Plant Physiology and Biochemistry

Computational prediction of NO-dependent posttranslational modifications in plants: current status and perspectives

--Manuscript Draft--

Manuscript Number:	
Article Type:	Review Article
Section/Category:	Reviews
Keywords:	computational prediction; nitric oxide; posttranslational modification; S-nitrosation; tyrosine nitration
Corresponding Author:	Zsuzsanna Kolbert, Ph.D. University of Szeged: Szegedi Tudomanyegyetem Szeged, HUNGARY
First Author:	Zsuzsanna Kolbert, Ph.D.
Order of Authors:	Zsuzsanna Kolbert, Ph.D.
	Christian Lindermayr, PhD
Abstract:	The perception and transduction of nitric oxide (NO) signal is achieved by NO-dependent posttranslational modifications (PTMs) among which S -nitrosation and tyrosine nitration has biological significance. In plants, 100-1000 S -nitrosated and tyrosine nitrated proteins have been identified so far by mass spectrometry. The determination of NO-modified protein targets/amino acid residues is often methodologically challenging. In the past decade, the growing demand for the knowledge of S -nitrosated or tyrosine nitrated sites has motivated the introduction of bioinformatics tools. For predicting S -nitrosation seven computational tools have been developed (GPS-SNO, SNOSite, iSNO-PseACC, iSNO-AAPAir, PSNO, PreSNO, RecSNO). Four predictors have been developed for indicating tyrosine nitration sites (GPS-YNO2, iNitro-Tyr, PredNTS, iNitroY-Deep), and one tool (DeepNitro) predicts both NO-dependent PTMs. The advantage of these computational tools is the fast provision of large amount of information. In this review, the available software tools have been tested on plant proteins in which S -nitrosated or tyrosine nitrated sites have been experimentally identified. The predictors showed distinct performance and there were differences from the experimental results partly due to the fact that the three-dimensional protein structure is not taken into account by the computational tools. Nevertheless, the predictors excellently establish experiments, and it is suggested to apply all available tools on target proteins and compare their results. In the future, computational prediction must be developed further to improve the precision with which S -nitrosation/tyrosine nitration-sites are identified.
Suggested Reviewers:	Mounira Chaki, Dr. mounira@ujaen.es She is an expert in plant nitric oxide-dependent posttranslational modifications.
	Marek Petrivalsky, Dr. marek.petrivalsky@upol.cz He is an expert in plant nitric oxide signalling.
	David Wendehenne, Dr. david.wendehenne@inra.fr He is an expert in plant nitric oxide signalling.

COVER LETTER

Dear Editorial Board of *Plant Physiology and Biochemistry*,

Hereby, please find our review manuscript entitled **„ Computational prediction of NO-dependent posttranslational modifications in plants: current status and perspectives”** written by Zsuzsanna Kolbert and Christian Lindermayr for consideration to publish in Plant Physiol Biochem. Previously, we contacted Professor Hiroshi Ezura, who has approved the proposal of this review topic.

This review collects, categorizes and characterizes the currently available online software tools for predicting nitric oxide (NO)-dependent posttranslational modifications (GPS-SNO, iSNO-PseACC, iSNO AAPair, SNOSite, RecSNO, PreSNO, GPS-YNO2, iNitro-Tyr, PredNTS, iNitroY-Deep DeepNitro). Additionally, the recently developed software tools are tested and their performances are compared on plant proteins for the first time. The aim of this work is to give a state-of-the-art overview for plant biologists about the computational prediction tools which are useful to establish and support laboratory experiments. Considerations for the future (e.g. what developments will be needed in the future) are also included. Previously two papers have been published in the topic by the authors. Chaki et al. (2014, PLOS ONE) evaluated *S*-nitrosation prediction software tools which were available at that time. In 2017, Kolbert et al. published a highly cited (32 independent citations) review paper in Plant Physiology and Biochemistry, in which they tested software tools for predicting protein tyrosine nitration on plant proteome. Compared to the previously published papers, this review paper would cover a wider topic evaluating both *S*-nitrosation and tyrosine nitration predicting software tools. The further novelty of this work is that it evaluates the recently developed algorithms (e.g. PreSNO, RecSNO, PredNTS) which hasn’t been tested on plant proteins so far.

We prepared the manuscript to our best knowledge and we are confident about its positive evaluation.

9th of June, 2021

Zsuzsanna Kolbert, PhD

Corresponding author

Christian Lindermayr, PhD

Corresponding author

Highlights:

- Eleven computational tools for predicting *S*-nitrosation and/or tyrosine nitration have been developed in the last ten years.
- On plant proteins, the predictors show distinct performances.
- The predictors can efficiently assign potentially modified amino acids in plant proteins.

Computational prediction of NO-dependent posttranslational modifications in plants: current status and perspectives

Zsuzsanna KOLBERT[1*], Christian LINDERMAYR[2*]

[1] Department of Plant Biology, University of Szeged, Közép fasor 52, 6726 Szeged, HUNGARY

[2] Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, German Research Center for Environmental Health, München/Neuherberg, Germany

* corresponding authors:

ordogne.kolbert.zsuzsanna@szte.hu

lindermayr@helmholtz-muenchen.de

Abstract

The perception and transduction of nitric oxide (NO) signal is achieved by NO-dependent posttranslational modifications (PTMs) among which *S*-nitrosation and tyrosine nitration has biological significance. In plants, 100-1000 *S*-nitrosated and tyrosine nitrated proteins have been identified so far by mass spectrometry. The determination of NO-modified protein targets/amino acid residues is often methodologically challenging. In the past decade, the growing demand for the knowledge of *S*-nitrosated or tyrosine nitrated sites has motivated the introduction of bioinformatics tools. For predicting *S*-nitrosation seven computational tools have been developed (GPS-SNO, SNOSite, iSNO-PseACC, iSNO-AAPAir, PSNO, PreSNO, RecSNO). Four predictors have been developed for indicating tyrosine nitration sites (GPS-YNO2, iNitro-Tyr, PredNTS, iNitroY-Deep), and one tool (DeepNitro) predicts both NO-dependent PTMs. The advantage of these computational tools is the fast provision of large amount of information. In this review, the available software tools have been tested on plant proteins in which *S*-nitrosated or tyrosine nitrated sites have been experimentally identified. The predictors showed distinct performance and there were differences from the experimental results partly due to the fact that the three-dimensional protein structure is not taken into account by the computational tools. Nevertheless, the predictors excellently establish experiments, and it is suggested to apply all available tools on target proteins and compare their results. In

the future, computational prediction must be developed further to improve the precision with which *S*-nitrosation/tyrosine nitration-sites are identified.

Keywords: computational prediction, nitric oxide, posttranslational modification, *S*-nitrosation, tyrosine nitration.

Introduction

Nitric oxide (NO), previously known as an air pollutant gas, has been shown to be an endogenously produced jack-off-all-trades plant signal molecule. In higher plants, nitrite is the major substrate for NO formation **(Astier et al., 2018)**, while in primitive algae, similar to animals, NO is primarily derived from the amino acid L-arginine (**Astier et al., 2021**), indicating that reductive pathways of endogenous NO formation have become dominant during the evolution of terrestrial plants (**Fröhlich and Durner, 2011**). NO is an integral regulator in a wide range of physiological processes such as vegetative-reproductive development (**Sánchez-Vicente et al., 2019**), photosynthesis (**Lopes-Oliveira et al., 2021**), stomatal movements (**Van Meeteren et al., 2020**), abiotic stress responses (**Fancy et al., 2017**), symbiotic interactions (**Berger et al., 2019**) and defence mechanisms against phytopathogens (**Lubega et al., 2021; Jedelská et al., 2021**). In biological systems, NO reacts among other things, with molecular oxygen, reactive oxygen species, glutathione, and amino acids to form the diverse group of reactive nitrogen species (RNS) including peroxynitrite ($ONOO^-$) and S-nitrosoglutathione (GSNO) as the most relevant ones. While the blood pressure regulating effect of NO in animals and humans is mediated by cGMP-dependent signalling and soluble guanylate cyclase (sGC) functions as a NO receptor (**Horst et al., 2019**), in plants NO-induced cGMP signalling seems to be unlikely (**Astier et al., 2019**). In recent years, the view has become prevalent that the transfer of NO's bioactivity is conveyed mainly through posttranslational modifications (PTMs) of specific protein targets. PTMs occurring following or during translation aim to increase the size and complexity of the proteome. Protein modifications result from enzymatic or nonenzymatic bounding of specific chemical groups to amino acid side chains (**Santos and Lindner, 2017**). Due to the alterations in the protein structure, protein activity, stability, localization, and molecular interactions may be modified (**Vu et al., 2018**). The biological function of more than 200 different enzymatic and non-enzymatic PTMs has been revealed so far (**Virág et al., 2020**). Among them, NO and its reaction products are responsible for the induction of non-enzymatic PTMs called nitration, *S*-nitrosation and metal nitrosylation. Nitration may covalently modify

tyrosine, tryptophan, cysteine and methionine (**Corpas et al., 2009**), *S*-nitrosation affects cysteine-containing proteins (**Hess et al., 2005**), and during metal nitrosylation NO reacts with metallo-enzymes (**Ignarro et al., 1999**). In biological systems, the most actively studied NO-dependent PTMs are *S*-nitrosation and tyrosine nitration affecting a large number of proteins thus having wide-ranging impact in the cells. Protein *S*-nitrosation has been established as a significant route by which NO transmits its ubiquitous cellular function **(Hess et al., 2005; Spadaro et al., 2010; Astier and Lindermayr, 2012),** while tyrosine nitration seems to have a major role as an irreversible modification leading to protein inactivation (**Kolbert et al., 2017**).

