer of autophagy. The generation of NO was accompanied by the accumulation of H_2O_2 and an increase in lipid peroxidation, which are indicators of oxidative stress. The search for a natural inducer of autophagy prompted us to test the effects of polyamines, members of the oxidizing pathway of NO generation in plants. We found that in wheat roots, spermine induces autophagosome formation, and this is significantly inhibited by the NO trap cPTIO suggesting the involvement of NO in this process. Autophagy is an energy dependent process. Treatment with nitrite and nitroprusside caused an energy deficit, a typical prerequisite of autophagy, which was indicated by a fall in mitochondrial potential and the inhibited activity of mitochondrial complexes. On the contrary, spermine sustained energy metabolism by upregulating the activity of genes that encode glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and SNF1-related protein kinase 1 (SnRK1). Considering the known synergy between NO and ROS, it can be suggested that NO donors, including the naturally occurring NO donors' polyamines, can trigger autophagy by inducing nitrosative and oxidative stress. More delicate control of some ATG proteins and other proteins, which are involved in autophagic cascades, could be governed via NO-mediated posttranslational modifications, such as protein S-nitrosylation. Taken together, our data suggest that NO plays the key roles in triggering autophagy in plants via diverse mechanisms, thus facilitating the removal of oxidized and damaged cellular constituencies.

Elevated CO_2 exacerbates nitric oxide production and modulates nitrogen metabolism in bread wheat

Lekshmy Sathee*, Sandeep Adavi B, Birendra K Padhan, and Sinto A

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Abstract

Wheat is a major staple food crop worldwide contributing approximately 20% of total protein consumed by mankind. The nitrogen and protein concentration of wheat crop and grain often decline as a result of exposure of the crop to elevated CO_2 (EC). The changes in nitrogen (N) assimilation, root system architecture, and nitric oxide (NO) mediated N signalling and expression of genes involved in N assimilation and high affinity nitrate uptake were examined in response to different nitrate levels and EC in wheat. Activity of enzyme nitrate reductase (NRA) was down regulated under EC both in leaf and root tissues. Plants grown under EC displayed enhanced production of NO and more so when nitrate supply was high. Based on exogenous supply of NO, inhibitors of NO production and NO scavenger, regulatory role of NO on EC mediated changes in root morphology and NRA was revealed. The enhanced NO production under EC and high N levels**,** negatively regulated the transcript abundance of *NR* and high affinity nitrate transporters (HATS). Elevated CO_2 also down regulated ammonia assimilation and nitrate signalling gene expression under high N availability, with a concomitant increase in nitrosothiol accumulation. There was conspicuous reduction in photorespiratory ammonia assimilation gene expression. Root nitrogen assimilation was less affected in comparison to shoot nitrate assimilation, thereby the proportion of root contribution towards total assimilation was higher. The results suggest that EC could alter and re-programme nitrogen assimilation and signaling in wheat seedlings. The effect of high reproductive stage N application on grain yield and NUE of wheat genotypes under EC was also evaluated. There was a conspicuous decline in leaf nitrogen metabolism by EC; depicted as a reduction in the activity of GS and GOGAT and grain protein content. The higher accumulation of RNS and ROS in the high nitrogen application re-confirmed the futility of applying excess N to alleviate the grain protein decline in EC.

Key words: Nitric Oxide (NO), Elevated CO_2, High Affinity Transport System (HATS), Nitrate signaling

Nitric oxide accumulates under iron deficiency in chloroplasts

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Abstract

Iron (Fe) is an essential cofactor of multiple enzymes, but to avoid its hazardous effects on oxidative stress, signalling of the Fe status, especially in plant cells organelles is essential, although hardly known. Since 80–90% of Fe in the mesophyll cells is targeted to the chloroplasts to operate the photosynthetic apparatus, the control over plastidial Fe status has prime importance. Sensing the cytoplasmic Fe is based on hemerythrin motif-containing E3 ubiquitin ligases that are negative regulators of Fe deficiency responses. Indeed, no mechanisms are described that monitor the Fe status of the organelles. In vitro chloroplast Fe uptake (Solti et al., 2012; Müller et al. 2019) and ferric chelate reductase enzyme studies (Solti et al. 2014; Sági-Kazár et al. 2021) suggests that Fe in the chloroplasts has a feedback regulatory effect on the affinity of the plastidial Fe acquisition machinery. Nitric oxide (NO) is long known to regulate Fe uptake of roots, but also generated in chloroplasts under increasing Fe availability. It is involved in post translation modification of proteins that directly affect the function of those. Since the synthesis and accumulation of NO in chloroplasts has not been confirmed so far, we aimed here to detect and quantify NO in response to altered Fe nutrition chloroplasts. Sugar beet (Beta vulgaris cv. Orbis) model was grown on Fe(III)-EDTA source. Fe deficiency (Fe-def) was achieved with a complete depletion of Fe in the four-leaf stage. Mesophyll cell protoplasts and chloroplasts were isolated. Abundance of the NO signal in the protoplast population and the subcellular NO locations in the protoplasts were analysed applying DAF-FM diacetate in fluorescence activated cell sorting and confocal microscopy, respectively. The proportion of DAF-FM positive protoplasts significantly increased under iron deficiency, together with a higher apparent DAF-FM signal intensity in the protoplasts. Applying BioXol membrane staining of the chloroplast envelopes, chloroplast stroma origin of the DAF-FM signal was approved. DAF-FM signal of isolated chloroplasts was analysed by epifluorescent microscope, also indicating an increased ratio and pixel density of DAF-FM positive chloroplasts under Fe-def compared to the control. Electron paramagnetic resonance (EPR) spectroscopy based semiquantitative NO determination, applying N-methyl-D-glucamine dithiocarbamate spin trap and 150 K environment indicated a concentration of NO in the Fe-def chloroplasts around the lower detection limit, that of attomol NO chloroplast-1 dimension. NO in the control chloroplasts remained below the detection limit. Tyr nitration pattern detected by anti-nitro-Tyr immunoblotting of total chloroplast protein showed no significant changes in the Tyr nitration status indicating that nitrosative stress level has not increased significantly.

S-nitrosoproteome Analysis in Brassica juncea from Apoplast to Nucleus Indicates Nitric Oxide (NO) and Cold Stress Cross-Talk in Stress, Signaling and Redox Related Pathways

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Abstract

Cold stress affects growth, development and crop yield. Nitric oxide (NO) is an important component of stress signaling. However, NO mediated signaling in cold stress is largely unknown. S-nitrosylation is a NO-dependent post-translational modification (PTM) involved in regulating stress responses. Due to the presence of abundant proteins, the list of regulatory/low abundant S-nitrosylated targets is still scanty. Therefore, 2 strategies, sub-cellular (apoplast and nucleus) proteome analysis and RuBisCO depletion were used for identifying these. Apoplastic and nuclear proteins were extracted from Brassica juncea seedlings using vacuum infiltration and sucrose density gradient method respectively, with negligible cytoplasmic and chloroplastic contamination. RuBisCO depleted fraction was purified by immunoaffinity purification. Cold stress induced nitrate reductase (NR) mediated NO production in crude and nucleus, and non-enzymatic nitrite reduction mediated production in the apoplast. Cold stress increased protein based thiols in apoplast, nucleus, crude and RuBisCO depleted fractions. Non-protein thiols increased in apoplast and nucleus, while decreased in crude and RuBisCO depleted fraction, indicating differential modulation of thiols in stress. Biotin switch technique and neutravidin agarose chromatography, detected 25, 32 and 78 cold responsive S-nitrosylated spots in apoplast, nucleus and RuBisCO depleted fractions respectively on the 2-D gel. Mass spectrometry identification showed the majority of targets with stress, signaling and redox related functions. S-nitrosylation of ascorbate-gluthathione cycle, cell wall modifying and proteolytic enzymes in the apoplast, while S-nitrosylation of enzymes of carbohydrate metabolism in the nucleus, indicated spatial role of S-nitrosylation. S-nitrosylation of enzymes associated with glucosinolate hydrolysis pathway, suggests a novel regulation of Brassicaceae specific pathway by NO. Cold stress enhanced dehydroascorbate reductase, superoxide dismutase and glutathione S-transferase activity by S-nitrosylation and reduced ROS level by ascorbate regeneration and hydrogen peroxide detoxification. Identification of 45 % novel S-nitrosylated proteins, provided the first evidence of NO mediated cold-stress signaling in the apoplast and nucleus.

Nitric Oxide maintained mitochondrial function in peach fruit during cold storage

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Abstract

Peaches were socked in NO solution (15 μmol L-1), c-PTIO (20 μmol L-1) solution, and double-distilled water (control). The effects of exogenous NO on mitochondrial function, mitochondrial oxidative damage, and DNA methylation in peach fruit during cold storage were studied. The results showed that exogenous NO significantly increased the endogenous NO content, inhibited the decline of storage quality, and delayed the browning and softening of peaches after harvest. Exogenous NO significantly inhibited the increase of mitochondrial ROS content, improved the activities of enzymes related to ROS metabolism, and alleviated the oxidative stress caused by cold stress on peaches. Exogenous NO treatment significantly inhibited the decrease of the mitochondrial membrane fluidity and mitochondrial respiration control rate, the opening of the mitochondrial permeability transition pore (MPTP), and the increase of mitochondrial oxygen consumption, which maintained mitochondrial function. Exogenous NO treatment significantly upregulated the expression level of PpmTERF18 and increased the mitochondrial DNA copy number in peaches, indicating that NO maintained the stability of mitochondrial DNA (mtDNA) by regulating mitochondrial copy number. Exogenous NO significantly upregulated the activity of total DNMT and related gene expression of methylase and the expression levels of PpCBF5, PpICE1, PpMYC2, and PpCOR in peaches. BSP analysis of CpG islands and promoter regions of the four transcription factors showed that NO signaling induces increased methylation of PpCBF5, PpICE1, and PpMYC2 promoters and demethylation of the PpCOR promoter region. Different CpG islands in the promoter region responded differently to NO regulation, and NO could modify cytosine C methylation sites. NO participated in the cold stress process by regulating the expression and methylation level of cold-tolerant transcription factors, indicating that NO, cold stress and DNA methylation were closely related. That explained that NO could enhance the cold tolerance of peach fruit through mediating DNA methylation.

Involvement of nitrate reductase-dependent nitric oxide production in magnetopriming-induced salt tolerance in soybean

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Abstract

The experiments were performed to investigate the role of nitric oxide (NO) in magnetopriming-induced seed germination and early growth characteristics of soybean (Glycine max) seedlings under salt stress in the present study. The NO donor (sodium nitroprusside, SNP), NO scavenger (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, CPTIO), inhibitors of nitrate reductase (sodium tungstate, ST) or NO synthase (N-nitro-L-Arg-methyl ester, LNAME) and NADPH oxidase inhibitor (diphenylene iodonium, DPI) have been used to measure the role of NO in alleviation of salinity stress by static magnetic field (SMF of 200 mT, 1h). Salt stress (50 mM NaCl) significantly reduced germination and early growth of seedlings emerged from non-primed seeds. Pre-treatment of seeds with magnetopriming positively stimulated the germination and consequently promoted seedling growth. ST, LNAME, CPTIO and DPI significantly decreased the growth of seedling, activities of α-amylase, protease and nitrate reductase (NR), hydrogen peroxide (H2O2), superoxide (O2•−) and NO content in roots of seedlings emerged from non-primed and SMF-primed seeds. However, the extent of reduction was higher with ST in seedlings of SMF-primed seeds under both the conditions; whereas SNP promoted all the studied parameters. Moreover, the generation of NO was also confirmed microscopically using a membrane permanent fluorochrome (4-5-diaminofluorescein diacetate (DAF-2 DA)). Further, analysis showed that SMF enhanced the NR activity and triggered the NO production and NR was maximally decreased by ST as compared to LNAME, CPTIO and DPI. The relative expression of NR (GmNR1) gene was increased by 3.86-fold in magnetoprimed seedlings over their unprimed controls under salinity. The 23.26-fold increase in relative expression of NR genes by the NO donor (SNP) was observed under salinity, while the NR inhibitor, ST caused maximum reduction in expression of NR genes as compared to other inhibitors (L-NAME and DPI). Thus, NO might be one of the important signalling molecules in magnetopriming-induced salt tolerance in soybean and NR may be responsible for SMF-triggered NO generation in roots of soybean.

Nitric oxide sustains root surface redox activity and growth under hypoxic conditions in barley root tip

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Abstract

Climate projection models suggest an increase in frequency and severity of flooding events in farming regions, which will limit plant growth and development by altering numerous morphological, physiological and biochemical processes; in very sensitive plants they may even evoke cell death. In order to gain more insight into the plant response to sudden flooding-induced hypoxic stress, we studied the involvement of an important signaling molecule - nitric oxide (NO), in various barley cultivars (cv.) at their early seedling stage during short-term partial submergence-induced stress. Sudden flooding stress induced a root growth arrest in cvs. Karmel and Levitus, accompanied by increased lipid peroxidation and cell death. By contrast, in more flooding-tolerant cvs. (Slaven and Valis) sudden flooding caused only reduced root growth rate, associated with elevated NO levels in the root tips. Meanwhile, the root tip surface redox activity decreased with the increasing timespan of flooding in all cvs.; however, this decrease in redox activity started earlier and was greater in the cvs. Levitus and Karmel in comparison with cvs. Valis and Slaven. Application of NO donors, sodium nitroprusside and S-Nitroso-L-glutathione, during flooding stress restored the root redox activity and eliminated the flooding-induced lipid peroxidation, accompanied by a partial restoration of post-flooding root growth even in the more sensitive cultivars. This indicates that NO plays a key role in maintaining the root tip surface redox activity in barley seedlings under sudden flooding stress, which is necessary to restrict lipid peroxidation and cell death in the root tips.

FLASH TALKS

Revealing the influence of nitric oxide signaling on drought tolerance mechanisms in 'Rangpur' lime

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Abstract

Citrus rootstocks exhibit varying performances under water deficit conditions, with 'Rangpur' lime standing out as an important rootstock due to its drought tolerance. This study delves into the correlation between drought tolerance and nitric oxide (NO) signaling in 'Rangpur' lime rootstock. We analyzed indicators of NO production, such as S-nitrosothiol and nitrite, in Valencia sweet orange plants grafted onto 'Rangpur' lime or Swingle citrumelo, another important citrus rootstock. Additionally, we evaluated oxidative damage, antioxidant metabolism, and leaf gas exchange in plants under both well-hydrated and water deficit conditions. The results revealed that plants grafted onto 'Rangpur' lime exhibited an increase in leaf S-nitrosothiol production during recovery, indicating elevated NO synthesis. Furthermore, 'Rangpur' lime roots showed higher levels of nitrite during periods of maximum stress and rehydration. In contrast, Swingle citrumelo had a faster recovery of stomatal conductance after rehydration. 'Rangpur' lime, however, demonstrated a more conservative water use strategy and a superior antioxidant capacity compared to Swingle citrumelo, particularly in the roots, characterized by higher superoxide dismutase activity during maximum stress and higher catalase activity during rehydration. Additionally, NO appears to regulate both root biomass accumulation and morphology in 'Rangpur' lime. These findings underscore the intricate relationship between NO signaling, water management, and antioxidant responses in 'Rangpur' lime under water deficit conditions, providing insights into the mechanisms of tolerance in this rootstock under low water availability.