S-nitrosation: mechanism, specificity, selectivity, identification in plants

The mechanism of *S*-nitrosothiol formation is an important issue for understanding the biological actions of NO. Often thiol-containing molecules like cysteine and glutathione have been used for *S*-nitrosation to yield low-molecular-weight *S*-nitrosothiols such as *S*-nitrosocysteine (CysNO) and GSNO and to study the *S*-nitrosation mechanism. However, the reactivity of NO with thiol groups is very low. Therefore, the formation of SNOs depends on the generation of reactive intermediates **(Hill et al., 2010; Broniowska and Hogg, 2012)**. As a free radical ($^{\bullet}$NO), it can lose or gain electrons to become oxidized nitrosonium cation (NO^+) or reduced nitroxyl anion (NO^-) species, each with different oxidation state for the nitrogen atom (+2, +3, and +1 respectively) **(Arnelle and Stamler, 1995)**. Moreover, in aerobic, biological milieu, NO can be oxidized to its +5 oxidation state to form non-reactive nitrate anion (NO_3^-). The existence of NO in different redox status multiplies the possibilities to form *S*-nitrosothiols *via* various pathways (**Fig 1)**. For instance, NO can be oxidized to the highly reactive dinitrogen trioxide (N_2O_3), which is an effective *S*-nitrosating agent. Moreover, the NO radical can react with highly electrophilic thiyl ($RS^{\bullet}$) radicals. Furthermore, redox-active metals, e. g. such as those present in heme groups, can catalyze SNO formation. Finally, *S*-nitrosothiols can transfer their NO moiety to cysteine thiol in a trans-nitrosation reaction. This is of special importance in context of the physiological NO donors CysNO and GSNO **(Hess et al., 2005; Smith and Marletta, 2012; Kovacs and Lindermayr, 2013)**. But also *S*-nitrosated protein cysteine residues can function as NO donors. Several nitrosated proteins are described to transferring their NO group to target proteins or low molecular weight thiols, e. g. hemoglobin **(Pawloski et al., 2001)**, thioredoxin **(Mitchell and Marletta, 2005; Mitchell**

et al., 2007; Wu et al., 2010), caspase-3 **(Nakamura and Lipton, 2013)**, cyclin-dependent kinase 5 **(Qu et al., 2011)**, glyceraldehyde 3-phosphate dehydrogenase **(Kornberg et al., 2010; Zaffagnini et al., 2013)**, and non-canonical catalase ROG1 **(Chen et al., 2020).**

The microenvironment around a cysteine residue defines its NO accessibility and reactivity. Cysteine residues exhibiting a low-pKa sulfhydryl group are particularly susceptible to certain types of redox modification **(Spadaro et al., 2010)**. In the past, different consensus motifs for *S*-nitrosation have been defined by comparing the amino acid sequences around identified target cysteine residues. In general, such NO sensitive cysteine residues are often located within an acid-base or hydrophobic motif **(Stamler et al., 2001)**, while **Greco et al. (2006)** supported the idea of extending the motif beyond the primary sequence including hydrophobic motifs nearby the target cysteine residues **(Greco et al., 2006)**. Based on amino acid sequence comparison of S-nitrosated proteins, several different consensus sequences for *S*-nitrosation have been described. **Stamler and colleagues (1997)** proposed an acid-base motif for protein *S*-nitrosylation and denitrosylation. The acid-base motif comprises flanking acidic (Asp (D), Glu (E)) and basic (Arg (R), His (H), Lys (K)) residues to the reactive thiol cysteine sites ([KRHDE]-C-[DE]). Moreover, a GSNO binding motif is described ([HKR]-C-[hydrophobic]X[DE]) **(Hess et al., 2005)**. Analysis of 1195 sequences of *S*-nitrosated peptides identified in *GSNOR-KO* plants **(Hu et al., 2015)** revealed 10 motifs, including EXC, EC, CD, CE, CXXE, CXD, CXE, DXXC, DC, and EXXXC, harboring conserved negatively charged amino acids glutamate (E) or aspartate (D) in close proximity of the *S*-nitrosated cysteine residue. Although such charged motifs have been shown to be predictive in a number of cases, the common features of acid-base motifs are still object of intense discussions and there are still no general rules, which can explain which cysteine residue is a target for NO.

In contrast, other studies have demonstrated on the peptide level that the sequence of the surrounding amino acids has no significant effect on the reactivity of cysteines towards *S*-nitrosation **(Taldone et al., 2005)**. Moreover, analysis of 70 *S*-nitrosation sites revealed that proximal acid–base motif, Cys pKa, sulfur atom exposure, and hydrophobicity in the vicinity of the modified cysteine do not predict *S*-nitrosation specificity. Instead, a revised acid-base motif that is located farther from the target cysteine and in which the charged groups are exposed has been identified **(Marino and Gladyshev, 2010)**. This emphasizes also the importance of the three-dimensional

folding, which needs to be considered whenever defining the NO sensitivity of a cysteine residue **(Kovacs and Lindermayr, 2013)**.

In recent two decades, much effort has been made to identify *S*-nitrosated proteins in plants. A number of indirect mass spectrometry (MS)-based proteomics approaches have been developed to identify *S*-nitrosated proteins and their modification sites from complex biological samples **(Jaffrey and Snyder, 2001; Hao et al., 2006, Camerini et al, 2007; Chouchani et al., 2010; Hu et al., 2015)**. The biotin switch technique (BST) is the most widely used method and is based on the conversion of *S*-nitrosated Cys to biotinylated Cys **(Jaffrey and Snyder, 2001)**. Such a labelling allows the detection of *S*-nitrosated proteins using specific anti-biotin antibodies and their enrichment by affinity chromatography using neutravidin matrices. Finally, the enriched proteins are identified by MS. Variants of the BST assay, including quantitative approaches and the use of protein microarrays have been reported and successfully used **(Torta et al., 2008; Astier et al., 2011; Seth and Stamler, 2011; Wang and Xian, 2011; Lee et al., 2014)**. Including a digest step before purification allows the enrichment of peptides containing NO-targeted cysteine residues (SNOSID) **(Hu et al., 2015)**. Modification of the BST method enabled quantification of *S*-nitrosated proteins *via* fluorescent labelling **(Santhanam et al., 2008)** or *via* the use of isobaric iodoacetyl tandem mass tags (iodoTMT) (**Qu et al., 2014)**. Furthermore, proteins can also react with a thiol-reactive resin allowing on-resin enzymatic digestion before MS analysis. This resin-assisted capture (SNO-RAC) requires fewer steps, detects high-mass *S*-nitrosated proteins more efficiently, and facilitates identification and quantification of *S*-nitrosated sites by mass spectrometry **(Forrester et al., 2009; Kolbert et al., 2019)**.

Until now, several hundreds of endogenously *S*-nitrosated proteins have been identified in proteome wide-scale studies in plants, whereas NO donor treatments are often used to increase the amount of *S*-nitrosated proteins. *S*-nitrosated proteins function in major cellular activities of the primary and secondary metabolism and regulate processes related to biotic and abiotic stress response **(Astier et al., 2012)**. However, these candidates need confirmation by candidate-specific approaches for the physiological relevance. This includes also the identification of the NO-sensitive cysteine residue(s) of these proteins.

Tyrosine nitration: mechanism, specificity, selectivity, identification in plants

Tyrosine is a moderately hydrophilic aromatic amino acid, which is therefore often on the surface of the protein and thus subject to modifications. Nitration reaction may be catalysed by $ONOO^-$ or by nitrogen dioxide radical formed in the reaction between hydrogen peroxide and nitrite in the presence of hemoperoxidase enzyme. Peroxynitrite is a strong oxidizing and nitrating agent resulting from the reaction between superoxide anion radical and NO, mainly at the sites of superoxide formation (**Radi et al., 2001**, **Szabó et al., 2007, Fig 1**). During nitration of tyrosine amino acid, a nitro group is attached to the hydroxyl group of the *ortho* carbon atom in the aromatic ring leading to the formation of 3-nitrotyrosine (YNO_2). The process takes place in two steps, since the attachment of the nitro group is preceded by a one electron oxidation of the tyrosine aromatic ring to tyrosyl radical. The major oxidants are hydroxyl radical and carbonate radical which originate from $ONOO^-$ due to diverse reactions (**Kolbert et al., 2017**). As a consequence of YNO_2 formation, the key physical and chemical properties including pKa, redox potential, hydrophobicity/hydrophilicity, molecular size of amino acids may be modified (**Sabadashka et al., 2021**). Due to these physico-chemical alterations, the structure and function of the target protein may be changed. In animal systems, accumulating evidence suggest the reversibility and consequently the signalling function of tyrosine nitration (**Sabadashka et al., 2021**). In contrast, most of the nitrated plant enzyme proteins examined in detail so far show activity loss indicating that tyrosine nitration may be a signal for degradation (**Kolbert et al., 2017**).

Protein tyrosine nitration is a relatively widespread PTM because it affects numerous proteins in different organs of plants grown under diverse conditions (both unstressed and stressed). At the same time tyrosine nitration can be considered as highly selective, since only 1-2% of the total tyrosine proteome (3% of the whole proteome) may be exposed to *in vivo* nitration (**Bartesaghi et al., 2007**). Consequently, the total yield (expressed as mole of 3-nitrotyrosine/mole tyrosine) is low, as was determined in hypocotyls of sunflower grown under physiological conditions (**Chaki et al., 2009**). Nitration of protein tyrosine is a selective process despite the fact that no consensus sequence ensuring selectivity has been convincingly confirmed (**Bartesaghi and Radi, 2018**). Rather, some common features appear to affect YNO_2 formation such as the presence of acidic residues next to the YNO_2 site, cysteine or methionine neighbouring the target tyrosine residue and the presence of loop-forming amino acids such as proline or glycine (**Souza et al., 2008**). Beyond the amino acid sequence, additional factors

influence the nitration process including the centrifugal-centripetal position of the tyrosine residue within the three-dimensional (3D) structure of the protein and the cellular and redox environment of the target protein (**Yeo et al., 2015**; **Bartesaghi and Radi, 2018**).

In plant studies, the one- and two-dimensional gel electrophoresis followed by immunochemical detection of nitrated proteins are frequently used approaches. Protein identification by regular MS/MS in combination with immuno-enrichment of tyrosine-nitrated peptides is possible. For detecting the nitrated peptides matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS and LC-MS/MS can be used (**Yeo et al. 2015**; **Batthyány et al., 2017**). In most plant studies, immune-affinity based approaches was optimized for identifying tyrosine nitrated-proteins (e.g. **Corpas et al., 2008; Lozano-Juste et al., 2011; Cecconi et al., 2009; Tanou et al., 2012; Begara-Morales et al., 2013ab**, **2019; Takahashi and Morikava, 2019**). However, false positive detection may happen due to non-specific antibody binding and the identified protein occasionally mismatch the protein database (**Corpas et al., 2013a**). Thus MS assays are being continuously improved in order to provide more accurate detection of tyrosine nitrated proteins and peptides (**Ng et al., 2013; Tsikas and Duncan, 2013; Yeo et al., 2015; Batthyány et al., 2017; Chaki et al., 2018**). To date, large-scale studies identified more than one hundred plant proteins as *in vivo* targets of tyrosine nitration in the organs of healthy and stressed plants. For most of these proteins, the YNO_2 site and the change in activity/function have not been studied.

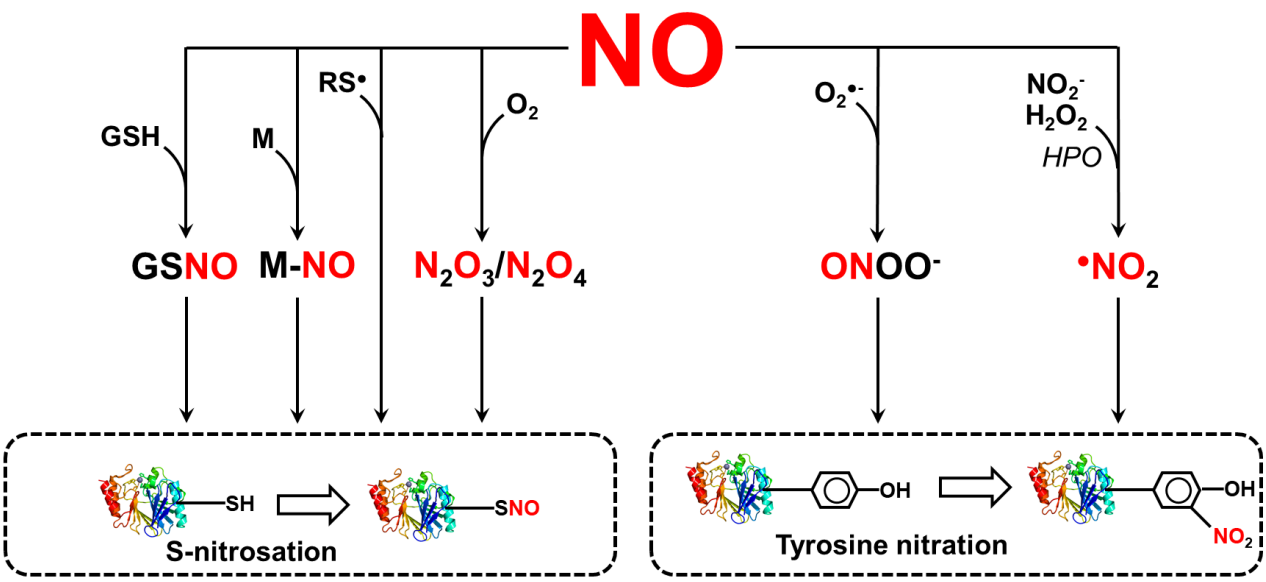

Fig 1. Reactions leading to the formation of reactive nitrogen species which are responsible for posttranslational modifications such as *S*-nitrosation and tyrosine nitration. See explanations in the text. Abbreviations: NO, nitric oxide; GSH, glutathione; GSNO, *S*-nitrosoglutathione; M, metal; $RS^{\bullet}$, thiyl radical; O_2, oxygen; N_2O_3, dinitrogen trioxide; N_2O_4, dinitrogen tetroxide, $O_2^{\bullet-}$, superoxide anion radical; $ONOO^-$, peroxynitrite; NO_2^-, nitrite; H_2O_2, hydrogen peroxide; HPO, hemoperoxidase; $^{\bullet}NO_2$, nitrogen dioxide radical.

Computational tools for predicting NO-dependent PTMs

Although many different experimental methods have been developed for accurate identification of NO target cysteine residues, these are often still associated with technical difficulties based on the instability of SNOs. For instance, direct detection of NO-modified thiols by MS or X-ray crystallography is still very challenging and only possible on recombinant proteins. Moreover, such approaches are time-consuming and cost-intensive. The situation is similar with the analytical determination of YNO_2, as there are methodological challenges during the detection: (i) endogenous levels of YNO_2 are very low, (ii) the vast excess of tyrosine in the samples disturbs the detection and quantification of YNO_2 (iii) special precautions must be taken since YNO_2 may be formed during sample preparation (**Tsikas and Duncan, 2013**). Therefore, the computational approach of screening proteins for NO sensitive cysteine or tyrosine residues is an attractive alternative since the recent progress of machine learning makes possible the efficient use of computational prediction preceding the laboratory experimentation. With the availability of a huge amount of amino acid sequences, it is possible to develop computational methods for predicting SNO or YNO_2 sites in proteins. Such kind of information is very useful for both basic research and application. **Table 1** summarizes the developed tools either for predicting SNO sites or YNO_2 sites, or both.

Table 1 List of software tools developed so far for predicting NO-dependent PTMs (*S*-nitrosation and tyrosine nitration). Modified from **Bignon et al. (2018)**.