Keywords: *S*-nitrosothiol; Nitrite; Oxidative stress; Leaf gas exchange; Citrus; Rootstocks.

Role of NO in modulating UV-B stress responses in the cyanobacterium *Synechococcus* PCC 7335

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Abstract

Nitric oxide (NO) is a highly diffusible antioxidant and signal molecule that modulates physiological responses in living organisms. NO can be produced by enzymatic (nitric oxide synthase, NOS, and nitrate reductase, NR) or non- enzymatic pathways. NO effects have been extensively studied in plants during growth, development, and stress responses including ultraviolet radiation (UV-B). UV-B is the part of the solar spectrum that ranges from 280 to 315nm. Although most of it is absorbed by the ozone layer, about 5% reaches Earth´s surface and induces photomorphogenesis or damage responses in terrestrial and aquatic organisms. In plants, NO is mainly produced by NR and triggers signaling responses, including tolerance to UV-B exposure. In the cyanobacteria *Nostoc muscurum* and *Anabaena* sp. pharmacological studies show that NOS- produced NO protects cell cultures from UV-B exposure. Nevertheless, this represents the sole report connecting NO and UV-B in these organisms. *Synechococcus* PCC 7335 is a marine free-living cyanobacterium with several adaptations to light fluctuations, such as complementary chromatic adaptation and far-red light photoacclimation. It also encodes a photolyase operon, suggesting high resistance to UV-B through coordinated regulation of enzymes involved in the repair of UV-B-damaged DNA. *S.* PCC 7335 present a non- canonical NOS, which produces both NO and nitrate using arginine as substrate. The aim of this work was to analyze the effect of UV-B on *S.* PCC 7335 cultures and the role of NO in this response. To this end, physiological and molecular studies were performed. Flow cytometry analysis showed that exposition to 4 hours of UV-B (3.4 $W.m^{-2}$) for 3 days did not cause cell death of the culture indicating that *S*. PCC 7335 is highly resistant to this stress. NO quantification demonstrated a continuously increase during 4 hours of UV-B exposure in *S.* PCC 7335. Treatment with tungstate, a non- specific inhibitor of NR, did not affect the raise of NO levels upon UV-B irradiation, suggesting that NR is no implicated in this process. Also, the rate of NO production with the addition of arginine was the same regardless of UV-B treatment. Moreover, RT-qPCR analysis showed a decrease in transcript levels of NOS and NR during UV-B compared to control condition. In addition, cyclobutane pyrimidine dimers in DNA generated by UV-B irradiation were not repaired after one hour of culture recovery in white light, despite an increase in transcript levels of photolyases operon. The upregulation of this operon by UV-B was not regulated by endogenous NO. Pigment quantification revealed a decrease in phycobiliproteins upon UV-B stress, which was not ameliorated by the addition of arginine before the irradiation treatment. Furthermore, UV-B promoted the dispersal of *S*. PCC 7335 biofilm, which was reversed by the scavenging of endogenous NO. Consistently, NO donors (such as GSNO and SNP) also facilitated biofilm dispersal. In summary, the results indicate that UV-B- induced NO is produced by non- enzymatic pathways, do not improve pigment degradation, nor participate in photolyase upregulation, however, is involved in biofilm dispersal as a strategy to avoid stressful environmental conditions.

Nanotechnological Interventions: Exploring the Impact of Nano-Nitric Oxide Donors in *Brassica napus*

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Abstract

In recent decades, nanotechnological approaches employed in agriculture have garnered increasing attention. Among these methods, the use of nano-nitric oxide (NO) donors, such as S-nitrosoglutathione (GSNO) nanoparticle (NP), has become prevalent. These NPs are often encapsulated in environmentally friendly and biodegradable polysaccharides, such as chitosan, influencing the release of NO. NO plays a dual role as a signaling molecule in the regulation of plant growth and development and in enhancing stress tolerance. Additionally, it serves as a primary representative of reactive nitrogen species (RNS). During the antioxidant reaction between NO and glutathione, GSNO is produced through a process known as S-nitrosylation, serving as a reservoir for NO and releasing it upon degradation. We employed free GSNO, GSNO-CHT, GSH-CHT, and empty CHT NPs for this study. Treatment concentrations included: control (distilled water only), 250 µM, 500 µM, 750 µM, 1000 µM. Treatments were applied on the 5th day with a 2-hour incubation period under light for Brassica napus. Fluorescent staining (NO, GSNO, peroxynitrite), and nitration were performed. We labeled chitosan nanoparticles with fluorescein isothiocyanate (FITC) to track their entry into plant cells. Encapsulated GSNO proved to be a more effective NO donor compared to free GSNO. Empty CHT treatments did not induce significant changes in RNS levels, and in seedlings, they did not trigger nitro-oxidative stress.

Keywords: *Brassica napus*, nanoparticles, nitric oxide, GSNO

Identification of Nitric Oxide Responsive Genes in Rice Upon Fungal Infection

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Abstract

Nitric oxide (NO) is a pivotal signalling molecule involved in diverse physiological processes, including plant defence responses. Nitric oxide (NO) is a free radical gas that can diffuse rapidly through biological membranes, allowing it to act as a transient, local, intra- and intercellular signalling molecule. NO is an important messenger helping in biotic stress alleviation. Lu et al., 2020 showed that after exogenous NO application, increased resistance against rice black-streaked dwarf virus infection was observed. **S**amalova et al 2013 showed that infection to rice plants is driven by NO produced by rice blast fungus *Magnaporthe oryzae*. In this study, we investigate the modulation of NO metabolism genes at transcriptional level in resistant and susceptible rice lines upon fungal infection. To achieve this, we performed meta-analysis approaches, to compare genome-wide transcriptional profiling datasets from resistant and susceptible rice lines upon infection with virulent and avirulent *Magnaporthae* fungal infection. Differentially expressed gene expression analysis was performed in the meta-analysis. It was observed that genes encoding nitrate reductase showed higher expression in both resistant and susceptible lines upon *Magnaporthe* infection. This shows that NO metabolism genes are induced upon fungal infection. The resistant NIL also shows increased expression of high affinity nitrate transporters upon infection. Our findings shed light on the complex regulatory network orchestrated by NO in rice plants during the defence response to fungal pathogens, providing valuable information for further understanding for improved disease resistance strategies.

Impacts of GSNOR manipulation on tomato heat stress responses

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Abstract

Enhancing plant thermotolerance is an urgent priority to sustain global food production, ensure food security, and mitigate the impacts of climate change on agriculture. Therefore, understanding the complex regulatory networks and metabolic adjustments triggered by elevated temperatures is key to generating the critically needed climate-smart, heat-resistant crops. Among other signaling molecules, exogenous nitric oxide (NO) supplementation has emerged as an effective alternative for modifying plant responses to heat stress and other abiotic stresses. Additionally, the manipulation of *S*-nitrosoglutathione reductase (GSNOR), a pivotal enzyme in NO and *S*-nitrosothiol homeostasis, has also been demonstrated to modulate stress responses across various plant species. In this study, we investigated the impacts of *SlGSNOR* overexpression (*SlGSNOR-OE*) and knockout (*Slgsnor*) on tomato (*Solanum lycopersicum*) plant responses to prolonged moderate heat stress. Nitrooxidative metabolism, photosynthesis, and biomass accumulation were compared across genotypes under control (25 °C/18 °C day/night) and warm (32°C/25 °C day/night) conditions. Overall, warm conditions negatively impacted most photosynthetic parameters across genotypes, except for the electron transport rates (ETR) and maximum quantum yield of photosystem II (Fv/Fm). Under warm conditions, the *Slgsnor* knockout mutant exhibited significantly higher net photosynthesis rates, carboxylation efficiency and water use efficiency compared to the wildtype. Although *SlGSNOR* loss-of-function intensified leaf hydrogen peroxide (H_2O_2) under warm conditions, no evidence of additional changes in leaf oxidative metabolism nor increments in cellular damage were detected in the mutant plants. These findings suggest a significant role for *SlGSNOR* in adjusting tomato plant responses to long-term mild heat stress conditions.

Mitochondrial alternative oxidase pathway helps in nitro oxidative stress tolerance in germinating chickpea

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Abstract

Mitochondrial alternative oxidase (AOX) is an important protein that can help in regulating reactive oxygen species and nitric oxide in plants. The role of AOX in regulation of nitro-oxidative stress in chickpea is not known. Using germinating chickpea as model system here we investigated the role of AOX in nitro-oxidative stress tolerance. NaCl treatment was used as an inducer of nitro-oxidative stress. Treatment of germinating seeds with 150 mM NaCl lead to reduced germination and radicle growth. AOX inhibitor SHAM caused further inhibition of germination and AOX inducer pyruvate improved growth of radicle under NaCl stress. Isolated mitochondria from germinated seeds under salt stress not only increased AOX capacity but also enhanced AOX protein expression. Measurement of superoxide levels revealed that AOX inhibition by SHAM can enhance superoxide levels. Whereas, AOX inducer pyruvate reduced the superoxide levels. Measurement of NO by gas phase chemiluminescence revealed an enhanced NO generation in response to NaCl treatment. Upon NaCl treatment there was enhanced tyrosine nitration which is an indicator of nitrosative stress response. Taken together, our results revealed that AOX induced under salinity stress in germinating chickpea can help in mitigating nitro-oxidative stress, thereby it can improve germination.

Host-derived nitrosative stress activates *Phytophthora infestans* HDAC3

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Abstract

Phytophthora infestans (Mont.) de Bary belongs to oomycetes and is one of the most essential phytopathogens in the agriculture sector worldwide, causing late blight disease in the *Solanaceae* family. Importantly, in oomycetes, there is a lack of 5mC DNA methylation but a large number of histone deacetylases (HDACs) orthologs in the genome of *Phytophthora* species suggests that reversible histone protein acetylation may play a crucial role in the transcriptional reprogramming of these fungal-like organisms. Importantly, *P. infestans* is a microorganism able to synthesize nitric oxide (NO) – a signaling molecule involved in epigenetic regulation of gene expression, as proved in animals and plants. Therefore, to verify whether and to what extent NO and other reactive nitrogen species (RNS) affect nuclear HDACs in the high-risk oomycete, we performed experiments on the avirulent (avr MP 946) and virulent (vr MP 977) *P. infestans* isolates in reference to the potato (*Solanum tuberosum* L.) cv. Sarpo Mira. Both *P. infestans* isolates were supplemented with specific RNS donors (400 µM GSNO – as NO donor and 5 mM SIN-1 – as peroxynitrite ($ONOO^-$) donor) to mimic the nitrosative stress condition to which the pathogen is usually exposed during *in planta* growth. Firstly, *in-silico* analysis of the distribution of motifs, conserved domains, and gene structure of HDACs in *P. infestans* were performed. The next stage of the investigation involved gene expression analyses encoding nuclear HDACs. Among 5 analyzed genes, we indicated 3 *i.e.*, *HDAC1, HDAC3*, and *HDAC5,* whose expression was RNS-dependent. The most substantial *ca.* 6-fold increase of transcript accumulation we noted for *HDAC3* in avr *P. infestans* structures at 72 h after GSNO treatment. We investigated the pattern of HDAC3 protein accumulation in response to both RNS donors and the host environment. Briefly, GSNO treatment provoked a *ca.* 40% increase in PifHDAC3 protein accumulation in both *P. infestans* isolates. While, during the contact with potato host tissues susceptible response, we noted the accumulation of HDAC3 in the later phase of plant colonization, starting from 72 h after the potato challenge with the pathogen. Moreover, *in-silico* analyses revealed that HDAC3 might be targeted by NO that modify their activity through tyrosine nitration and S-nitrosylation. Summarizing, nitrosative stress can regulate HDAC3 at transcript and protein accumulation levels in avr/vr *P. infestans* isolates, and changes in HDAC3 expression were dependent on the pathogen virulence pattern. Moreover, PifHDAC3 protein accumulation under *in planta* conditions indicates its engagement in the pathogen's offensive strategy.

Cytosolic alkalinization of guard cell pH facilitates the increase in nitric oxide levels and stomatal closure in *Arabidopsis thaliana*

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Abstract

Stomatal closure by abscisic acid (ABA) is a common response of plants under abiotic or biotic stress conditions. Such closure by ABA regulates the rate of photosynthesis/transpiration and provides immunity to plants. ABA-induced closure was initiated by alkalinization of cytosol, followed by an increase in reactive oxygen species (ROS), and nitric oxide (NO) in guard cells of *Arabidopsis thaliana*. External addition of methylamine (MA, a weak base) caused alkalinization of guard cells and promoted stomatal closure, suggesting pH changes were important events. Both ABA and MA elevated the fluorescence levels corresponding to ROS and NO during closure. The presence of NO modulators (cPTIO, L-NAME, and tungstate) nullified the effects of ABA and MA, confirming the importance of NO. Concanamycin A1, a vacuolar H^+-ATPase (V-ATPase) inhibitor, restricted ABA-/MA-induced stomatal closure. Further, concanamycin A1, reduced the levels of cytosolic pH, and NO, suggesting that NO generation depended on the V-ATPase activity. Similarly, the inability of ABA and MA to induce stomatal closure in Arabidopsis mutants deficient in V-ATPase (*vha*) nitrate reductase (*nia1*, *nia2*, and *nia1/2*) and nitric oxide associated1 (*noa1*) confirmed the functional need for V-ATPase, NR and NOA for NO generation during stomatal closure. Taken together, our results suggest that, V-ATPase might mediate the cytosolic alkalinization followed by the generation of NO during stomatal closure induced by ABA.

Effects of red light, far red light and NO on embryo germination of *Sorbus pohuashanensis*

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Abstract

Seed dormancy release and germination is a complex physiological and biochemical process, which is determined by the dormancy level of seeds and external environmental factors. Seed germination may require other conditions such as light or nitrates in addition to the most basic requirements of adequate water, sufficient oxygen and suitable temperature. These factors cooperate with and antagonize each other to control seed dormancy and germination. Seeds of different species have different sensitivity to light, which are regulated by light signal transduction pathway mediated by photoreceptor. After the light signal is detected by the photoreceptor, the molecular morphology will undergo corresponding changes, and then nuclear transport will be carried out, and then regulate plant growth and development by activating or inhibiting the expression of downstream genes. Nitric oxide (NO) is also an important signaling molecule affecting seed dormancy and germination, and a certain concentration of NO can promote seed germination. In recent years, it has been found that there are interactions between NO and photoreceptors especially phytochrome during seed germination, but the physiological and molecular responses and mechanisms of the interaction between the two have not been reported on *Sorbus pohuashanensis*, and the pathway are still unclear.Therefore, in this study, mature zygote embryos of *Sorbus pohuashanensis* were treated with red light, far red light and exogenous NO donor (NO) alone or in combination, and the mechanism of NO and photochrome on embryo germination of *Sorbus pohuashanensis* was explored through the analysis of embryo phenotype, endogenous hormones, enzymes related to NO synthesis and active oxygen metabolism system. In addition, real-time quantitative PCR was used to determine the transcription levels of key genes in the process of seed germination, and the key genes in the regulation of embryo germination by their interaction were mined, which provided scientific basis for elaborating the biological mechanism and molecular regulation mechanism of the interaction between NO and photochrome in the process of embryo germination, and helped to further understand the signal reception and transduction mechanism of plants to the environment.

S-nitrosylation of proteins during stress-induced autophagy in wheat *Triticum aestivum*

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Abstract

Autophagy is a catabolic process, which is involved in growth, development and stress responses of plants, contributes to the programmed death of individual cells and the survival of the whole organism. Reactive nitrogen species are signaling molecules implicated in autophagy in eukaryotic cells. Post-translational modification (PTM) of proteins *via* S-nitrosylation is a key mechanism of NO-mediated signaling. In animal cells, this PTM can switch the activity of signaling proteins that trigger autophagy. In plants, however, information on the role of protein S-nitrosylation in autophagy is extremely scarce. The aim of current work was to analyze S-nitrosylation of proteins during stress-induced autophagy in the wheat roots. The results of the current work indicate that in plant cells autophagy is characterized not only by the changes in the expression level of *ATG* genes and proteins, but also accompanied by an increase in the level of S-nitrosylated proteins with NO-mediated PTM. Therefore, S-nitrosylation of proteins, which are involved in the formation of autophagosomes and autophagic signaling cascades, is one of the fine mechanisms regulating autophagy in plants.

Role of nitric oxide on trans-resveratrol production in grapevine cells elicited with cyclodextrins and methyl jasmonate

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Abstract

Grapevine cell cultures are characterized by their ability to provide a sustainable source of natural bioactive compounds, among which is included *trans*-resveratrol (tR). The most effective strategy to improve tR production in grapevine cell cultures involves the use of elicitors that induce its biosynthesis or the activation of transcription factors, thus increasing the expression of genes involved in the tR biosynthetic pathway. Furthermore, the molecular mechanisms triggered in the grapevine cells upon recognition of the elicitor, leading to the production of this specialized metabolite, include the mobilization of signaling molecules such as reactive oxygen and nitrogen species. NO acts as a signaling molecule that plays a key role in many physiological processes such as xylogenesis, defense against pathogens, stomatal closure, and programmed cell death, among others. Belchí-Navarro et al. (2013) described that tR production could be dependent on NO production in grapevine cells induced by cyclodextrins (CD) and methyl jasmonate (MJ). However, little is known about the levels of NO produced in grapevine cells in the presence of CD and MJ. The aim of this work is to elucidate the role of NO in MJ- and CD-induced tR production in cell cultures of *Vitis vinifera* cv. Monastrell. On this basis, the present work focuses on studying the elicitation mechanism to check if it induces changes in NO homeostasis, and if this leads to alterations in tR production. To achieve these objectives, we have analyzed NO levels in grapevine cell cultures using confocal laser scanning microscope (CLSM) under four conditions: control (C), CD, MJ, and the combination of CD and MJ (CD+MJ). Intracellular NO levels were specifically detected using the fluorogenic dye 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM). Additionally, to determine the impact of NO on tR production, we have analyzed the effect of exogenous NO application using NO donors (such as diethylamine NONOate (DEAN) and S-Nitrosoglutathione (GSNO)), as well as the NO scavenger Carboxy-PTIO (CPTIO) on cell cultures elicited with CD+MJ. High-performance liquid chromatography (HPLC) was used to quantify tR at 24h after elicitation with CD+MJ alone or in combination with DEAN, GSNO and CPTIO. Cell viability was also evaluated by fluorescein diacetate and propidium iodide staining techniques in grapevine cells exposed to CD+MJ, DEAN, GSNO and CPTIO. The results showed an involvement of NO in tR production. CLSM studies revealed an early NO burst induced in CD-treated cells, with a fourfold increase in CDMJ-treated cells within the first hour and a half after elicitation, followed by a decreased in NO levels to basal values at 24h from elicitation. Furthermore, tR production was doubled in CD+MJ-elicited cells in combination with NO donors, and significantly decreased in CPTIO-treated cells. Viability studies showed that the addition of CD+MJ, DEAN, GSNO or CPTIO to the culture medium induced a small reduction of approximately 30% in cell viability after 24 h. The results obtained in this study confirm the involvement of NO in CD-induced tR production, alone or in combination with MJ, in grapevine cell cultures. These data allow us to improve our knowledge about the elicitation mechanism of grapevine cell cultures treated with CD and MJ, in particular the changes that these cells undergo when NO levels increase due to the presence of stress factors.

Low abundant myrosinases S-nitrosation suggest myrosinases involvement in nitic oxide signalling in *Brassica juncea*.

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Abstract

Nitric oxide, an important gaseous signalling molecule. It regulates every aspect of biological function in plants from cell proliferation, organ development, response to stimulus, flowering, ripening, and senescence in plants. NO's regulatory machinery operates mainly via S-nitrosation, a post-translational modification of target proteins. Captivating the role of NO in different biotic and abiotic stress could help in manipulating crops to be tolerant/resistant against stress conditions. *Brassica juncea*, Indian mustard an important oilseed and vegetable crop suffers from cold stress leading to yield loss. Exploration of cold stress (4°C) responsive targets identified myrosinases to be S-nitrosated. The glucosinolate-myrosinase system is activated after tissue damage leading to hydrolysis of glucosinolates by myrosinases producing isothiocyanates, nitriles, thiocyanates, and epithionitriles with isothiocyanates being the most bioactive and abundant form. Myrosinases are localized in the vacuoles of myrosin cells, guard cells, parenchyma cells of phloem and mesophyll tissues. Interestingly, myrosinases were identified in the nucleus, apoplast and cuticle of *B. juncea*. Along with the vacuolar form myrosinases in apoplast and nucleus were differentially S-nitrosated during cold stress. To decipher the role of NO on the glucosinolate-myrosinase system, myrosinases were purified from seeds and seedlings, apoplast and nucleus of seedlings. Multiple isozymes of myrosinases (8-23) were purified for the first time using a combination of anion exchange, hydrophobic interaction, and affinity chromatography. These are heterodimers of 62 & 63 kDa with pI 5.3-6.7 and constituted the most abundant form. Surprisingly, these heterodimers were not S-nitrosated. Further investigation of low abundant myrosinases (65, 70 & 75 kDa) that exist as dimers, trimers, tetramers, and complex forms which were present along with the purified heterodimers in seeds and seedlings showed 65 and 70 kDa sub-units to be S-nitrosated. Reversible regulation of these myrosinases by NO donor suggested role in NO signalling. The diversity of genes and protein isoforms is a huge challenge in determining whether the S-nitrosated forms are part of dimers, trimers, tetramers, or complex form and how S-nitrosation affect the tertiary and quaternary structure of proteins. However, S-nitrosated myrosinases in apoplast (65 kDa) exist as protein complex along with myrosinase binding proteins (18/20/22 kDa) while in nucleus it exists as trimers of 65 kDa. Deformation of cuticle and cell wall architecture in knock-down mutants of *Arabidopsis thaliana*, presence of myrosinases in apoplast and cuticle and differential S-nitrosation of specific low abundant myrosinases suggest myrosinases intersecting the biotic and abiotic stress signalling leading to stress resistance or tolerance. Understanding this signalling pathway would be of great significance in crop manipulation against stresses.

PHYTOGLOBIN - Boon or Bane during *Botrytis* fungus infection in Arabidopsis and Tomato

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Phytoglobin (*PGB*) is proteinaceous enzyme which regulates Nitric oxide homeostasis in plants. Nitric oxide is a Reactive Nitrogen Species which activates defense signalling pathway during stress condition. In presence of broad spectrum necrotrophic fungus such as *Botrytis cinerea,* pathogenicity is mainly due to various strategies develop by the fungus to infect and colonize over the host surface. In our studies, we check the Phytoglobins regulated NO mediated defense signaling in plants in presence of *Botrytis*. In our observation we elucidate that overexpression (OE) of *PGB* with intercede NO signaling, leads to susceptibility whereas *PGB* antisense (AS) leads to higher tolerance against *Botrytis* in both Arabidopsis (PGB) and Tomato (*nsHb*). PGB OE (*nsHb* OE) lines shows sensitivity against the pathogen by prompt appearance of necrotic symptoms, elevated ROS levels as compared to AS lines. Interestingly, we found that NO regulates elevated levels of Ethylene/Jasmonic acid mediated defense (*PAL1*, *ERF1*, *ASC2*, *ACS6*, *MYC2*) genes in AS line on the contrary in the OE lines Salicylic mediated (*PR1*, *PR2*, *PR5* and *NPR1*) genes were upregulated. hence, from our results we conclude that reduced NO levels leads susceptibility against *Botrytis* and it can assist both SA and JA/ET mediated pathways are deferentially activated in OE (*nsHb* OE) and AS lines (*nsHb* RNAi). RNAi lines of tomato also shows higher modulation in levels of primary metabolite than OE lines in response to pathogen which ultimately responsible for higher tolerance against pathogen.

Leucine aminopeptidase (LAP) activity from sweet pepper fruits is modulated during ripening and by NO and reducing events

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Abstract

Leucine aminopeptidase (LAP) is an essential metalloenzyme that cleaves N-terminal leucine residues from proteins but also metabolizes dipeptides and tripeptides. LAPs play a fundamental role in cell protein turnover and participate in plant physiological processes such as defense mechanisms against biotic and abiotic stresses, although little is known about their involvement in fruit physiology. This study aims to identify and characterize genes encoding LAP in pepper (*Capsicum annuum* L.) fruits and to evaluate their role during the ripening and under a nitric oxide (NO) enriched environment. Using a data mining approach of the pepper plant genome and the fruit transcriptome (RNA-seq), two LAP genes, designated *CaLAP1* and *CaLAP2*, were identified. The time course expression analysis of these genes during different ripening stages showed that whereas *CaLAP1* decreased, *CaLAP2* was upregulated in ripe red fruits. However, after exogenous NO treatment of fruits, both genes were downregulated. On the other hand, it was shown that fruit ripening provoked an increase of the LAP activity about 80%. Additionally, *in vitro* assays of the LAP activity in the presence of different modulating compounds including peroxynitrite, NO donors, and reducing agents was carried out. Thus, peroxynitrite and reducing compounds provoked around 50% inhibition of the LAP activity in green immature fruits. To our knowledge, this is the first characterization of LAP in pepper fruits as well as of its regulation by diverse modulating compounds. Based on the capacity of LAP to metabolize dipeptides and tripeptides, it could be hypothesized that the LAP might be involved in the GSH recycling during the ripening process in an NO- and a reducing-modulating manner.

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The function of peroxynitrite in rice roots under waterlogging stress

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Abstract

Plants under waterlogging stress face anoxygenic conditions which reduce their metabolism and induce several adaptations. The formation of aerenchyma is of paramount importance for the survival of plants under waterlogging conditions. Though some studies have shown the involvement of ethylene in aerenchyma formation under waterlogging conditions, the implication of peroxynitrite ($ONOO^-$) in such a developmental process remains elusive. Here, we report an increase in aerenchyma formation in rice roots exposed to waterlogging conditions which (the number of aerenchyma cells and their size) was further enhanced in response to exogenous ethephon (a donor of ethylene) or SNP (a donor of nitric oxide) treatment. Application of epicatechin (a peroxynitrite scavenger) to waterlogged plants inhibited the aerenchyma formation, signifying that $ONOO^-$ might have a role in aerenchyma formation. Interestingly, epicatechin and ethephon co-treated waterlogged plants were unable to form aerenchyma, indicating the necessity of $ONOO^-$ in ethylene-mediated aerenchyma formation under waterlogging conditions. Taken together, our results highlight the role of $ONOO^-$ in ethylene-mediated aerenchyma formation in rice, and could be used in the future to develop waterlogging stress-tolerant varieties of rice.

Keywords: Cell death; Crop yield; Epicatechin; Ethephon; Ethylene; Nitric oxide; Stress tolerance

Mechanisms derived from titanium dioxide nanoparticles and nitric oxide interaction to alleviate salinity stress in wheat seedlings

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Abstract

Modern agriculture faces a serious challenge from salinity which inhibits and impairs crop growth and productivity. A conceivable strategy to improve the crop performance, growth and productivity in such challenging circumstances is the use of nanotechnology. Titanium dioxide nanoparticles (TiO_2NPs) have been reported to impose positive impact on plant growth in terms of morphological, physiological, and biochemical characteristics and tolerance against different abiotic stressors. Therefore, this study was carried out to evaluate the alleviatory potential TiO_2NPs (100µM) against salinity stress (150mM, sodium chloride, NaCl) in wheat (*Triticum aestivum L.*) seedlings and to test the involvement of nitric oxide in TiO_2 NPs-induced changes. A significant decline was observed in morphological parameters including length and fresh-dry biomass of root and shoot of wheat seedlings upon exposure to salt stress with respect to control. However, a prominent increase was observed in these parameters in TiO_2 NPs-treated wheat seedlings in comparison with control. The combined application of TiO_2 NPs and NaCl to wheat seedlings showed improvement in these parameters as compared with NaCl-treated wheat seedlings and showed decrease in uptake of Na+ ions. Similar pattern was observed in case of chlorophyll florescence (Fv/Fm, qP and NPQ), protein and carotenoids content under NaCl alone, TiO_2 NPs alone and TiO_2 NPs + NaCl treatments respectively. The oxidative stress markers including superoxide radical (SOR), hydrogen peroxide (H2O2) and malondialdehyde (MDA), and antioxidant enzymes including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) were also analyzed under the salt stress alone and together with TiO_2 NPs. Further, N(ω)-nitro-L-arginine methyl ester (L-NAME, inhibitors of nitric oxide synthase (NOS) activity) and sodium nitroprusside (SNP, NO donor) were used to examine the involvement of NO, as a signaling molecule, in TiO_2 NPs-mediated mitigation of salt stress in wheat seedlings. The results depicted that TiO_2 NPs alone are capable to minimize salt stress-induced negative impact on wheat seedlings through interaction with endogenous NO, but there is no requirement of exogenous NO supplementation.