Name	Year	Availibility	Link	number of citations (1st of June 2021)	Citation	Note
SNO prediction						

GPS-SNO	2010	web server, standalone	http://sno.biocuckoo.org/	157	Xue et al., 2010	
SNOSite	2011	web server	http://csb.cse.yzu.edu.tw/SNOSite/	69	Lee et al., 2011	link doesn't work
iSNO-PseACC	2013	web server	http://app.aporc.org/iSNO-PseAAC/index.html	345	Xu et al., 2013a	
iSNO-AAPAir	2013	web server	http://app.aporc.org/iSNO-AAPair/	249	Xu et al., 2013b	
PSNO	2014	web server	http://59.73.198.144:8088/PSNO/	82	Zhang et al., 2014	link doesn't work
PreSNO	2019	web server	http://kurata14.bio.kyutech.ac.jp/PreSNO/	21	Hasan et al., 2019	
RecSNO	2021	web server	http://nsclbio.jbnu.ac.kr/tools/RecSNO/	1	Siraj et al., 2021	
YNO_2 prediction						
GPS-YNO2	2011	web server, standalone	http://yno2.biocuckoo.org/	66	Liu et al., 2011	
iNitro-Tyr	2014	web server	http://app.aporc.org/iNitro-Tyr/	209	Xu et al., 2014	
PredNTS	2021	web server	http://kurata14.bio.kyutech.ac.jp/PredNTS/	1	Nilamyani et al., 2021	
iNitroY-Deep	2021	webserver	http://3.15.230.173/	0	Naseer et al., 2021	link does

						n't work
Both SNO and YNO_2 prediction						
DeepNitro	2018	web server	http://deepnitro.renlab.org	33	Xie et al. 2018	

Tools for computational prediction of *S*-nitrosation sites and testing their performance

The algorithms developed to identify NO-sensitive cysteine residues include GPS-SNO, SNOSite, iSNOPseAAC, iSNO-AAPair, RecSNO, PreSNO, and DeepNitro **(Lee et al., 2011; Xu et al., 2013a; Xu et al., 2013b; Xue et al., 2010; Hasan et al., 2019; Xie et al., 2018; Siraj et al., 2021; Zhang et al., 2014)**. A big disadvantage of these computational methods is still the non-consideration of the 3D structure of the proteins. Cysteine residues, which might be predicted as target for *S*-nitrosation could be buried inside the protein and in this way inaccessible for NO. Moreover, for calculating the NO-sensitivity of a cysteine residue, the algorithms consider only amino acids, which are nearby a cysteine residue in the primary structure. However, in the folded protein amino acids, which are far away in the primary structure, could get in close vicinity of a cysteine residue and affect its microenvironment.

The first released online tool for SNO-site prediction was GSP-SNO 1.0 in 2010 **(Xue et al., 2010)**. The leave-one-out validation and 4-, 6-, 8-, 10-fold cross-validations were calculated to evaluate the prediction performance and system robustness. The GPS 3.0 algorithm performed quite well with an accuracy of 75.70%, a sensitivity of 55.32% and a specificity of 80.11% under the low threshold. The online service and local packages of GPS-SNO 1.0 were implemented in JAVA 1.4.2 and freely available at: http://sno.biocuckoo.org/.

One year later, the software tool SNOSite was presented **(Lee et al., 2011)**. The authors used a total of 586 experimentally identified *S*-nitrosation sites from *S*-nitroso-L-penicillamine (SNAP)/L-cysteine-stimulated mouse endothelial cells for an informatics analysis on *S*-nitrosation sites including structural factors such as the flanking amino acids composition, the accessible surface area and physicochemical properties, i.e. positive charge and side chain interaction parameter. Maximal dependence

decomposition (MDD) has been applied to obtain statistically significant conserved motifs. Support vector machine (SVM) is applied to generate predictive model for each MDD-clustered motif. According to five-fold cross-validation, the MDD-clustered SVMs could achieve an accuracy of 0.902, and provides a promising performance in an independent test set. The MDD-clustered model was adopted to construct an effective web-based tool, named SNOSite (http://csb.cse.yzu.edu.tw/SNOSite/), for identifying *S*-nitrosation sites on the uncharacterized protein sequences. At the time of writing this review, SNOSite is not available.

In 2013, a new predictor, called iSNO-PseAAC, was developed for identifying the SNO sites in proteins by incorporating the position-specific amino acid propensity (PSAAP) into the general form of pseudo amino acid composition (PseAAC) **(Xu et al., 2013a)**. The predictor was implemented using the conditional random field (CRF) algorithm. The overall cross-validation success rate achieved by iSNO-PseAAC in identifying nitrosylated proteins on an independent dataset was over 90%, indicating that the new predictor is quite promising. A web server for iSNO-PseAAC is available at http://app.aporc.org/iSNO-PseAAC/, where users can easily obtain the desired results without the need to follow the mathematical equations involved during the process of developing the prediction method. Then same group published another prediction tool called iSNO-AAPair **(Xu et al., 2013b)**. This algorithm was developed by considering the coupling effects for all the pairs formed by the nearest residues and the pairs by the next nearest residues along protein chains. A web server for iSNO-AAPair was established at http://app.aporc.org/iSNO-AAPair/.

In 2014, Zhang and co-workers presented a new bioinformatics tool, named PSNO, to identify SNOs from amino acid sequences **(Zhang et al., 2014)**. They explored various promising sequence-derived discriminative features, including the evolutionary profile, the predicted secondary structure and the physicochemical properties and used the relative entropy selection and incremental feature selection approach to select the optimal feature subsets. Afterwards, they trained their model by the technique of the *k*-nearest neighbour algorithm. Using both informative features and an elaborate feature selection scheme, the PSNO method achieved good prediction performance with a mean Mathews correlation coefficient (*MCC*) value of about 0.5119 on the training dataset using 10-fold cross-validation. The PSNO web server was established at http://59.73.198.144:8088/PSNO/, but at the time of writing this review it is not accessible.

Four years later, Xie and colleagues developed a computational tool for predicting nitration and nitrosation sites in proteins **(Xie et al., 2018)**. They constructed positional amino acid distributions, sequence contextual dependencies, physicochemical properties, and position-specific scoring features, to represent the modified residues. Based on these encoding features, they established a predictor called DeepNitro using deep learning methods for predicting *S*-nitrosation. Using n-fold cross-validation, the evaluation shows great AUC value for DeepNitro, of 0.70 for cysteine nitrosation, demonstrating the robustness and reliability of the predictor. The application of deep learning method and novel encoding schemes, especially the position-specific scoring feature, seems to improve the accuracy of *S*-nitrosation site prediction. DeepNitro is implemented in JAVA and PHP and is freely available for academic research at http://deepnitro.renlab.org.

A novel predictor PreSNO has been developed that integrates multiple encoding schemes by the support vector machine and random forest algorithms **(Hasan et al., 2019)**. The PreSNO achieved an accuracy and MCC value of 0.752 and 0.252 respectively in classifying between SNO and non-SNO sites when evaluated on the independent dataset, outperforming the existing methods. The web application of the PreSNO and its associated datasets are freely available at http://kurata14.bio.kyutech.ac.jp/PreSNO/.

The latest SNO-site prediction tool is called RecSNO and was published in 2021 by Siraj and colleagues **(Siraj et al., 2021)**. They proposed an end-to-end deep learning based *S*-nitrosation site predictor with an embedded layer and bidirectional long short-term memory. This method uses amino acid sequences as inputs without any need for complex features interventions. This sequence-based protein prediction method is associated with a significant improvement in identification of *S*-nitrosation sites. The best prediction of the proposed architecture showed an improvement of in MCC 3% on 5-fold cross validation and 5% on an independent test dataset. The user-friendly publicly available web server is accessible at http://nsclbio.jbnu.ac.kr/tools/RecSNO/.

It has to be emphasized that the prediction tools GPS-NO and DeepNitro have both an option for selecting a threshold (low, medium, high) allowing to altering the stringency of the SNO site prediction. Similarly, a threshold between 0 and 1.0 can be selected in RecSNO. All other available SNO site prediction tools work with a fixed stringency.