Keywords: Salt stress, TiO_2 nanoparticles, Oxidative stress, Antioxidative defense, Wheat

The Essential Role of Nitric Oxide in Zinc-induced cadmium stress resistance in rice roots

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Abstract

The global environmental issue of metal toxicity has fortified notably in recent decades primarily as a result of expeditious technological developments, industrialization, and anthropogenic activities of the ceaselessly mushrooming world population, and it ultimately jeopardizes ~~the~~ food security. Cadmium (Cd) is one such non-essential metal that ~~which~~ displays toxicity in plants even at relatively low concentrations. Despite being an essential nutrient, zinc (Zn) also engenders phytotoxicity at high concentrations. This research was envisaged to delineate the interaction between Zn and Cd in rice (*Oryza sativa* L.) roots at their toxic doses (100 µM each) and to investigate the involvement of nitric oxide (NO) in the underlying signaling processes. The results interestingly indicated a beneficial impact of Zn against Cd stress even at high concentrations. Cd alone treatment reduced plant biomass, cell viability, and photosynthetic parameters and caused oxidative stress due to inhibition of the ascorbate-glutathione cycle. Rice roots treated with Zn alone also displayed similar toxic effects, however, combined application of Zn with Cd exhibited improvement in these parameters. application also fine-tuned the ascorbate-glutathione cycle which in turn lessened the Cd-induced oxidative stress in rice roots. Further, the application of a higher concentration of Zn against Cd significantly reduced Cd uptake in rice roots while increasing Zn accumulation. In addition, the expression of the *PLANT CADMIUM RESISTANCE1* (*OsPCR1*) transporter gene was observed to be higher in rice roots exposed to Cd only than in the case of roots treated with Zn alone hinting at its role in Cd stress tolerance in rice roots. Moreover, Zn minimizes Cd uptake in Zn+Cd treated rice roots as the expression of *OsPCR1* in these roots was found to be higher than that observed in roots treated with Cd or Zn alone. Further, N^G-nitro L-arginine methyl ester (L-NAME, an inhibitor of nitric oxide synthase-like activity) application prominently suppressed the beneficial impacts of Zn against Cd stress, which may be due to the inhibition of NO biosynthesis. Whereas, the supplementation of sodium nitroprusside (SNP, an NO donor) significantly reversed the effect of L-NAME suggesting that NO signaling is essential for Zn-mediated cross-tolerance against Cd stress via modulation of Cd uptake, expression of *OsPCR1* transporter genes, and ROS homeostasis. We further aim to utilize the results of this study to develop new varieties of rice through genetic modifications which will be of great significance for maintaining crop productivity in Cd-contaminated areas throughout the world.

Keywords: Zinc, Cadmium, Nitric oxide, Ascorbate-glutathione cycle; Cell viability; Cross tolerance; Redox buffers

Nitric Oxide Regulates Antioxidant Machinery, Enhances PSII Efficiency, Improves Chloroplast Ultrastructure, and Promotes Artemisinin Biosynthesis in *Artemisia annua* Under Cadmium Stress

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Abstract

Soil contamination by heavy metals presents a significant environmental challenge, with existing remediation technologies facing practical obstacles in the field. As a result, there is a need to explore alternative solutions to minimize harm to plants. This study focuses on investigating the potential of nitric oxide (NO) in alleviating cadmium (Cd) toxicity in *A. annua* plants. While NO is known for its crucial role in plant growth and development, information on its contribution to reducing abiotic stress in plants remains limited. During this study, *A. annua* plants were subjected to 20 and 40 mg/kg soil Cd concentrations, followed by the exogenous sodium nitroprusside (SNP) supplementation, a NO donor, at a concentration of 200 µM. The results indicate that SNP treatment enhances plant growth, photosynthesis, chlorophyll fluorescence, pigment content, and artemisinin production. Simultaneously, it reduces Cd accumulation and enhances membrane stability in *A. annua* under Cd stress. The findings demonstrate that NO effectively counteracts Cd-induced damage in *A. annua* by modulating the antioxidant system, maintaining redox homeostasis, and improving photosynthetic performance, as indicated by various fluorescence parameters such as *Fv/Fm*, *ΦPSII*, *qP,* and *ETR*. The supplementation of SNP significantly improves chloroplast ultrastructure, stomatal behavior, and attributes related to glandular secretory trichomes, resulting in a 14.11 % increase in artemisinin production in plants exposed to Cd stress at 20 mg/kg. These results underscore the potential utility of NO in mediating the repair of Cd-induced damage in *A. annua*, suggesting a critical role in plant signaling networks and enhancing plant adaptability to Cd stress. The implications of these findings are crucial for developing novel strategies to mitigate the adverse effects of environmental contaminants on plant health and, ultimately, the ecosystem.

Exogenous application of nitric oxide alleviates arsenic-induced toxicity and enhances antioxidant defense mechanisms in *Ocimum basilicum*

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Abstract

Arsenic (As), a metalloid, significantly inhibits plant growth and presents health hazards for humans when transmitted through the food chain due to its toxicity. The beneficial effects of nitric oxide (NO) on enhancing plant resilience against various stresses, including metal toxicity, are well-documented, still there remains limited understanding regarding its involvement in conferring As tolerance in hyperaccumulator plants. This study explored the potential and protective effects of exogenously applied NO, in the form of sodium nitroprusside (SNP), against As toxicity in *Ocimum basilicum*. Exposure to toxic concentrations of As increases NO and causes damage to the cell membrane. This damage is evidenced by the elevated levels of hydrogen peroxide and malondialdehyde concentrations, ultimately reducing plant growth. Nevertheless, the supplementation of SNP enhances growth and mitigates As induced oxidative stress by boosting the activity of superoxide dismutase, ascorbate peroxidase, glutathione reductase, glutathione S-transferase, as well as increasing glutathione, proline, and thiol concentrations. The outcome confirms the advantageous role of NO in enhancing tolerance to As stress. This study suggests that the exogenous application of NO imparts tolerance to *O. basilicum* against As toxicity and exerts a beneficial ameliorating effect in alleviating As-induced stress.

Key Words: Arsenic, Nitric oxide, *Ocimum basilicum,* Stress, Toxicity.

Nitric oxide boosts photosynthetic nitrogen and sulfur-use efficiency and the ascorbate-glutathione cycle to mitigate high-temperature stress in rice plants

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Abstract

The study investigated the effect of the application of 100 µM sodium nitroprusside (SNP, NO donor) on the performance and high-temperature stress mitigation capacity in rice (*Oryza sativa* L. cv. Taipie-309). Under high temperatures (40°C for 6 hours), plants experienced reduced photosynthesis, nitrogen and sulfur-use efficiency, and an increase in oxidative stress markers, hydrogen peroxide (H_2O_2), and thiobarbituric acid reactive substances (TBARS). However, SNP treatment to plants in the presence of high-temperature stress reduced levels of H_2O_2 and TBARS and increased the levels of proline and activity of the ascorbate-glutathione cycle, collectively enhancing the tolerance of Taipie-309 to high temperature. The application of SNP also maintained higher levels of photosynthetic nitrogen use efficiency (p-NUE) and photosynthetic sulfur use efficiency (p-SUE), along with the activity of Rubisco and gas exchange parameters. Additionally, it boosted the production of reduced glutathione (GSH), aiding in oxidative stress management. The use of 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide (cPTIO, NO scavenger) confirmed the crucial role of NO in these processes. The study suggests that the application of NO can help rice plants better withstand high-temperature stress by reducing oxidative damage and improving photosynthetic efficiency and growth.

Keywords: Sodium nitroprusside, antioxidants, photosynthesis, heat stress, rice

Effect of nitric oxide-releasing nanoparticles on the growth and physiology of *Cecropia pachystachya* Trécul seedlings subjected to moderate water deficit

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Abstract

Nitric oxide (NO) is a signaling molecule related to the protection of plants against water deficit (WD). Its application is hindered due to its unstable chemical nature. The nanoencapsulation of NO donor molecules constitutes a strategy to promote gradual NO release, increasing its effectiveness. Thus, this study aimed to evaluate and compare the effects of treatments with chitosan nanoparticles (NPs) containing the NO donor S-nitrosoglutathione (GSNO), GSNO in its free form, and NPs without NO, all at 50 µM, on *Cecropia pachystachya* Trécul seedlings subjected to moderate WD in a greenhouse. The seedlings in WD were treated with nanoformulations in the substrate three times throughout the experiment, with a ten-day interval between applications. In addition, there were two control treatments, which did not receive formulation: one with plants kept in WD and another with plants kept at field capacity. The stomatal conductance (g_s) of the plants was measured daily. After thirty days of WD, the stem water potential, the relative water content (RWC) of the leaves, the photosynthetic rate, and the biomass of the plants were measured. The treatment with NPs containing the GSNO donor led to an increase in the g_s of the plants in WD, without negatively affecting the leaf RWC. The increase in g_s was observed during the morning and early afternoon. In addition, a positive effect on the water potential, photosynthetic rate, and root and leaf biomass. In general, the results show that the application of nanoencapsulated GSNO at 50 µM can increase the tolerance of *C. pachystachya* to moderate WD. The results indicate the potential of applying NO-releasing NPs to obtain seedlings of tree species that are more tolerant to WD, with a view to using them in reforestation programs.

A discrete strategy of AOX during hypoxia to increase nitric oxide and ATP synthesis

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Abstract

Alternative oxidase (AOX) is an integral part of the mitochondrial electron transport chain (ETC) which is known to play multifaceted roles in plants including regulation of nitric oxide in mitochondria. Here we assessed the roles of AOX by imposing stress under normoxic and hypoxic conditions using AOX over expressing (AOX OE) and anti-sense (AOX AS) transgenic Arabidopsis seedling roots. Under normoxic conditions stress was induced with the defence elicitor flagellin (flg22). AOX OE reduced NO production whilst this was increased in AOX AS, consistent with the classical role of AOX as a NO scavenger even when exposed to biotic stress. AOX–AS also exhibited an increase in superoxide and therefore peroxynitrite and tyrosine nitration. In contrast, during hypoxia AOX was demonstrated to generate NO. Thus, the NO produced during hypoxia, was enhanced in AOX OE and suppressed in AOX AS. Additionally, treatment of WT or AOX OE with the AOX inhibitor SHAM inhibited hypoxic NO production. The enhanced levels of NO correlated with expression of non-symbiotic haemoglobin, increased NR activity and ATP production. This suggests that hypoxic AOX production has a discrete role by feeding into the haemoglobin-NO cycle to drive energy efficiency under conditions of low oxygen tension.

S-nitrosoglutathione-loaded chitosan nanoparticles improve the physiological performance and reduce the mortality of neotropical tree seedlings after transplanting to the field

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Abstract

Environmental stresses have negative effects on the development and survival of tree seedlings after transplanting to the field, decreasing the success of reforestation initiatives. A technique that could be applied to silvicultural practices is the supplementation with nitric oxide (NO) donors, that could be efficiently carried by polymeric nanoparticles. In the present study, we evaluated the effects of the application of free and nanoencapsulated S-nitrosoglutathione (GSNO) on the physiology, initial growth and mortality of tree seedlings after transplantation to a reforestation field. Nursery-grown seedlings of two neotropical Fabaceae species were used: *Hymenaea courbaril* L. (jatobá-da-mata) and *Amburana cearensis* (Allemão) A.C.Sm (amburana). The seedlings were treated with the following formulations in the substrate one day before transplanting: water (control), free GSNO 200 μM (GSNO), chitosan/tripolyphosphate nanoparticles loaded with GSNO 200 μM (GSNO-CS-NPs), and chitosan/tripolyphosphate nanoparticles without the NO donor. After the transplanting to field, the seedlings showed transient decreases in stomatal conductance and photosystem II activity over time. GSNO-CS-NPs was the treatment that induced the best physiological responses of the seedlings of both species, as indicated by the highest values of CO_2 assimilation, stomatal conductance, and photosystem II activity. All formulations decreased the mortality of the seedlings, although the growth parameters were not affected. Again, the effects of GSNO-CS-NP treatment stood out in comparison with the other formulations, as it induced the sharpest decreases in the mortality of *H. courbaril* (from 50 to 0%) and *A. cearensis* seedlings (from 62.5 to 12.5%). Overall, these results suggest that the application of GSNO-CS-NPs is a promising strategy to increase the resistance of tree seedlings to post-transplanting stress with a consequent mortality reduction, thereby improving the success of reforestation programs.

Keywords: *Amburana cearensis*, *Hymenaea courbaril*, nanotechnology, nitric oxide, photosynthesis, reforestation.

Chitosan-GSNO nanoparticles: a positive modulator of drought stress tolerance in soybean

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Abstract

Chitosan biopolymer is an emerging non-toxic and biodegradable plant elicitor or bio-stimulant. Chitosan nanoparticles (CSNPs) have been used for the enhancement of plant growth and development. On the other hand, NO is an important signaling molecule that regulates several aspects of plant physiology under normal and stress conditions. Here we report the synthesis, characterization, and use of chitosan-GSNO nanoparticles for improving drought stress tolerance in soybean. The CSGSNONPs released NO gas for a significantly longer period and at a much lower rate as compared to free GSNO indicating that incorporation of GSNO in CSNPs can protect the NO-donor from rapid decomposition and ensure optimal NO release. CS-GSNONPs improved drought tolerance in soybean plants reflected by a significant increase in plant height, biomass, root length, root volume, root surface area, number of root tips, forks, and nodules. Further analyses indicated significantly lower electrolyte leakage, higher proline content, higher catalase, and ascorbate peroxidase activity, and reduction in MDA and H_2O_2 contents after treatment with 50 µM CS-GSNONPs under drought stress conditions. Quantitative real-time PCR analysis indicated that CS-GSNONPs protected against drought-induced stress by regulating the expression of drought stress-related marker genes such as GmDREB1a, GmP5CS, GmDEFENSIN, and NO-related genes GmGSNOR1 and GmNOX1. This study highlights the potential of nano-technology-based delivery systems for nitric oxide donors to improve plant growth, and development and protect against stresses.

Unraveling the interplay between Melatonin and Nitric Oxide during the PSII/PSI gene dynamics under single and combined abiotic stresses

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Abstract

In the intricate world of plant physiology, the regulation of photosynthesis stands as one of nature's most fascinating orchestrations. Central to this symphony are the delicate interactions between various signaling molecules, among which melatonin and nitric oxide (NO) emerge as key conductors. Melatonin governs numerous physiological processes in plants, ranging from growth regulation to stress response. In parallel, nitric oxide operates as a ubiquitous signaling entity, modulating diverse pathways across the plant kingdom. However, the interaction between these two molecules have garnered increasing attention, particularly in the context of plant cell signaling mechanisms under abiotic stress. In this work, we show how tomato plants defective in GSNOR protein are more sensitive to single or combined stresses, presenting a significant reduction of the PSII efficiency and ETR, due to and over-accumulation of H_2O_2 and the downregulation of RbcS and some of the PSII-related genes (PsbC, PsbB, PsbP and PsbQ) and PSI-related genes (PsaA and PsaB). On the other hand, overexpressing GSNOR lines, showed an upregulation of these genes under single or combined salinity and heat stress. More importantly, exogenous application of melatonin significantly improved PSII and PSI gene expression, leading to a better performance of PSII and ETR, with the concomitant ROS detoxification under abiotic stress. Here we demonstrate an interplay between melatonin and nitric oxide in the regulation of the photosynthetic machinery. This interaction not only underscores the interconnectedness of diverse signaling pathways but also hints at the potential for harnessing these mechanisms to optimize photosynthetic efficiency and enhance crop productivity in agricultural settings.