NO-sensitive cysteine residues can be experimentally identified/verified by MS or by generation and analysis of cysteine mutants. Although MS allows the direct identification of the modified cysteine residues, cysteine mutants are often additionally analysed, especially if the physiological function of the *S*-nitrosated protein needs to be characterized. In this case, knock-out/knock-down plants of the NO-sensitive protein is complemented with corresponding cysteine mutants to get hints to the physiological function of the *S*-nitrosated proteins and to verify the NO-sensitivity of the cysteine residue(s) *in vivo*. This approach is the gold standard for characterisation of protein *S*-nitrosation. However, because of different reasons such as *in vivo* analyses are not always possible, e. g. if knock-out/knock-down lines are not available. In this case, recombinant proteins of the cysteine mutants can be produced and analysed for their NO-sensitivity, provided, that enzymatic or functional assays are available. Until now, 32 NO-sensitive cysteine residues have been identified/verified in 26 plant proteins by MS or by generation and analysis of cysteine mutants **(Table 2)**. We have chosen these 26 proteins to compare the prediction efficiency of the available SNO site prediction software. **Table 2** shows that the different computational programs have predicted SNO sites in the selected proteins with different efficiency. GPS-SNO, iSNO-PseAAC, iSNO-AAPair and RecSNO identified between 20 and 22 of the 26 analysed proteins as targets for S-nitrosation, whereas DeepNitro and PreSNO identified 15 and 10, respectively. Moreover, the first published online tool for SNO site detection, GPS-SNO, as well as the newer tools DeepNitro and PreSNO predict 31, 24 and 16 putative SNO sites, respectively, including 13 (GPS-SNO) and 9 (DeepNitro and PreSNO) verified SNO sites. These three prediction tools have a hit rate (number of matched SNO sites divided by the total number of predicted SNO sites) of 42% (GPS-SNO), 38% (DeepNitro) and 56% (PreSNO). The other computational tools, such as iSNO-PseAAC, iSNO-AAPair or RecSNO predict a much higher number of putative NO-sensitive cysteine residues - 83, 39, and 60, respectively – whereas only 11 (iSNO-PseAAC), 7 (iSNO-AAPair) and 10 (RecSNO) are matching with experimentally identified/verified SNO sites. This quite high rate of mis-prediction is making these three tools less useful. The prediction efficiency of the different online tools is further characterized by calculating their sensitivity (Sn), specificity (Sp) and accuracy (AC) as described by **Nilamyani et al. (2021)** (**Table 3).** Sensitivity is the proportion of true positives that are correctly identified by the prediction algorithm, specificity is the proportion of the true negatives correctly identified by the software and

accuracy is the proportion of true results, either true positive or true negative, in a population **(Wihinen, 2012)**.

Table 2 List of plant proteins in which the *S*-nitrosated cysteine residues have been experimentally identified. *S*-nitrosated sites in the listed proteins were computationally predicted using GSP-SNO 1.0, iSNO-PseAAC, iSNA-AAPair, DeepNitro, PreSNO and RecSNO software. Bold indicates matched cysteine residue.

Protein name	**Accession number**	**Total number of Cys**	**Identified by LC-MS/MS or mutation**	**Predicted by GPS-SNO 1.0 (2010) (medium threshold)**	**Predicted by iSNO-PseAAC (2013)**	**Predicted by iSNO-AAPair (2013)**	**Predicted by DeepNitro (2018) (medium threshold)**	**Predicted by PreSNO (2019)**	**Predicted by RecSNO (2020) 0.6 threshold**	**Citation**
NPR1	At1g64280	17	**C156**	**C156**, C385	C212, C306	C223, C306, C394, C457	non	non	C82, C212, C216, C223, C378, C394, C457	Tada et al., 2008
SAMS1	At1g02500	8	**C114**	**C114**	C161	C31, C90, C161	non	**C114**	C45, C73, C90, C161	Lindermayr et al, 2006
OST1 (SnRK2.6)	At4g33950	7	**C137**	non	C107, C159, C203	C131, C203	non	**C137**	C159,	Wang et al., 2015
ASK1	At1g75950	3	**C37, C118**	**C118**	non	C59, **C118**	**C118**	non	C59, **C118**	Iglesias et al., 2018
SCE1	At3g57870	4	**C139**	non	C94, **C139**	**C139**	**C139**	non	non	Skelly et al., 2019
SRG1	At3g46080	7	**C87**	**C87**	**C87**	C28	non	non	C18, C28,	Cui et al., 2018
AHP1	At3g21510	4	**C115**	non	C104	**C115**	non	non	non	Feng et al., 2013

cALD2	At2g36460	6	**C173**	C68, C326	C326	C208	C326	non	C197, C208, C326	van der Linde et al., 2011
TIR1	At3g62980	23	**C140**	C516, C551	C34, C53, C121, **C140**, C155, C210, C269, C288, C311, C405, C480, C491	C121, **C140**, C405, C551	C53, C516	non	C53, C121, C551	Terrile et al., 2012
MC9	At5g04200	7	**C147**	C17, **C147**	C17, C29	C117	C17, C29, **C147**	**C147**	C17, C29, C117, **C147**, C251	Belenghi et al., 2007
PRXII E	At3g52960	2	**C121**	**C121**	**C121**, C146	**C121**	**C121**	**C121**	**C121**, C146	Romero-Puertas et al., 2007
GAPDH	At1g13440	2	**C156, C160**	**C156, C160**	non	non	**C156, C160**	**C156, C160**	**C156**	Holtgrefe et al., 2008
SABP3	At3g01500	7	**C280**	C34, C173, **C280**	C230, C257	C34	non	non	C34, C173, C230	Wang et al., 2009
NADPH Oxidase (RBOHD)	At5g47910	10	**C890**	non	C208, C387, C433, C480, C695	C412, C480, C695, **C890**	C695, **C890**	non	C433, C695, **C890**	Yun et al., 2011

TGA1	At5g65210	4	**C172, C287**	**C172**	non	non	non	C260, C266	non	Lindermayr et al., 2010
CDC48	Q1G0Z1 *Nicotiana tabacum*	14	**C110, C526, C664**	C426, C576	C74, C82, **C110**, C526, C539, C576, **C664**, C699	C74, C426, C539, C576	**C110**, C419, C539, **C664**	**C526**, C539	C74, C82, **C110**, C272, C539, C695	Astier et al., 2012
MYB30	At3g28910	7	**C53**	C6	C6, C7, C49, **C53**, C257, C289	C6, C7	C49	C49, **C53**	non	Tavares et al., 2014
PDK1	Q5I6E8, *Solanum lycopersicum*	4	**C128**	C214	C128, C214, C244, C466	non	non	non	non	Liu et al., 2017
GSNOR	At5g43940	15	**C10**	**C10**, C283	**C10**, C59, C77, C117, C125, C189, C283, C296, C385	C59	non	non	**C10**, C382, C385	Guerra et al., 2016; Zhan et al., 2018
ROG1	At1g20620	7	**C343**	non	C230, C370, C402, C420	C402	C230	non	C86, C230, C370, C402	Chen et al., 2020
cFBP1	AAD10213, *Pisum sativum*	7	**C153**	C173	C178	C92, C306	C306	non	C49, C92, C306	Serrato et al., 2018

APX1	At1g07890	5	**C32**	C119	**C32**, C138	C19, **C32**	C138	**C32**, C49	**C32**, C49, C138	Yang et al., 2015
ABI5	At2g36270	4	**C153**	**C153**, C440	C56, C440	non	C440	non	**C153**, C293	Albertos et al., 2015
PRMT5	At4g31120	12	**C125**	**C125**	C17, C70, **C125**, C141, C189, C238, C260, C610, C611	C238, C260	non	non	**C125**, C160	Hu et al., 2015
GAPC1	At3g04120	2	**C149**	C156, C160	non	non	C156, C160	C156, C160	C156	Zaffagnini et al., 2013
VND7	At1g71930	4	**C264, C340**	C320	C58, C153, **C264**, C320	non	non	non	non	Kawabe et al., 2013
Number of proteins predicted as targets for NO			**26**	**21**	**22**	**20**	**15**	**10**	**20**	
Predicted SNO sites			**32**	**31**	**83**	**39**	**24**	**16**	**60**	
Cys match (verified vs.				**13 (42%)**	**11 (13%)**	**7 (18%)**	**9 (38%)**	**9 (56%)**	**10 (17%)**	

predicted)										

384

Table 3 Values of sensitivity, specificity and accuracy of SNO predicting software tools. Metrics were calculated based on the predictions in 26 experimentally identified *S*-nitrosated plant proteins listed in Table 2.

Software	Sensitivity (Sn, %)	Specificity (Sp, %)	Accuracy (Acc, %)
GPS-SNO (medium threshold)	46.07	67.48	61.59
iSNO-PseAAC	42.66	45.14	46.89
iSNO-AAPair	26.00	63.92	56.64
DeepNitro (medium threshold)	24.66	47.96	43.38
PreSNO	29.20	29.96	29.77
RecSNO (0.6 threshold)	33.20	42.69	44.00

Tools for computational prediction of tyrosine nitration sites and testing their performance

The first software for predicting YNO_2 sites in proteins using the FASTA format of peptide sequence was GPS-YNO2 1.0 which was published in 2011 by **Liu and co-workers (Liu et al, 2011)**. The algorithm is based on the biochemical properties of neighbouring amino acids and it showed promising performance (accuracy of 76.51%, sensitivity of 50.09%, specificity of 80.18%) using leave-one-out validation and 4-, 6-, 8-, 10-fold cross-validations. The tool can be used online or as a local package both implemented in JAVA. It is freely available at: http://yno2.biocuckoo.org/.

In 2014, a novel predictor algorithm called iNitro-Tyr was developed (**Xu et al., 2014**). It is based on the incorporation of the position-specific dipeptide propensity into the general pseudo amino acid composition which allows the proper discrimination of the YNO_2 sites from the non-nitrated ones. It was demonstrated by the rigorous jackknife tests that iNitroTyr shows higher success rate and stability and is less noisy than GPS-YNO_2. This algorithm indicates the total number of tyrosine residues within the protein sequence which is useful information. iNitroTyr is freely available online at: http://app.aporc.org/iNitro-Tyr/.

In 2018, DeepNitro a predictor simultaneously identifies sites of *S*-nitrosation, tyrosine nitration and tryptophan nitration has been developed (**Xie et al., 2018**).

One of the most recent computational predictors for identifying YNO_2 sites is PredNTS published by **Nilamyani et al. (2021)**. The algorithm was developed by integrating multiple sequence features including K-mer, composition of k-spaced amino acid pairs, AAindex and binary encoding schemes. Using a comprehensive dataset,

PredNTS outperformed the previously developed predictors. The software is freely available at: http://kurata14.bio.kyutech.ac.jp/PredNTS/.

The other recently developed predictor is iNitroY-Deep which uses pseudo amino acid compositions and deep neural networks (DNNs) **(Naseer et al., 2021)**. Using widely-accepted model evaluation measures, iNitroY-Deep outperformed the previously published nitrotyrosine predictor tools. The web server was established at http://3.15.230.173/, but at the time of writing this review it is not accessible.

In order to evaluate the performance of the available tyrosine nitration predicting tools, we performed *in silico* analysis of proteins with nitrated tyrosine residues identified by LC-MS/MS. Among those, 11 proteins were tested by GPS-YNO_2 and iNitro-Tyr in our previous work (**Kolbert et al., 2017**) and the list has been supplemented by recently identified proteins **(Table 4)**. Of the 15 nitrated proteins, 14 were identified as candidates by GPS-YNO_2 software, 12 by iNitro-Tyr, 13 by DeepNitro and 15 by PredNTS. In the 15 proteins, 36 YNO_2 sites have been experimentally identified and the number of YNO_2 sites predicted by the software tools was variable. The DeepNitro tool assigned 27 tyrosine amino acids as candidates for being nitrated (which is the 75% of the experimentally identified sites), while the recently developed PredNTS indicated 104 sites in 15 proteins, which is 3-fold more than the experimentally identified sites. Both GPS-YNO_2 and iNitro-Tyr predicted 41 YNO_2 sites in 15 proteins. The highest number of YNO_2 sites were assigned by PredNTS, and accordingly this tool showed the highest match rate, since one or more predicted nitrated sites matched the experimentally identified ones for 12 of the 15 proteins. When we calculated the hit rate, we found that those are relatively low, and DeepNitro had the highest hit value (26%). It has to be noted that of the 36 MS-identified YNO_2 sites only 18 sites matched the predictions of one of the programmes indicating 50% agreement between *in silico* and experimental results. This number was significantly lower (only 4 out of 26, 15%) when two software tools (GPS-YNO_2 and iNitro-Tyr) were tested (**Kolbert et al., 2017**). It can be concluded that all available tools are advisable to use for a certain protein in order to predict as many YNO_2 sites as possible.

Table 4 List of plant proteins in which the nitrated tyrosine residues have been experimentally identified. Nitration sites in the listed proteins were computationally predicted using GSP-YNO_2 1.0, iNitro-Tyr, DeepNitro, PredNTS software. Bold indicates matched tyrosine reidue.

Protein name	Accession number	Total number of Tyr	Identified by LC-MS/MS or mutation	Predicted by GPS-YNO2 (2011) (medium threshold)	Predicted by iNitro-Tyr (2014)	Predicted by DeepNitro (2018) (medium threshold)	Predicted by PredNTS (2021)	Citation
MS1	At5g17920	26	Y287	Y463, Y469, Y698	Y141, Y623, Y650	Y141, **Y287**, Y463	Y8, Y132, Y141, Y161, Y188, Y226, Y243, **Y287**, Y453, Y463, Y581, Y740	Lozano-Juste et al., 2011
OASA1	At4g14880	7	Y302	Y158	non	**Y302**	Y20, Y91,Y143, Y158, Y192, Y203, **Y302**	Álvarez et al., 2011
psbA	AtCg00020	12	Y262	Y73, Y107, Y237, Y246	Y246	Y237, Y246	**Y262**	Galetskiy et al., 2011
IDH (NADP)	Q6R6M7 *Pisum sativum*	14	Y392	Y69, Y210, Y221, Y274	Y172, Y185, Y221, Y233,Y241, Y259, Y274	Y274	Y43, Y69, Y141, Y172, Y185, Y210, Y221, Y233,	Begara-Morales et al., 2013a

							Y274, **Y392**	
APX, cytosolic	P48534 *Pisum sativum*	7	Y5, Y235	**Y5**	**Y5**, Y93	non	**Y5**, Y12, Y224, Y235	Begara-Morales et al., 2013b
HPR, peroxisomal	At1g68010	11	Y97, Y108, Y198	Y10, **Y108**, Y150	Y10,Y150, Y251	**Y97**, Y180	**Y97**	Corpas et al., 2013b
PYR1	At4g17870	4	Y23, Y58, Y120	non	non	**Y23**	**Y23, Y58, Y120**, Y143	Castillo et al., 2015
MnSOD1, mitochondrial	At3g10920	10	Y38, Y40, Y63, Y67, Y198, Y199, Y202	**Y63**, Y226	**Y63, Y67,** Y226	**Y63**	**Y63, Y67**, Y209, Y221, Y226	Holtzmeister et al., 2015
Leghemoglobin-1	P02232 *Vicia faba*	3	Y25, Y30, Y133	Y134	non	non	**Y25,Y30**, Y134	Sainz et al., 2015
MDHAR	Q66PF9 *Pisum sativum*	22	Y213, Y292, Y345	Y154, Y34	Y7, Y192, **Y292**	**Y292**, Y383	Y7, Y44, Y53, Y89, Y114, Y143, Y154, Y172, **Y292**, Y305, Y383	Begara-Morales et al., 2015

PSBO1	At5g66570	8	Y9	Y94, Y102, Y328	Y236	Y94, Y102, Y131, Y236	Y94, Y102, Y169, Y328	Takahashi et al., 2015
NADP-MAE1	At2g19900	25	Y73	Y129, Y204, Y235, Y248, Y522, Y528, Y550	Y235, Y263, Y286, Y550, Y580	Y235, Y248, Y550	Y66, Y92, Y99, Y114, Y235, Y148, Y263, Y343, Y522, Y528, Y550, Y573, Y577, Y580	Begara-Morales et al., 2019
CDKA1	A0A3L6F4W4 *Zea mays*	11	Y15, Y19	Y11, Y178, Y222	Y78, Y231	**Y15**	Y11, **Y15**, Y73, Y78, Y178, Y194	Méndez et al., 2020
NIA1	At1g77760	30	Y548, Y614, Y714, Y771,	Y10, **Y548**, Y908	Y10, Y83, Y431, Y851, Y908	Y241, Y266, Y395, Y624	Y10, Y62, Y82, Y83, Y266, Y286, Y330, Y331, Y333, Y390, Y395, Y397, **Y548**, **Y614**, Y624, **Y714,**	Costa-Broseta et al., 2021

							Y771, Y802, Y851, Y862, Y908	
NIA2	At1g37130	34	Y545, Y714, Y771	Y10, Y77, **Y545**, Y908	Y77, Y392, Y428, Y568, Y802, Y908	Y182, Y271	Y10, Y76, Y77, Y99, Y182, Y271, Y328, Y387, Y382, Y394, **Y545**, Y611, Y621, **Y771**, Y802, Y819, Y822, Y843, Y851, Y908	Costa-Broseta et al., 2021
Number of proteins predicted as targets for NO			15	14	12	13	15	
Predicted YNO2 sites			36	41	41	27	123	

Tyr match (verified vs. predicted)				5 (12%)	4 (10%)	7 (26%)	21 (17%)	

Furthermore, based on the previously identified 15 nitrated proteins, sensitivity, specificity and accuracy were calculated in order to evaluate the performance of the software tools **(Table 5)**. The highest Sn value (~62%) was obtained in case of PredNTS, but the Sp and AC values of this tool were relatively low. The highest AC value was shown by the DeepNitro software supporting its better performance compared to the other programmes. In general, the above mentioned values are relatively low which indicates that the agreement of the *in silico* predictions with experimental data is moderate. This is partly due to the limitations of MALDI based methods used for identifying YNO_2 sites in proteins (**Ytterberg and Jensen, 2010**) and to the fact that prediction algorithms do not consider the 3D structures of the proteins which greatly affect the sensitivity to tyrosine nitration.