9TH PLANT NITRIC OXIDE INTERNATIONAL MEETING

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KEY NOTE TALK

A new oxidative pathway of nitric oxide production from oximes in plants

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Abstract

Nitric Oxide (NO) is an essential reactive oxygen species and a signal molecule in plants and animals. In humans, NO is synthesized by oxidation of the amino acid arginine via nitric oxide synthase (NOS), and regulates crucial aspects such as cardiovascular health or inflammatory processes. Although several works have proposed the occurrence of oxidative NO production in land plants, the NOS enzyme is not present and only reductive routes for NO production, such as the nitrate reductase pathway, were known in land plants. However, plants grown axenically with ammonium as a sole source of N exhibit contents of nitrite (NO_2^-) and nitrate (NO_3^-), evidencing the existence of a metabolic pathway for oxidative production of NO.

We hypothesised that oximes, such as the indole-3-acetaldoxime (IAOx), a precursor to the auxin indole-3-acetic acid, are intermediate oxidation products in the oxidative pathways to NO from aminoacids. Thereby, we have synthetized several oximes including IAOx, we have detected the production of NO from IAOx and the other oximes in vitro only when catalysed by peroxidase enzyme (POD) using both DAF-FM fluorescence and chemiluminescence as detection methods. Flavins stimulated the reaction, while superoxide dismutase inhibited it evidencing a role for the superoxide anion in the reaction. Interestingly, mouse NO synthase can also use IAOx to produce NO, at a lower rate than POD. We also studied the possible reaction mechanism by Density-Functional Theory (DFT) calculations. We have been able to describe a full mechanism for POD-dependent NO production from oxime, consistent with the experimental data and supported by the free energy calculations.

We also found that the addition of IAOx to extracts from *Medicago truncatula* increased the *in vitro* production of NO, while *in vivo* supplementation of IAOx and other oximes increased

the number of lateral roots, in the same way as shown for NO donors, and a >10-fold increase in IAOx dehydratase expression. Also, *in vivo* supplementation of IAOx increased the NO production in *Arabidopsis thaliana* wild-type plants, while *prx33-34* mutant plants, defective for POD33-34, had reduced production. Our data show that the release of NO by IAOx, together with its auxinic effect, explains the so-called *superroot* phenotype. Altogether, we demonstrate that plants produce NO utilising molecules such as oximes, POD, and flavins, which are widely distributed in the plant kingdom, thus introducing a long-awaited oxidative pathway to NO production in plants. This knowledge has essential implications to understand signalling in biological systems.

The oximes are a new group of adjustable NO donors with properties to be studied in clinical application in the treatment in cardiovascular diseases and other. The reaction described uses superoxide radical, which is thus scavenged. In contrast to current NO donor that produce NO as soon as they are in solution, oximes are stable in solution, and they will exert its function only in the presence of a widespread enzyme, the POD. Furthermore, very different types of molecules can be attached to oximes to perform or to modulate specialized functions, which foresees a plethora of clinical uses.

Nitric oxide signaling and its role in mitochondrial biogenesis in plants

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Abstract

Nitric oxide is free radicle signal molecule that play role in plant growth, development and stress responses. NO is generated by oxidative and reductive pathways. The reductive pathway is mainly operated by nitrate reductase and mitochondrial electron transport chain. The site of nitric oxide (NO) production in mitochondrial cytochrome *c* oxidase and the role of NO in mitochondrial biogenesis are not known in plants. By imposing osmotic stress and recovery process on *Arabidopsis* seedlings, we investigated the site of NO production and its role in mitochondrial biogenesis. We found that imposing osmotic stress leads to reduced growth and mitochondrial number while increasing NO production. During the recovery phase the mitochondrial number drastically increased and this increase was higher in WT and the high NO-producing Phytoglobin silencing line in comparison to the NO-deficient nitrate reductase double mutant (*nia1/nia2*). Application of nitrite as substate accelerated NO production and mitochondrial number in the *nia1/nia2* mutant. Osmotic stress induced *COX6b-3* and *COA6-L* genes encoding important subunits of COX. Strikingly the mutants *cox6b-3* and *coa6-l* were impaired both in NO production and mitochondrial number during stress to recovery suggesting the involvement of these subunits in nitrite-dependent NO production. Transcripts encoding the mitochondrial protein import machinery (MPIM) displayed reduced expression in *cox6b-3* and *coa6-l* mutants. Interaction studies revealed *COX6b-3* and *COA6-L* interacted with the VQ27 motif-containing protein in the presence of NO donor SNP and nitrite. The *vq27* mutant was impaired in mitochondrial biogenesis and recovery from stress. Our results suggest the involvement of COX derived NO in mitochondrial biogenesis

Identification and functional characterization of S-nitrosated proteins during salt stress in *Klebsormidium nitens*

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Abstract

Nitric oxide (NO) is a major ubiquitous component of cell signaling triggering part of its effets through protein S-nitrosation. This post-translational modification impacts protein activities, their subcellular localization and ability to form protein complexes. Therefore, the characterization of S-nitrosated proteins is of major interest in elucidating NO functions. Previously, we provided evidences that land plants lack NO Synthase (NOS), the main NO-synthesizing enzyme in metazoans (Jeandroz et al., 2016). In contrast, few algal species possess NOS isoforms, thus questioning their biochemistry and roles in these organisms. We focused on the identification of S-nitrosated proteins in Klebsormidium nitens, a freshwater algal species possessing two NOS isoforms and considered as a model to study plants adaptation to land (Chatelain et al., 2022). 43 candidate proteins with significantly higher levels of S-nitrosation under salt stress condition were identified. Orthology analysis was performed against Arabidopsis thaliana to determine the potential function of these proteins. Among them, we selected Inositol Polyphosphate Multikinase 2 (IPK2), which is potentially involved in cell signaling and stress response. KnIPK2 is able to phosphorylate as well as, more surprisingly, dephosphorylate certain inositol phosphates (InsP). Confirming our in silico predictions, in vitro S-nitrosation of KnIPK2 by exogenous NO inhibits its activity, suggesting the involvement of NO in the regulation of InsP metabolism. This modulation could play a role in cell signaling, as some InsP are major intracellular messengers interacting with calcium signaling. This project will provide a better understanding of NO functions in an algae possessing a NOS.

Role of Nitric Oxide in Stress-Induced Somatic Embryogenesis in Trees

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Abstract

Osmotic stress promotes somatic embryogenesis of Fraxinus mandshurica, which leads to accumulation of reactive oxygen species. The substantial osmotic stress was essential for Manchurian ash somatic cells to obtain embryogenic competence. The explant cells displayed hallmarks of programmed cell death (PCD), chromatin condensation, and DNA fragmentation to oligonucleotides during somatic embryogenesis. The PCD occurred during tissue browning and death of some explant cells during somatic embryogenesis. The hydrogen peroxide (H2O2) and nitric oxide (NO) were important endogenous regulator factors influencing the PCD of somatic embryogenesis in F. mandshurica synergistically. H2O2 and NO could be used as the cells indicative signal of PCD during the somatic embryogenesis. Exogenous H2O2 and NO regulated of intracellular H2O2 metabolism and NO synthesis of somatic embryogenesis in F. mandshurica, and changed the activity of enzymes of superoxide dismutase, peroxidase and polyphenol oxidase related to the intracellular H2O2 metabolism, and as well as the activity of Nitric oxide synthase (NOS) and nitrate reductase (NR) related to NO enzymes, and finally changed the content of intracellular H2O2 and NO. As the important endogenous signal factors, H2O2 and NO induced the PCD in F.mandshurica synergistically. NO maybe located in the upstream of H2O2 signaling pathways. In order to maintain the balance of NO/H2O2 in F. mandshurica and relieve the stress of low oxygen or oxygen, both the enzymes of antioxidant and NO synthase and content of H2O2 should be regulated. There were two ways to compound the intracellular NO in somatic embryogenesis of F. mandshurica through NOS and NR. But the two ways varied in the times of each leading role and and functions in somatic embryogenesis. The NOS and NR played an important role in the early time of culture and somatic transformed to embryonic stage, respectively.

Keywords: Nitric oxide, Osmotic stress, Somatic embryogenesis, Reactive oxygen species, Programmed cell death

An update to the model of "nitrosative door" in seed biology

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Abstract

Seeds constitute mobile plant organs enabling the extension of a given species (the biodiversity maintenance). The physiological fate of seeds depends on two opposing physiological conditions: dormancy state and germination process. Seed dormancy enables survival of unfavorable external conditions due to the very low metabolic activity. Contrary, seed germination is a multi-pathway process leading to the embryo activation. Both, seed dormancy and germination are under the control of many regulators, including hormones and reactive compounds. Low metabolic activity during dormancy establishment is achieved by water loss and cytoplasmic glassy state formation. Despite these changes and low enzymatic activity, other small compounds may react with basic cell molecules. Among them, nitric oxide (NO), known as a gasotransmitter is considered to be implicated in seed vigour modulation. Reactive nitrogen species (RNS), the derivatives of NO, are pivotal compounds of dual action in living organisms. This double, molecular function of NO depends on concentration, described by "nitrosative door" model (Krasuska and Gniazdowska 2012). The concept was based on the "oxidative window" (Bailly et al. 2008) linked to the reactive oxygen species (ROS) action in seeds. Similarly to ROS, NO low concentration is not efficient to "wake up" seeds. Germination starts when NO reaches "nitrosative door" level. Too high NO concentration usually is harmful or even lethal to seeds (Ciacka et al. 2020). However, this description refers to non-pathological conditions. Seed ageing, especially accelerated seed deterioration is a pathophysiological process not entirely matched to the model. Seed ageing is accompanied by the lowering of NO level (Dębska et al. 2013). Thus, the application of NO may restore vigour of aged seeds. The aim of this presentation is to update the "nitrosative door" conception by inclusion of our recent observation. We assume that NO action in aged seeds is mostly linked to the modulation of ROS level (the maintenance of ROS homeostasis), lowering of transcripts of the central regulators of lifespan and ageing (e.g. TOR - the target of rapamycin) (Ciacka et al. 2022) and regulation of proteolytic activity (e.g. preventing of toxic protein aggregates accumulation).

Nitric oxide is complemented by reactive oxygen species and calcium during stomatal closure and plant defence against abiotic and biotic stress

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Abstract

Whenever plants are exposed to stress, the guard cells sense and respond through a series of steps that include the production of ROS and NO, followed by a rise in Ca^{2+} and the modulation of ion channels. These events promote the efflux of cations and anions from guard cells. As a result, guard cells lose turgor, leading to stomatal closure. Such closure by ABA is essential to plant adaptation to stress factors. We propose that the initial stomatal closure response triggers many defensive strategies to adapt to abiotic and biotic stress pathogens. We describe the follow-up of events limiting pathogen spread and emphasise stomata & its role in ensuring plants & its long-term adaptation against microbes. Using ABA as a typical stress signal, we highlight the importance of reactive oxygen species (ROS), nitric oxide (NO), and Ca^{2+} in guard cells as key signalling components during the ABA-mediated short-term plant defence reactions. Stomatal closure also has long-term consequences and can promote additional events in long-term adaptive measures, including gene expression, accumulation of compatible solutes to protect the cell, hypersensitive response (HR), and programmed cell death (PCD). In all these stress-adaptive events, the role of NO is complemented by ROS and Ca^{2+}. The restricted CO_2 supply to the mesophyll cells lowers the rate of photosynthesis and stimulates photorespiration and associated H_2O_2 production. The elevated levels of NO, along with H_2O_2, H_2S, and Ca^{2+}, can upregulate genes involved in HR, PR, and PCD to prevent the spread of pathogens within the leaf. These reactive molecules also promote the accumulation of antimicrobial secondary metabolites. Parallelly, reduced transpiration creates mineral deficiency and limits microbial growth. We suggest that stomatal closure is a trigger to set off long-term events involved in prolonged plant disease resistance. Stomatal guard cells are quite sensitive to environmental stress and are considered sound model systems for signal transduction studies. Further research on the ABA-induced stomatal closure mechanism can help us design strategies for plant/crop adaptations to stress.

NO and ROS promote hypocotyl cell growth under shade in plants

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Abstract

In the last 25 years since its discovery as a molecular signal in plants, nitric oxide (NO) has been associated to several plants responses. However, its role in synergism with reactive oxygen species (ROS) in the regulation of cell growth under environmental changes is not completely deciphered. Shade promotes the growth of the stem (hypocotyl) to facilitate plant access to light in crowded environments. Our results show that auxin produced in response to shade triggers the accumulation of ROS and NO in the hypocotyl, causing a more oxidative environment, which enhanced the promotion of polar cell growth in this organ. Under shade, NO bioactivity is efficiently transferred and stored within the cell through its co-valent attachment to specific reactive cysteine thiols of proteins, increasing S-nitrosylation. We proposed that a major role for the rapid, transcient and largely parallel increment of ROS and NO in the hypocotyl is to engage a specific set of NO-responsive proteins. We demonstrate the S-nitrosylation of central regulators of light signaling. Impairing these modifications reduced protein stability, its ability to interact with some partners and its biological activity under shade. Disabling this regulation also generated transversal asymmetries in hypocotyl growth, indicating poor coordination among different cells, which resulted in random bending and predictably low ability to compete with neighbours.

Nitric oxide-releasing polymeric nanoparticles in plants recent progress, perspective and challenges

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Abstract

Nitric oxide (NO) controls several important physiological roles in plants, ranging from plant defense against abiotic stress to postharvest fruit quality control. As a gaseous free radical, NO administration faces practical limitations. Although NO donors have partially overcome this issue, their direct application is still impaired by limited bioavailability and instability. Recently, nanotechnology offered a promising strategy to enhance targeted NO delivery to plants. In fact, nanomaterials allied to NO donors are effective to enhance tissue NO levels, increasing positive effects in plants. In this direction, our research group has been developing chitosan (a biodegradable and biocompatible biopolymer) containing NO donors (S-nitrosothiols, such as S-nitrosoglutathione and S-nitroso-mercaptosuccinic acid) and applying these nanoparticles in several plant species (such as sugarcane, soybean, maize, wheat, coffee, common beans, and neotropical tree species, among others). We also conducted tests utilizing various treatment methods, including seed priming, foliar spraying, substrate watering, fruit spraying and immersion, across a range of stress conditions such as drought, salinity, and metal contamination. Overall, we have observed an enhancement in the physiological performance of plants under stressful conditions such as enhancement of photosynthesis and chlorophyll content, increase in the activities of important antioxidant enzymes, decrease of reactive oxygen formation, and increase of S-nitrosothiol levels in plant tissues. Interestingly, by using a fluorescent marker, we tracked the nanoparticle uptake by plants. Taken together, NO-releasing polymeric nanoparticles can find important and diverse applications in plant science/agriculture to increase food production/preservation and to reforestation of degraded areas.

Unmasking the Hero: How the GSH-GSNO Module Defeats Iron Deficiency in Plants

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Abstract

Iron (Fe) is an essential micronutrient for plant growth and development. It is involved in a number of important processes, including photosynthesis, respiration, and nitrogen fixation. However, Fe deficiency is a common problem in many soils, particularly in calcareous soils. The importance of plant adaptation to Fe deficiency in soil is twofold. First, it allows plants to grow and develop normally even in soils that are low in iron. Second, it helps to ensure that humans have a reliable source of food. Many important food crops, such as wheat, rice, and soybeans, are sensitive to Fe deficiency. Without plant adaptation, these crops would not be able to grow in many parts of the world. Maintaining Fe homeostasis under Fe deficiency is crucial for plant survival. Here, we explored the glutathione (GSH)-mediated regulation of Fe homeostasis during Fe deficiency in Arabidopsis. The GSH-depleted mutants, *cad2-1* and *pad2-1*, displayed increased sensitivity to Fe deficiency with a lower expression of the vacuolar Fe exporters, *AtNRAMP*3 and *AtNRAMP*4, and the chloroplast Fe importer, *AtPIC*1. Moreover, the mutants accumulated higher Fe content in the vacuole and lower in the chloroplast compared with Col-0 under Fe limited condition. Further analysis revealed that the GSH-GSNO module induced transcription of these genes via the *S*-nitrosylated transcription factors, *At*bHLH29, *At*bHLH38 and *At*bHLH101. This finding has important implications for our understanding of how plants regulate Fe homeostasis, and could lead to the development of new strategies for improving Fe nutrition in plants.

Canavanine-induced decrease in NO synthesis alters cell cycle regulation

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Abstract

Canavanine (CAN), a non-proteinogenic amino acid, arginine analog synthesized by some legume species, serves as a multifaceted bioactive compound. Originally identified as a defense compound against herbivorous insects in seeds and young seedlings, CAN has gained attention for its cytotoxic properties and its specific inhibition of the inducible nitric oxide (NO) synthase activity in mammalian cells, rendering it a potential candidate for anti-cancer therapies (Staszek et al. 2017). Previous studies have demonstrated CAN's impact on tomato (*Solanum lycopersicum* L.) physiology, revealing its inhibitory effects on root growth, reduction of arginine-dependent nitric oxide production, and disturbance of reactive oxygen species (ROS) and reactive nitrogen species (RNS) metabolism (Staszek et al., 2019; Staszek and Gniazdowska, 2020). In this study, tomato seedlings were exposed to CAN at concentrations of 10 and 50 µM for 24 or 72 h to investigate the molecular mechanisms underlying root growth inhibition.Flow cytometry analysis of cell cycle progression unveiled that exposure to 50 µM CAN resulted in a significant decrease in the number of cells in the S phase at the root growth tip, accompanied by an increase in cells in the G2M phase. Remarkably, CAN induced endocycles in cells the root elongation zone, further substantiating its impact on cell cycle dynamics.Transcriptomic analysis of genes associated with cell cycle regulation provided insights into the molecular basis of CAN-mediated modifications. Higher concentrations of CAN led to the overexpression of genes encoding CycA3.2 and CycD1.1 cyclins, subsequently altering the expression profiles of cyclin-dependent kinases CDKA and CDKB. Furthermore, induction of endoreduplication was confirmed through the upregulation of genes encoding Kip-related proteins (KRP), known as cyclin-dependent kinase inhibitors. Western blot analysis revealed dynamic changes in the quantity of CycB, as after short-term treatment with 50 µM CAN, its level was higher than in the control. However, after 72 hours, this trend reversed.In summary, the inhibitory effect of CAN on NO synthesis on root growth can be linked to disruptions in cell divisions within the root apical meristem. The modulation of cell cycle progression, coupled with alterations in the expression of key genes involved in cell cycle regulation, provides insights into molecular mechanisms of CAN mode of action in plants and probably animal cells.

Molecular regulation of bZIP67 by *S*-nitrosylation impacts on fatty acid storage during seed development

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Abstract

Seed development can be divided into two main stages, namely embryo morphogenesis and seed maturation. The latter is characterized by the acquisition of dormancy, desiccation tolerance and accumulation of storage compounds, mainly triacylglycerols. This step is essential for seed germination and seedling establishment, until photosynthesis makes the plant autotroph. Regulation of fatty acid synthesis and metabolism involves a complex network that implies external signals, hormone signaling events, and transcription factors. In this context, bZIP67 is a bZIP transcription factor (TF) previously described to regulate lipid accumulation during seed development (Mendes et al., 2013). Our results highlight that bZIP67 is posttranslationally modified by nitric oxide (NO) through *S*-nitrosylation of Cys residues, and reversed by PRXIIE redoxin activity, finally controlling the correct seed fatty acid accumulation (Sánchez-Vicente et al., 2024). Additionally, this TF is susceptible to be regulated by nitro fatty-acids, important biomolecules that connect NO and fatty acid metabolism and signaling (Mata-Pérez et al., 2016). Environmental conditions impact on seed yield and composition, influencing deposition of storage compounds. It has been published that bZIP67 accumulation is also influenced by cold conditions (Bryant et al., 2019). By using transgenic lines overexpressing bZIP67 and bZIP67w/oCys, we observed different patterns in bZIP67 accumulation during plant acclimation to different temperatures, highlighting the relevance of Cys residues and the potential role of NO in the control of this responses. All together, these results point to bZIP67 as a TF able to integrate external and internal signals, to modulate the fatty acid composition of Arabidopsis seeds.

FLASH TALKS

Exploring new insights into the role of nitric oxide synthase in nitrogen metabolism among photosynthetic organisms

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Abstract

Nitric oxide synthase (NOS) plays a crucial role in catalyzing the conversion of L-arginine into L-citrulline and nitric oxide (NO). Existing evidence suggests the absence of NOS in higher plants; however, they have been identified in numerous photosynthetic microorganisms. In our laboratory, we are investigating the functionality of the NOS in the cyanobacterium *Synechococcus* PCC 7335 (syNOS) and the algae *Ostreococcus tauri* (otNOS). The objective of our research is to assess whether NOS is involved in primary nitrogen metabolism, given that arginine serves as a major organic nitrogen source. Our findings indicate that *O. tauri* can sustain a consistent growth rate when L-arginine is the sole nitrogen source, despite lacking conventional pathways for metabolizing this amino acid into assimilable nitrogen. The transcript level of genes involved in nitrogen uptake and metabolism were increased in nitrogen-starved condition while the addition of L- Arg reduced their induction. Notably, levels of NO increased in *O. tauri* cells growing in L-arginine, suggesting the potential involvement of the otNOS enzyme in L-arginine metabolism. Conversely, syNOS exhibits an additional domain, distinct from the classical oxygenase and reductase, encoding for a globin. Consequently, syNOS can facilitate the oxidation of Arg to citrulline and NO, followed by the further oxidation of NO to nitrate. To decipher the function of syNOS, we expressed the syNOS protein in *Synechococcus* PCC 7942, a strain lacking NOS. Preliminary results indicate that syNOS expression enhances bacterial growth under nitrogen scarcity. Our working hypothesis posits that syNOS expression contribute to degrade internal arginine pools dealing with nitrogen starvation. An early indicator of nitrogen deficiency is the rapid degradation of phycobiliproteins in cyanobacteria. The expression of syNOS in *S.* PCC 7942 accelerates this process, suggesting that this strain may perceive nitrogen deficiency more rapidly. Our current results strongly imply the involvement of NOSs in the growth and primary nitrogen metabolism of photosynthetic microorganisms. These findings encourage us to evaluate the effect of NOS expression in higher plants. Transgenic Arabidopsis and potato lines expressing syNOS display higher yield respect to wild type plants in growing either higher or low nutrient conditions. The metabolic profile of nitrogen organic compounds, including proline and serine, experienced a notable decrease in wild-type plants under nitrogen deficiency. In contrast, these compounds showed relatively little change in potato transgenic lines under the same conditions. In summary, this research expands our understanding of nitrogen recycling and metabolism in alga and cyanobacteria and holds potential applications for enhancing nitrogen use efficiency in higher plants.

Study of the interaction of a free and nanoencapsulated nitric oxide donor with soybean roots

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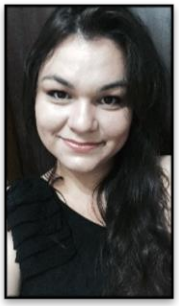

Abstract

Nitric oxide (NO) is a molecule that participates in several processes of plant growth and development, in addition to acting in defense against stress. The exogenous application of NO in plants requires the use of NO donors, such as S-nitrosoglutathione (GSNO). However, these compounds present relative instability. Thus, the use of nanocarriers appears as a promising alternative for the application of NO donors in agriculture. However, there is still a gap related to the interaction mechanisms between nanomaterials and plants. Therefore, the objective of this study was to evaluate the effects of free and nanoencapsulated GSNO in altering the bioavailability of NO in the roots of soybean plants. The experiment was conducted in hydroponics using Glycine max L. Merr. (BRS 257). The treatments were control (distilled water), chitosan nanoparticles containing GSNO (NP CS-GSNO), and free GSNO for each time interval (30 min and 24 h). The nanoparticles were labeled with rhodamine B to allow their detection in the roots by confocal spectral microscopy. The NO levels in the roots were measured simultaneously with the use of the fluorescent probe DAF-2DA. As a control for NO, fluorescence was quenched by the NO scavenger cPTIO. After 30 min, it was possible to detect 46% of co-localization between the nanoparticles and NO in the root maturation zone and more than 60% in the root branching zone for the NP CS GSNO treatment. A lower co-localization percentage was detected for GSNO in the root maturation zone (34%) and root branching zone (25%). The co-localization for this formulation occurs because GSNO emits a low fluorescence in the rhodamine wavelength range. At 24 h, the percentage of co-localization was reduced to 7% in the maturation zone and 13% in the branching zone for NP CS-GSNO. The low IF detected in roots incubated with cPTIO confirmed the specificity of the DAF-2DA probe for NO in this protocol. Moreover, the treatment with NP CS-GSNO induced higher and more persistent increases in NO (particularly in the 30 min time-point) and S-nitrosothiol levels in soybean roots compared to the free GSNO Overall, these results suggest that the roots absorbed the NP CS-GSNO, resulting in a more efficient NO delivery to the tissues than the free GSNO.

The effect of plasma-activated water on wheat seedling development and the levels of *in planta* reactive oxygen and nitrogen species

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Abstract

Seed priming is a technique with which we can enhance the growth and resilience of plants resulting in better germination, yield and stress response. We used plasma-activated water [PAW], and a novel form of PAW with zinc-ions [PA(W+Zn)] added in order to stabilize the reactive oxygen and nitrogen species (RONS) ensuring a longer lifespan. The long-term goal of our research is to enhance the drought tolerance of wheat. Current experiments focus on finding the optimal priming circumstances to induce seedling growth. Two wheat cultivars were used, GK Szilárd (drought sensitive), and GK Szereda (drought tolerant). Distilled water was used as control agent (hydroprimed, HP). The treatments were the followings: HP, PAW, PA(W+Zn) and a 50% solution of PAW and PA(W+Zn) (P50). The RONS concentration of PAW was 11 mg/L hydrogen peroxide (H_2O_2), 30 mg/L nitrate with a pH of 4.4. The PA(W+Zn) contained 18 mg/L H_2O_2, 12 mg/L nitrite, 20 mg/L NO_3, 15mg/L zinc with a pH of 5.9. Seeds were surface sterilized and were imbibed in the priming solutions for 24h in the dark following a 5-days-long cultivation in Petri dishes at 24 °C. The fresh weight, shoot and root length of 5-days-old seedlings were measured, and *in planta* RONS were detected in the root tips using DAF-FM DA (for nitric oxide, NO), APF (for peroxynitrite, $ONOO^-$), and Amplex Red (for H_2O_2). In case of GK Szilárd, the best priming agent was the P50, that had a significant positive impact on fresh weight, root and shoot length. The PAW caused the most significant induction of all the examined RONS (NO, $ONOO^-$, H_2O_2) in the root tips of GK Szilárd. Moreover, in case of NO, the PA(W+Zn) and the P50 solution also resulted in a significant promotion. The P50 induced root elongation of GK Szereda compared to control; however, only PAW treatment caused elevated NO levels compared to HP. The treatments (mainly P50) resulted in growth induction of both wheat cultivars (especially in GK Szilárd), which might be partly the consequence of the increased *in planta* NO, $ONOO^-$ and H_2O_2 contents. These results form the basis of future experiments during which we try to find the best PAW concentrations providing the strongest endurance against drought stress.

Ecotype-Dependent Responses to Nickel Oxide Nanoparticles in Ni Hyperaccumulator *Odontarrhena lesbiaca*: Unraveling the Impact Across Cellular, Tissue, Organ, and Molecular Level

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Abstract
The increasing industrial use and environmental release of nickel oxide nanoparticles (NiO NPs) highlight the importance of understanding their interactions with plants comprehensively. This study explores the responses of three ecotypes of the Ni hyperaccumulator *Odontarrhena lesbiaca* at the cellular, tissue, organ, and molecular levels under elevated concentrations of NiO NPs (250 mg/L and 500 mg/L). Despite similar Ni accumulation in the presence of nano Ni, the translocation of Ni from roots to shoots was lower with NiO NPs compared to bulk Ni. Root cell walls played a crucial role in sequestering NiO NPs, serving as a cellular defense mechanism. NiO NP exposure induced anatomical changes in roots, such as increased cortex thickness and depositions of lignin-suberin and pectin, acting as tissue-level defense against excessive Ni. Biomass parameters showed ecotype-dependent variations, with Olympos displaying increased parameters. Free Ni salt had more detrimental effects on biomass than the nanoform, suggesting that the observed effects of NiO NPs are linked to Ni ion release. Nitric oxide and peroxynitrite levels varied among ecotypes in response to NiO NPs. Protein analysis revealed the impact of NiO NPs on *S*-nitrosoglutathione reductase, indicating posttranslational regulation. Protein tyrosine nitration was slightly intensified by NiO NPs, with variations correlating with ecotype biomass production. This study sheds light on the multi-level tolerance mechanisms of *Odontarrhena lesbiaca* ecotypes to NiO NPs, involving compositional modifications at the cellular level, anatomical changes at the tissue level, subtle adjustments in biomass production at the organ/organism level, and modifications in reactive nitrogen species metabolism and induced nitrosative protein modification at the molecular level.