Table 5 Values of sensitivity, specificity and accuracy of YNO_2 predicting software tools. Metrics were calculated based on the predictions in 15 experimentally identified nitrated plant proteins listed in Table 2.

Software	Sensitivity (Sn, %)	Specificity (Sp, %)	Accuracy (Acc, %)
GPS-YNO2 (medium threshold)	10.00	65.70	57.36
iNitro-Tyr	7.40	60.66	52.36
DeepNitro (medium threshold)	24.21	77.11	62.86
PredNTS	61.76	39.15	49.38

Conclusion and future perspectives

Both *S*-nitrosation and tyrosine nitration are NO-dependent PTMs affecting plant proteins of various kinds from structural proteins to transporters and enzymes. *S*-nitrosation is directly involved in cell signalling while tyrosine nitration is thought to result in protein instability and degradation and it may indirectly affect signal transduction. Both PTMs are selective and specific, since not every Cys/Tyr is nitrosated/nitrated in a

protein's amino acid chain and not every Cys/Tyr-containing proteins are targets of these modifications. In the case of *S*-nitrosation various consensus amino acid sequences have been suggested; however, there is still no general rule explaining which cysteine residue is a target for NO. Similarly, there is no amino acid motif or any definite pattern in the protein structure which determines the target tyrosine for nitration. For both NO-dependent PTMs, some common physico-chemical features have been revealed. In the future, intensive effort should be directed on revealing the high-resolution structure of the microenvironment around each cysteine/tyrosine residue to get information about the physicochemical features that determine *S*-nitrosation/tyrosine nitration specificity.

In order to assign the target Cys and Tyr residues within a certain protein, specific computational tools have been developed. In the last ten years, 11 computational tools for predicting *S*-nitrosation, tyrosine nitration or both based on different algorithms have been created. In Table 1, the number of references indicates that these tools are frequently used by the scientific community. This is not surprising, since the predictors rapidly generate extensive information, while the laboratory experiments are lengthy and often technically cumbersome. Our tests on plant proteins showed that there are discrepancies between the experimentally confirmed and the predicted PTM sites, which may be due in part to the fact that the algorithms don't take into account the 3D protein structure.

Therefore, computational prediction of SNO or YNO_2 sites can't substitute laboratory work but can provide a starting point for experimental verification and the combination of computer-based prediction and experimental verification represents still a promising approach for a better understanding of the molecular mechanisms and the regulatory functions of protein *S*-nitrosation and tyrosine nitration. Before planning experiments, it is advisable to use all the available tools on the proteins of interest and compare the results of the predictions. Based on our analyses on plant proteins, *S*-nitrosation sites can be predicted by the available tools with higher confidence compared to the sites of tyrosine nitration. However, computational prediction still must be developed further to improve the precision with which *S*-nitrosation/tyrosine nitration-sites are identified. In this context, probably machine learning systems (artificial intelligence) based on experimentally verified *S*-nitrosated cysteine residues and nitrated tyrosine residues and 3D protein structures could provide a step further to successful prediction of NO-dependent PTM sites. But all these prediction approaches can finally not replace

the experimental analysis of the function of *S*-nitrosated or tyrosine nitrated proteins, including recombinant proteins, site-directed mutagenesis and *in vivo* experiments.

Funding: NO research in the Kolbert Lab is supported by the National Research, Development and Innovation Fund (Grant no. K135303). ZsK receives funding from János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

References

Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O (2015) S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. Nat Commun 6, 8669; https://doi.org/10.1038/ncomms9669

Álvarez C, Lozano-Juste J, Romero LC, García I, Gotor C, León J (2011) Inhibition of Arabidopsis O-acetylserine(thiol)lyase A1 by tyrosine nitration. J Biol Chem 286: 578-586.

Arnelle DR, Stamler JS (1995) NO^+, NO, and NO^- donation by *S*-nitrosothiols: implications for regulation of physiological functions by *S*-nitrosylation and acceleration of disulfide formation. Arch Biochem Biophys 318: 279-285.

Astier J, Rasul S, Koen E, Manzoor H, Besson-Bard A, Lamotte O, Jeandroz S, Durner J, Lindermayr C, Wendehenne D (2011) *S*-nitrosylation: An emerging post-translational protein modification in plants. Plant Sci 181: 527-533.

Astier J, Lindermayr C (2012) Nitric oxide-dependent posttranslational modification in plants: an update. Int J Mol Sci 13: 15193–15208.

Astier J, Besson-Bard A, Lamotte O, Bertoldo J, Bourque S, Terenzi H, Wendehenne D (2012) Nitric oxide inhibits the ATPase activity of the chaperone-like AAA+ ATPase CDC48, a target for *S*-nitrosylation in cryptogein signalling in tobacco cells. Biochem J 447: 249–260.

Astier J, Gross I, Durner J (2018) Nitric oxide production in plants: an update. J Exp Bot 69: 3401–3411.

Astier J, Mounier A, Santolini J, Jeandroz S, Wendehenne D (2019) The evolution of nitric oxide signalling diverges between animal and green lineages. J Exp Bot 70: 4355-4364.

Astier J, Rossi J, Chatelain P, Klinguer A, Besson-Bard A, Rosnoblet C, Jeandroz S, Nicolas-Francès V, Wendehenne D (2021) Nitric oxide production and signalling in algae. J Exp Bot 72: 781–792.

Bartesaghi S, Ferrer-Sueta G, Peluffo G, Valez V, Zhang H, Kalyanaraman B, Radi R (2007) Protein tyrosine nitration in hydrophilic and hydrophobic environments. Amino Acids 32: 501-15.

Bartesaghi S, Radi R (2018) Fundamentals on the biochemistry of peroxynitrite and protein tyrosine nitration. Redox Biol 14: 618–625.

Batthyány C, Bartesaghi S, Mastrogiovanni M, Lima A, Demicheli V, Radi R (2017) Tyrosine-nitrated proteins: proteomic and bioanalytical aspects. Antiox Redox Signal 26: 313–328.

Begara-Morales JC, Chaki M, Sánchez-Calvo B, Mata-Pérez C, Leterrier M, Palma JM, Barroso JB, FJ Corpas (2013a) Protein tyrosine nitration in pea roots during development and senescence. J Exp Bot 64: 1121–1134.

Begara-Morales JC, Sánchez-Calvo B, Chaki M (2013b) Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and *S*-nitrosylation. J Exp Bot 65: 527–538.

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Mata-Pérez C, Valderrama R, Padilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB (2015) Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and *S*-nitrosylation. J Exp Bot 66: 5983-5996.

Begara-Morales JC, Sánchez-Calvo B, Gómez-Rodríguez MV, Chaki M, Valderrama R, Mata-Pérez C, López-Jaramillo J, Corpas FJ, Barroso JB (2019) Short-term low temperature induces nitro-oxidative

stress that deregulates the NADP-Malic enzyme function by tyrosine nitration in *Arabidopsis thaliana*. Antioxidants 8, 448; https://doi.org/10.3390/antiox8100448

Belenghi B, Romero-Puertas MC, Vercammen D, Brackenier A, Inze D, Delledonne M, Van Breusegem F (2007) Metacaspase activity of *Arabidopsis thaliana* is regulated by *S*-nitrosylation of a critical cysteine residue. J Biol Chem 282: 1352–1358.

Berger A, Boscari A, Frendo P, Brouquisse R (2019) Nitric oxide signaling, metabolism and toxicity in nitrogen-fixing symbiosis. J Exp Bot 70: 4505–4520.

Bignon E, Allega MF, Lucchetta M, Tiberti M, Papaleo E (2018) Computational structural biology of *S*-nitrosylation of cancer targets. Front Oncol 8, 272; https://doi.org/10.3389/fonc.2018.00272

Broniowska KA, Hogg N (2012) The chemical biology of *S*-nitrosothiols. Antiox Redox Signal 17: 969–980.

Camerini S, Polci ML, Restuccia U, Usuelli V, Malgaroli A, Bachi A (2007) A novel approach to identify proteins modified by nitric oxide: the HIS-TAG switch method. J Proteome Res. 6: 3224–3231.