Keywords: Ampeliko, ecotypes, Loutra, nanoparticles, nickel oxide, nitrosative signalling *Alyssum lesbiacum*, Olympos

Can HCN be converted to NO under physiological conditions?

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Abstract

Hydrogen cyanide (HCN) is a small gaseous molecule that for a long time was considered as a poison. However, depending on the concentration, HCN can act as a cytotoxic and a signalling compound. Although, some aspects of the activity and effects of HCN, especially in plants, are still not fully understood. HCN is a compound produced in plants. In some, known as cyanogenic plants, HCN is accumulated in a bonded form of cyanogenic glycosides. Fumigation of biological material with HCN results in the emission of nitric oxide (NO). After HCN treatment of apple embryos, NO emission from the embryonic axes was observed however, the mechanism of this phenomenon is unknown. Under controlled aerobic conditions, HCN *in vitro* could be oxidized to N-containing species such as NO. The chemical kinetic mechanisms of such reactions are well known. The process of HCN conversion into NO has not been observed under physiological conditions. In our studies, we determined the *in vitro* NO_2^- formation from acidified $NaNO_2$ (50 and 100 mM) or acidified KCN (75 mM and 150 mM) by spectrophotometric assay with a modified Griess reagent. We also determined the content of 3-nitrotyrosine (3-NT) and *S*-NO groups in bovine serum albumin (BSA) after treatment with $NaNO_2$ (50 and 100 mM) or acidified KCN (75 mM and 150 mM) by spectrophotometric method. Our data indicate that HCN may be a source of NO_2^- (*in vitro*) and HCN *in vivo* leads to the formation of 3-NT and *S*-NO groups in BSA. We propose that HCN is likely converted to NO in the presence of free radicals (e.g. •OH or $O_2^{-\bullet}$).

Keywords: hydrogen cyanide, nitric oxide, nitration, *S*-nitrosation

Mechanism of nitric oxide alleviating alkali-induced inhibition of embryo germination in *Sorbus pohuashanensis*

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Abstract

Plant seed is an important part of plant breeding and maintaining species continuity. Currently, several studies have confirmed that different concentrations of saline and alkaline stress caused a decrease in seed germination, and with the increase of salt concentration, the decrease in seed germination increased, and the inhibitory effect of alkaline salt was stronger than that of neutral salt. Nitric oxide (NO), as an important biosignaling molecule in plants, plays an important role in plant growth, development and biotic/abiotic stress responses. However, the physiological response of NO to alkaline salt stress and its associated molecular response mechanisms in *Sorbus pohuashanensis* have not been reported, and how NO interacts with other signaling molecules to regulate plant stress tolerance is still unclear. Therefore, in this study, mature zygote embryos of *Sorbus pohuashanensis* were used as experimental materials, and SNP was used as exogenous NO donor. The phenotype characteristics, physiological and biochemical differences of *Sorbus pohuashanensis* under different treatments, as well as changes in reactive oxygen species (ROS) metabolism system caused by alkaline salt stress were analyzed, so as to explore the mechanism of exogenous NO alleviating alkali stress on seed germination inhibition of *Sorbus pohuashanensis*. In addition, RNA-seq was used to explore the gene regulatory network in response to sodium carbonate and NO, and explore the key genes in the process of seed germination regulated by NO and the interaction between genes, so as to elucidate the physiological and molecular mechanism of exogenous NO alleviating alkali stress, with a view to providing theoretical guidance for improving seed germination rate and seedling yield.

Trichoderma supplementation enhances defense responses under low nitrate nutrition in Arabidopsis

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Abstract

Nitrogen (N) is essential for growth, development and defense but, how low N affects defense and the role of Trichoderma in enhancing defense under low nitrate is not known. Low nitrate fed Arabidopsis plants displayed reduced growth and compromised resistance responses when infected with both avirulent and virulent *Pseudomonas syringae* DC3000. These responses were enhanced in the presence of Trichoderma. The mechanism of increased resistance mediated by Trichoderma involved increased N uptake and enhanced protein levels via modulation of nitrate transporter genes. The *nrt2.1* mutant is compromised in local and systemic acquired resistance responses suggesting a link between enhanced N transport and defense. Enhanced N uptake was mediated by Trichoderma elicited nitric oxide (NO). Low NO producing *nia1,2* mutant and nsHb+ over expressing lines were unable to induce nitrate transporters and thereby compromised defense in the presence of Trichoderma under low N suggesting a signaling role of Trichoderma elicited NO. Trichoderma also induced SA and defense gene expression under low N. The SA deficient NahG transgenic line and the npr1 mutant were also compromised in Trichoderma-mediated resistance responses. Collectively our results indicated that the mechanism of enhanced plant defense under low N mediated by Trichoderma involves NO, ROS, SA production as well as the induction of NRT for increased resistance.

Brassica juncea has multiple forms of S-nitrosoglutathione reductase (GSNOR) suggesting multiple roles

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Abstract

S-nitrosoglutathione reductase (GSNOR) is extensively studied and recognized as a key regulator of nitric oxide (NO) homeostasis in plants. GSNOR plays a crucial role in controlling the concentration of S-nitrosoglutathione (GSNO) by facilitating its conversion into the oxidized form of glutathione, namely glutathione disulphide (GSSG), and ammonia, utilizing NADH as a co-factor. Experimental evidences have shown the significance of GSNOR in normal plant development and tolerance in both biotic and abiotic stresses. Genome-wide identification yielded 4 GSNOR genes in *Brassica juncea*. Multiple copies were confirmed by PCR amplification at gene level. At protein level, 4 immunopositive spots at 41.5 kDa (pl 5.79 and 6.78) and 43 kDa (pl 6.16 and 6.23). GSNOR purification via anion-exchange chromatography resulted in the isolation of two distinct pools, namely GSNOR-A and GSNOR-B. Following this, affinity-purification led to the identification of one polypeptide in GSNOR-A and two polypeptides in GSNOR-B. Size exclusion-HPLC analysis confirmed the presence of three GSNORs with molecular weights of 87.48±2.74 KDa (GSNOR-A), 87.36±3.25 KDa, and 82.74±2.75 KDa (GSNOR-B). GSNO (500 µM concentration) decreased the relative activity of purified GSNORs. The reversal of GSNOR activity after DTT (10 mM) treatment further confirmed redox regulation of GSNORs. Biotin Switch Technique (BST) showed S-nitrosylation in both GSNOR-A and GSNOR-B validating the suppression of GSNOR activity due to S-nitrosylation. Comparative analysis of immunoblot probed with anti-biotin antibody showed denitrosylation of most SNO-proteins by GSNOR-A and GSNOR-B with NADH as cofactor. Among the two, BjGSNOR-B showed lesser intensities of SNO-proteins indicating higher denitrosylation efficiency. This research paves the way for unraveling the physiological functions of multiple GSNORs in *B. juncea*. However, conducting additional experiments, such as differential expression analysis and exploring S-nitrosylation targets, is essential for gaining a deeper understanding of the specific roles and functional significance of each form of GSNOR.

The effect of seed priming with plasma activated water on germination and RONS content of *Arabidopsis thaliana* L.

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Abstract

Nowadays, due to global climate change, plants are under more and more significant environmental stress. Seed priming is a widely used procedure, which can be used to increase the resistance of plants against biotic and abiotic stress factors. For seed priming we used plasma-activated water (PAW). With plasma-based technologies, priming agents can be produced cost-effectively with high efficiency and low environmental impact. PAW is made with cold plasma, which increases the amount of reactive oxygen and nitrogen species (RONS) in the treatment material. The RONS concentration can be stabilized by adding zinc (Zn) to the liquid [1] which may increase the efficiency of PAW as a priming agent.The seeds of wild type *Arabidopsis thaliana* L. (Col-0) were surface-sterilized and incubated in different treatment solutions for 24h in the dark at 24 $^{\circ}$C. Treatments were the followings: unprimed (UP), hydroprimed (HP), PAW, PA(W+Zn) and 50 $^{V}/_{V}$ % mixture of PAW and PA(W+Zn) (P50). The PAW contained 38 mg/L nitrate (NO_3^-), 0 mg/L nitrite (NO_2^-) and 8 mg/L hydrogen peroxide (H_2O_2), while in PA(W+Zn) the concentration of NO_3^-, NO_2^- and H_2O_2 were 22, 12 and 16 mg/L, respectively. Primed seeds were planted on agar media and cultivated for 5 days. Root length of Arabidopsis seedlings was measured and the levels of nitric oxide (NO), superoxide radical anion ($O_2^{\bullet-}$), H_2O_2, and peroxynitrite ($ONOO^-$) were visualized in the root tips. We found that PA(W+Zn) resulted in the longest roots compared to other treatments, presumably because Zn created the proper ratio of RONS in the solution, that can ensure growth. The PA(W+Zn)-induced root elongation may not be obviously associated with the changes in RONS levels due to additional physiological factors (*e.g.* hormones) affecting root development. As for P50, the ratio of RONS could be beneficial but the root length doesn't confirm this. The effects of PAW on seedling viability and endogenous NO signaling will be studied in detail in the near future.

Concentration-based regulation of dormancy-emergence mechanisms in *Sorbus pohuashanensis* embryos by nitric oxide

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Abstract

Although the proper timing of seed dormancy and germination is extremely important for the adaptation of trees to various environments, it makes forestry production difficult. The inability to accurately control the germination process of dormant seeds is a bottleneck problem that affects seedling emergence and seedling quality after sowing. Nitric oxide (NO), an important biosignaling molecule in plants as well as a component of wildfire smoke, significantly promotes plant seed germination. However, the exact mechanism by which NO regulates seed dormancy lifting has not been elucidated, and it is mostly believed to be related to reactive oxygen species signaling and abscisic acid degradation. *Sorbus pohuashanensis* seeds have a dormant habit, and low concentrations of exogenous NO can significantly promote the lifting of dormancy in *S. pohuashanensis* embryos. In our study, we found that NO regulation of dormancy lifting in *S. pohuashanensis* embryos was related to ethylene synthesis and proved that it was involved in seedling development, but the mechanism of its action was not yet understood. We hypothesized that NO concentration-dependent regulation of ethylene synthesis through hydrogen peroxide signaling controls abscisic acid signaling and thus determines embryo dormancy or germination. To confirm this hypothesis, we pretreated *S. pohuashanensis* embryos with exogenous NO donors, replicated the dormancy lifting and maintenance model, and analyzed the interactions among nitric oxide signaling, hydrogen peroxide signaling, and hormones, such as ethylene, at physiological and transcriptional levels. This study will elucidate the mechanism of nitric oxide concentration-based regulation of plant embryo dormancy and germination from a new perspective, and provide a theoretical basis for the establishment of seedling seed dormancy precise regulation lifting technology.

NO modulates in pepper fruits the gene expression of tryptamine 5-hydroxylase (*T5H*), a gene involved in the serotonin generation

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Abstract

Serotonin is an intermediate compound in the biosynthesis of melatonin that is generated from tryptophan in two-step reactions catalyzed by the enzymes tryptophan decarboxylase (TDC) and tryptamine 5-hydroxylase (T5H). Pepper (*Capsicum annuum* L.) fruit is a horticultural product worldwide consumed and, to our knowledge, there is no information about the *T5H* gene in this fruit. Previously, we have characterized the *TDC* gene in pepper fruits [1]. Therefore, in this study, we focused on the identification of *T5H* genes in pepper and how they could be modulated during the ripening of fruits and by exogenous NO treatment. Four *CaT5H* genes, named *CaT5H1* to *CaT5H4*, were identified in the complete genome of pepper, but only *CaT5H1* and *CaT5H3* were expressed in fruits. During ripening both genes were upregulated, but the NO treatment exerted a negative impact on their expression. Taken together, these data provide the first evidence of how these *CaT5H* genes are modulated by ripening and NO, and open new questions about the physiological relevance of serotonin in the physiology of pepper fruits.

Nitric oxide sensing by TGA transcription factors is essential for the root stem cell niche preservation

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Abstract

Root development is a rigorously controlled process which depends on the proper coordination among different cell types located in the stem cell niche (SCN). The gasotransmitter nitric oxide (NO) has pivotal roles in various plant biological processes[1,2,3], and is particularly essential for shaping root architecture and meristem organization within the root apical meristem (RAM) [4,5]. Furthermore, among the key regulators of SCN maintenance, TGA transcription factors bears particular relevance, where PERIANTHIA (PAN/TGA8) has been described as a master regulator of quiescent centre (QC) cell functioning[6]. Nevertheless, our understanding of the specific molecular targets of NO in maintaining homeostasis within the SCN remains limited. We have demonstrated the involvement of TGA transcription factors in mediating the NO-dependent regulation of the root SCN, through phenotypic, transcriptomic and biochemical approaches. Our findings reveal that NO significantly influences primary root length due to an aberrant effect in SCN organization. Our genetic evidences point to a prominent role of TGA family in NO sensing during root growth. Through transcriptomic approaches, we observed that these TGA transcription factors are responsible for the gene regulation during NO sensing in the SCN. Additionally, biochemical studies by *S*-nitrosylation show that NO induces the posttranslational modification of TGA-bZIP transcription factors, thereby inhibiting their capacity to bind DNA and associated transcriptional regulation. Moreover, our molecular data underscore the interaction among various TGA members and the key gene regulatory network involved downstream these transcription factors. These discoveries establish a molecular framework where NO modulates the function of TGA transcription factors, thereby highlighting them as pivotal regulators of root SCN development and maintenance.

Unraveling the dynamics of the ascorbate-glutathione cycle enzymes in pepper fruits during ripening and under a NO-enriched atmosphere

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Abstract

The intricate interplay between nitric oxide (NO) signaling and the Ascorbate-Glutathione (AsA-GSH) cycle, as pivotal components of the plant stress response, remains a relatively unexplored area in fruit ripening. This study investigates the influence of a NO-enriched atmosphere on the expression of genes (RNA-Seq), protein content (iTRAQ), and enzyme activity associated with the AsA-GSH cycle in pepper fruits during the ripening process. Building upon our previous characterization of ascorbate peroxidase (APX) from pepper (*Capsicum annuum* L.) fruits [1,2], this study focuses on the other enzymatic components within the AsA-GSH cycle. Specifically, we investigated the dynamics of monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DAR), and glutathione reductase (GR) during fruit ripening and after exogenous NO treatment. Our findings suggest a multifaceted regulatory network where NO modulates the expression of the AsA-GSH cycle genes, which subsequently impacts the respective protein abundance and enzymatic activities. The comprehensive integration of RNAseq, iTRAQ, and enzyme activity data depicts a holistic understanding of how NO influences the AsA-GSH cycle during pepper fruit ripening. This research provides valuable insights into the molecular and biochemical mechanisms governing the interaction between NO and the AsA-GSH cycle, shedding light on the complex nitroxidative metabolism exhibited by pepper fruits during the ripening process

Phytoglobin-NO cycle and AOX pathway play a role in anaerobic germination and growth of deepwater rice

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Abstract

Rice has a noteworthy characteristic in that it may germinate in anoxic environments. Although several biochemical adaptive processes are crucial in the anaerobic germination of rice but the function of the phytoglobin-nitric oxide cycle and alternative oxidase is unknown, therefore in this study we investigated the role of these pathways in anaerobic germination. Under anoxic conditions, deepwater rice germinated much higher and rapidly than aerobic condition and the anaerobic germination and growth were much higher in the presence of nitrite. The addition of nitrite stimulated NR activity and NO production. Important components of phytoglobin-NO cycle such as methaemoglobin reductase activity, expression of Phytoglobin1, NIA1 were elevated under anaerobic conditions in the presence of nitrite. The operation of phytoglobin-NO cycle also enhanced anaerobic ATP generation, LDH, ADH activities and in parallel ethylene levels were also enhanced. Interestingly nitrite suppressed the ROS production and lipid peroxidation. The reduction of ROS was accompanied by enhanced expression of mitochondrial alternative oxidase protein and its capacity. Application of AOX inhibitor SHAM inhibited the anoxic growth mediated by nitrite. In addition, nitrite improved the submergence tolerance of seedlings. Our study revealed that nitrite driven phytoglobin-NO cycle and AOX are crucial players in anaerobic germination and growth of deepwater rice.

Emergence and Initial Development of Seedlings of Atlantic Forest Tree Species in Response to Seed Priming with Nanoencapsulated Nitric Oxide Donor

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Abstract

The Atlantic Forest is one of the most threatened biomes in the world. Lowering tree seedling production costs is essential for reforestation aiming at climate change mitigating and biodiversity conservation. Seed nanopriming is a promising technique that can improve germination rates, seedling vigor, and stress resistance at the initial phases of plant development. Furthermore, nitric oxide (NO) plays important roles as a signaling molecule in plant development processes. Seed nanopriming with NO-releasing nanoparticles (NPs) represents a promising approach to improve the efficiency of seedling production for reforestation. We investigated the effects of seed priming with chitosan NPs containing the NO donor S-nitrosoglutathione (GSNO) on the early development of tree species native to the Atlantic Forest commonly used in reforestation programs: *Heliocarpus popayanensis* Kunth (Malvaceae), *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae), and *Schinus terebinthifolia* Raddi (Anacardiaceae). For the seed priming process, various concentrations of chitosan NPs containing GSNO were used (0.1, 0.25, 0.5, 2.5, and 5 mM), as well as hydropriming and non-primed controls. Seeds were immersed in the formulations for 10 minutes under continuous stirring. Then, they were dried in the dark at room temperature for 24 hours before being sown in pots with sand in a greenhouse. The measured parameters were emergence percentage, mean emergence time, emergence speed index, main root, shoot and lateral root length, number and density of lateral roots, vigor index, and root and shoot fresh and dry masses. For H. popayanensis, the optimal dose of nanoencapsulated GSNO was close to 2.5 mM, which increased by 102.88% and 109.03% the emergence percentage and emergence speed index, respectively, compared to hydropriming. The same treatment positively affected the fresh mass of both roots and shoots. Conversely, the 5 mM concentration had a negative impact on the main root length. Most parameters of C. estrellensis were not responsive to GSNO-loaded NPs, but the doses 1, 2.5 and 5mM exhibited harmful effects on shoot and lateral root length, which demonstrates the dualistic nature of NO. In the case of S. terebinthifolia, nanoencapsulated GSNO (0.25 mM) increased the length of shoot and lateral roots, but it decreased the number of lateral roots. These results highlight the potential use of the seed priming with NO-releasing NPs to improve tree seedling emergence and initial development for reforestation programs. However, the technique's effectiveness varies across species. Therefore, it is essential to develop more research with seeds of diverse Atlantic Forest species and adapt the technique according to their characteristics.

Keywords: Reforestation, S-Nitrosoglutathione, Chitosan nanoparticles.

Status of nitric oxide during fungal disease development in plants

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Abstract

Nitric oxide (NO) is emerged as important signaling molecule in plants. It is involved in different pathophysiological processes in plants from germination to plant disease resistance. Until now, the exact source of NO in plants is under question mark. Plants are facing different types of biotic and abiotic stresses throughout life. Among different signalling molecules involved in stress tolerance, NO is very important. In plant biotic stresses, fungal diseases are major concern. Establishment of fungal disease in plants and its spreading actually reduce the crop yield, which ultimately raises a question about food security. Therefore, to check the fungal disease it is an immediate precondition to understand the exact mechanism of disease developmental processes. The present work is an amalgamation of different aspects of fungal disease development in relation to NO generation in different host plants. Finally, the status of plant defense machinery and NO content must be unravelled in different plant-fungi interaction.

Understanding the putative role of Nitric Oxide in growth and development of *Dioscorea alata* tubers.

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Abstract

Plants have to cope with a large variety of biotic and abiotic stresses encountered in their ever-changing environment. The dynamicity in the proteome is critical for responding rapidly and efficiently to emerging threats. Nitric oxide (NO) is a lipophilic, free radical, regulating a plethora of physiological processes in plants, such as development, ripening and senescence, balancing cellular redox status and defense. The mechanism of how NO regulates physiological responses in tubers has not been well understood in case of tubers. Protein S-nitrosylation, a redox-based modification of cysteine thiol by NO, is known to be one of the important post-translational modifications to regulate the activity and interactions of proteins. Thus to elucidate NO function in plants, proteomic analysis of S-nitrosylated proteins in *Dioscrea alata* tuber was analyzed. The aim of the study was to analyze the contribution of NO in the four contrasting stages of tuber growth i.e S1 (root initiation), S2 (vegetative growth), S3 (new tuber formation) and S4 (mature tuber) to elucidate the existence of NO signalling in *D. alata* tuber. NO production monitored using NO measuring system (inNO-T,Innovative instruments Inc.) showed 3.7 folds higher NO content in mature tuber (S4) than the germinating tuber (S1). NO combines with GSH (the main reductant in the cell) or thiol group(s) in proteins to produce GSNO and SNOs respectively. A higher SNO content (4.5 fold) during tuber maturation (S4) in comparison with the germinating tuber (S1) was observed. Increased SNO leading to S-nitrosylation of dioscorin was also confirmed by Biotin switch assay across all the stages of tuber growth. To understand if the higher SNO content in the mature stages of the tuber growth has an effect on the activities associated with dioscorin the effect of GSNO (a NO donor) was analyzed. GSNO negatively regulated MDHAR (4.5 fold in S3 & S4), APx (1.81 & 5 fold in S3 & S4 respectively) and GR (1.3 fold in S3 & S4) activities. Another NO donor, CysNO negatively regulated the DHAR (4.8 & 5.1 fold in S3 & S4 respectively) activity. The study shows NO mediated regulation of dioscorin. This information may be useful to understand the role of reactive nitrogen species (RNS) in growth and development of tuber. Recently, it has been established that NO is required to delay senescence and extend the shelf life of fruits by competitively inhibiting ethylene-responsive components. These observations clearly offer the prospect of managing post-harvest handling and storage of the tubers.

Unraveling the involvement of Nitric oxide synthase in mitigating oxidative burst in cyanobacterium *Aphanizomenon flos-aquae 2012/KM1/D3*

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Abstract

Nitric oxide synthase (NOS) in mammals is recognized for its essentialities in several metabolism including blood vascular relaxation and nerves transmission etc. yet their functionalities in photosynthetic prokaryotes, including cyanobacteria, is lesser appreciated. Pioneering studies unveiled the existence of NOS homologs not only in bacteria but also in photosynthetic organism, exhibiting the essentiality of NOS in these life forms. Besides, NOS seems to have global functions that differ from their mammalian counterparts. Therefore, in our study, the distribution, abundance and phylogeny of NOSs among cyanobacteria were evaluated. Further, physiological and biochemical importance of NOS-derived nitric oxide (NO) production was investigated in *Aphanizomenon flos-aquae* 2012/KM1/D3 during its progression from the exponential to stationary growth phase. *The accumulation of NO was dramatically reduced upon NOS inhibitor* L-N^{G}-Nitro arginine methyl ester *(L-NAME) supplementation, exhibiting significant NO synthesis by NOS, whereas addition of L-arginine increase NOS derived NO* in a dose dependent manner. Moreover, *the reduction in the growth and metabolic activities of the cyanobacterium were evident upon L-NAME treatment that possibly pertained to decline in photopigments, PSII efficiency,* impaired *membrane fluidity and cellular integrity due to oxidative burst which culminated into cell death. This suggests that NOS inhibition renders the cells more susceptible to deleterious accumulation of ROS, as evidenced by distinctive cytoplasmic alterations, compromised antioxidative system, protein oxidation and exacerbated oxidative DNA fragmentation*. Further, NOS elicitation bestows increased tolerance by maintaining ATP/ADP, Asc/DHAsc and GSH/GSSG ratio — essential markers of redox homeostasis — ultimately fostering redox balance and energy status. Besides, our findings forecasted coordinated action between GSNOR and NOS in dynamically regulating NO flux. These findings provide considerable evidences to support the vitality of NOS derived NO production in neutralizing the oxidative burst, thereby maintaining stable cyanobacterial growth. The present study strengthens the foundation to access biological significance of NOS-derived NO in relation to stress tolerance and physiology of cyanobacteria.

Nitric oxide stimulates seed germination via regulating the respiration in chickpea.

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Abstract

The process of seed germination is vital for the plant's life cycle and is controlled by a sequence of metabolic, biochemical and molecular processes to release dormancy. Following water absorption, the seed initiates germination, generating diverse reactive oxygen species (ROS). If not eliminated, ROS can harm cellular components including membranes and facilitate protein breakdown, thereby impeding the germination process. Nitric oxide (NO), a gaseous free radical, is an important regulator of plant developmental processes including germination and mitochondrial function. In this study, we examined the involvement of NO in the germination process of two chickpea varieties with contrasting germination capacities: Kabuli, characterized by a low germination rate and delayed germination, and Desi, known for its enhanced germination rate. Desi had higher levels of NO production compared to Kabuli and demonstrated lower respiratory rates. Due to the elevated rates of respiration, Kabuli exhibited decreased internal oxygen concentration and increased levels of ROS such as superoxide radicals and hydrogen peroxide. Administration of NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) caused a decrease in respiration in Kabuli and a reduction in ROS levels, leading to an increase in the speed of germination. These findings indicate that NO plays a crucial role in the germination of Kabuli. SNAP elevated the expression of transcripts encoding enzymes implicated in glucose metabolism and the cell cycle. Furthermore, the concentrations of amino acids and organic acids in Kabuli were elevated due to the use of SNAP. 1H-nuclear magnetic resonance research indicated that Kabuli possesses a greater ability for glucose oxidation compared to Desi. A homozygous hybrid, derived from a recombinant inbred line population resulting from a cross between Desi and Kabuli, exhibited accelerated germination, elevated NO levels, and decreased accumulation of ROS in comparison to Kabuli. Collectively, our results emphasize the significance of NO in controlling respiration, internal oxygen levels, and ROS homeostasis, all of which play critical roles in seed germination.

RNS and ROS alterations in *Nepenthes x ventrata*

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Abstract

Carnivorous plants attract animals, trap and kill them, then absorb nutrients from the digested bodies. This adaptation is crucial for their survival in low-nutrient environments. Pitcher plants (*Nepenthes*) belong to carnivorous plants that develop passive pitcher-shaped traps at the end of the main nerve of the leaf blade (Mithöfer, 2011). The traps are filled with digestive fluid in which digestive enzymes, reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been identified (Chia et al., 2004, Wal et al., 2022). In plant physiology ROS, together with RNS, have a regulatory and signalling role (Krasuska et al., 2023). **The aim of this study was to determine alterations in ROS and RNS level metabolism in *Nepenthes x ventrata* during the development and digestive process.** The material was digestive fluid or trap tissue, which were collected at different stages of development and 1, 2 and 4 days after introducing the protein source and protein source with the addition of donor NO_x. As the control, non-fed traps were used. We measured the nitric oxide (NO) and peroxynitrite $ONOO^-$ levels in digestive fluid with fluorescent markers and the electrochemical sensor for NO. To localize $O_2^{\cdot-}$ production histochemical staining was made. We observed differences in the levels of various forms of RNS (NO and $ONOO^-$) during development and after NO_x application. The addition of NO_x modulated the homeostasis of ROS (mostly radical forms). ROS and RNS play a crucial role in digestion, and NO_x may have both antioxidant and signalling functions.

LEAD TALK

Organomercury-based capture of S-nitrosated proteins links protein quality control to fertility defects

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Abstract

S-nitrosation, the selective posttranslational modification of protein cysteine residues to form S-nitrosocysteine (-SNO), is one of the molecular mechanisms by which nitric oxide (NO) influences diverse biological functions and can alter protein function, interactions and location. Despite the biological importance of this posttranslational modification, significant gaps exist regarding the identification of this modification on a global scale. To investigate the *in vivo* nitrosoproteome of *Arabidopsis thaliana* we employed an organomercury-based approach that reacts directly, efficiently, and specifically with S-nitrosocysteine, enabling precise identification of S-nitrosocysteine–containing peptides and S-nitrosated proteins. Reaction of phenylmercury compounds with S-nitrosocysteine results in the formation of a relatively stable thiol–mercury bond. The method comprises three basic steps: (1) blocking reduced cysteines with methyl methanethiosulfonate (MMTS), (2) capture S-nitrosated proteins or peptides with *p*-aminophenylmercuric-acetate coupled to agarose beads, and release with mild performic acid, and (3) liquid chromatography/tandem MS analysis. The performic acid also oxidizes cysteine thiols to sulfonic acid, creating a unique MS signature that permits site-specific identification of the modified cysteines. We applied this method for the first time in plants, using floral tissues of an *A. thaliana* mutant lacking a central regulator of NO-homeostasis, S-nitrosoglutathione reductase (GSNOR, *hot5-2*). We identified 927 endogenously S-nitrosated proteins, including proteins previously described as targets for this kind of posttranslational modification, validating this new methodology. Additionally, we were able to capture 660 proteins that haven't been reported so far. To date, this is the largest data set of S-nitrosated proteins described in *A. thaliana*.

Keywords: *Arabidopsis thaliana*, proteomics, S-nitrosation, S-nitrosylation, organomercury, GSNOR, *hot5-2*.

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Thank You