Castillo M-C, Lozano-Juste J, González-Guzmán M, Rodriguez L, Rodriguez PD, León J (2015) Inactivation of PYR/PYL/RCAR ABA receptors by tyrosine nitration may enable rapid inhibition of ABA signaling by nitric oxide in plants. Science Signal 8, ra89; https://doi.org/10.1126/scisignal.aaa7981

Cecconi D, Orzetti S, Vandelle E, Rinalducci S, Zolla L, Delledonne M (2009) Protein nitration during defense response in *Arabidopsis thaliana*. Electrophoresis 30: 2460–2468.

Chaki M, Valderrama R, Fernández-Ocaña A, Carreras A, López-Jaramillo J, Luque F, Palma JM, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Gómez-Rodríguez MV, Corpas FJ, Barroso JB (2009) Protein targets of tyrosine nitration in sunflower (*Helianthus annuus* L.) hypocotyls. J Exp Bot 60: 4221–4234.

Chaki M, Sánchez-Calvo B, Carreras A, Valderrama R, Begara-Morales JC, Corpas FJ, Barroso JB (2018) Identification of tyrosine and nitrotyrosine with a mixed-mode solid-phase extraction cleanup followed by liquid chromatography–electrospray time-of-flight mass spectrometry in plants. In: Mengel A., Lindermayr C. (eds) Nitric Oxide. Methods in Molecular Biology, vol 1747. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-7695-9_13

Chen L, Wu R, Feng J, Feng T, Wang C, Hu J, Zhan N, Li Y, Ma X, Ren B, Zhang J, Song CP, Li J, Zhou JM, Zuo J (2020) Transnitrosylation mediated by the non-canonical catalase ROG1 regulates nitric oxide signaling in plants. Develop Cell 53: 444–457.

Chouchani ET, Hurd TR, Nadtochiy SM, Brookes PS, Fearnley IM, Lilley KS, Smith RA, Murphy MP (2010) Identification of S-nitrosated mitochondrial proteins by S-nitrosothiol difference in gel electrophoresis (SNO-DIGE): implications for the regulation of mitochondrial function by reversible S-nitrosation. Biochem J 430: 49–59.

Corpas FJ, Chaki M, Fernandéz-Ocaña A, Valderrama R, Palma JM, Carreras A, Begara-Morales JC, Airaki M, del Río LA, Barroso JB (2008) Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. Plant Cell Physiol 49: 1711–1722.

Corpas FJ, Chaki M, Leterrier M, Barroso JB (2009) Protein tyrosine nitration. A new challenge in plants. Plant Signal Behav 4: 920–923.

Corpas FJ, Palma JM, del Río LA and Barroso JB (2013a) Protein tyrosine nitration in higher plants grown under natural and stress conditions. Front Plant Sci 4, 29; https://doi.org/0.3389/fpls.2013.00029

Corpas FJ, Leterrier M, Begara-Morales JC, Valderrama R, Chaki M, López-Jaramillo J, Luque F, Palma JM, Padilla MN, Sánchez-Calvo B, Mata-Pérez C, Barroso JB (2013b) Inhibition of peroxisomal hydroxypyruvate reductase (HPR1) by tyrosine nitration. Biochim Biophys Acta 1830: 4981-4989.

Costa-Broseta Á, Castillo MC, León J (2021) Post-translational modifications of nitrate reductases autoregulates nitric oxide biosynthesis in *Arabidopsis*. Int J Mol Sci 22, 549; https://doi.org/10.3390/ijms22020549

Cui B, Pan Q, Clarke D, Villarreal MO, Umbreen S, Yuan B, Shan W, Jiang J, Loake GJ (2018) S-nitrosylation of the zinc finger protein SRG1 regulates plant immunity. Nat Commun 9, 4226; https://doi.org/10.1038/s41467-018-06578-3

Fancy NN, Bahlmann A-K, Loake GJ (2017) Nitric oxide function in plant abiotic stress. Plant Cell Environ 40: 462-472.

Feng J, Wang C, Chen Q, Chen H, Ren B, Li X, Zuo J (2013) S-nitrosylation of phosphotransfer proteins represses cytokinin signalling. Nat Commun 4, 1529; https://doi.org/10.1038/ncomms2541

Fröhlich A, Durner J (2011) The hunt for plant nitric oxide synthase (NOS): is one really needed? Plant Sci 181: 401-404.

Galetskiy D, Lohscheider JN, Kononikhin AS, Popov IA, Nikolaev EN, Adamska I (2011) Mass spectrometric characterization of photooxidative protein modifications in Arabidopsis thaliana thylakoid membranes. Rapid Commun Mass Spectrom 25: 184–190.

Greco TM, Hodara R, Parastatidis I, Heijnen HF, Dennehy MK, et al. (2006) Identification of *S*-nitrosylation motifs by site-specific mapping of the *S*-nitrosocysteine proteome in human vascular smooth muscle cells. Proc Natl Acad Sci U S A 103: 7420–7425.

Guerra D, Ballard K, Truebridge I, Vierling E (2016) S-nitrosation of conserved cysteines modulates activity and stability of s-nitrosoglutathione reductase (GSNOR). Biochem 55: 2452–2464.

Hao G, Derakhshan B, Shi L, Campagne F, Gross SS (2006) SNOSID, a proteomic method for identification of cysteine *S*-nitrosylation sites in complex protein mixtures. Proc Natl Acad Sci U S A 103: 1012–1017.

Hasan MM, Manavalan B, Khatun S, Kurata H (2019) Prediction of *S*-nitrosylation sites by integrating support vector machines and random forest. Mol Omics 15: 451-458.

Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein *S*-nitrosylation: purview and parameters. Nature Reviews. Molecular Cell Biology 6: 150–166.

Hill BG, Dranka BP, Bailey SM, Lancaster JR Jr, Darley-Usmar VM (2010) What part of NO don't you understand? Some answers to the cardinal questions in nitric oxide biology. J Biol Chem 285: 19699–19704.

Holtgrefe S, Gohlke J, Starmann J, Druce S, Klocke S, et al. (2008) Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications. Physiol Plant 133: 211–228.

Holzmeister C, Gaupels F, Geerlof A, Sarioglu H, Sattler M, Durner J, Lindermayr C (2015) Differential inhibition of *Arabidopsis* superoxide dismutases by peroxynitrite-mediated tyrosine nitration. J Exp Bot 66: 989-99.

Horst BG, Yokom AL, Rosenberg DJ, Morris KL, Hammel M, Hurley JH Marletta MA (2019) Allosteric activation of the nitric oxide receptor soluble guanylate cyclase mapped by cryo-electron microscopy. eLife 8, e50634; https://doi.org/10.7554/eLife.50634

Hu J, Huang X, Chen L, Sun X, Lu C, Zhang L, Wang Y, Zuo J (2015) Site-specific nitrosoproteomic identification of endogenously *S*-nitrosylated proteins in *Arabidopsis*. Plant Physiol 167: 1731–1746.

Iglesias MJ, Terrile MC, Correa-Aragunde N, Colman SL, Izquierdo-Álvarez A, Fiol DF, París R, Sánchez-López N, Marina A, Calderón Villalobos LIA, Estelle M, Lamattina L, Martínez-Ruiz A, Casalongué CA (2018) Regulation of SCFTIR1/AFBs E3 ligase assembly by S-nitrosylation of Arabidopsis SKP1-like1 impacts on auxin signalling. Redox Biol 18: 200-210.

Ignarro LJ, Cirino G, Casini A, Napoli C (1999) Nitric oxide as a signaling molecule in the vascular system: an overview. J Cardio-vasc Pharmacol 34: 879–886.

Jaffrey SR, Snyder SH (2001) The biotin switch method for the detection of *S*-nitrosylated proteins. Sci STKE 2001, pl1; https://doi.org/10.1126/stke.2001.86.pl1

Jedelská T, Luhová L, Petřivalský M (2021) Nitric oxide signalling in plant interactions with pathogenic fungi and oomycetes. J Exp Bot 72: 848-863.

Kawabe H, Ohtani M, Kurata T, Sakamoto T, Demura T (2018) Protein S-nitrosylation regulates xylem vessel cell differentiation in Arabidopsis. Plant Cell Physiol 59: 17-29.

Kolbert Zs, Feigl G, Bordé Á, Molnár Á, Erdei L (2017) Protein tyrosine nitration in plants: Present knowledge, computational prediction and future perspectives. Plant Physiol Biochem 113: 56-63.

Kolbert Z, Molnar A, Oláh D, Feigl G, Horváth E, Erdei L, Ördög A, Rudolf EE, Barth TK, Lindermayr C (2019) S-nitrosothiol signaling is involved in regulating hydrogen peroxide metabolism of zinc-stressed *Arabidopsis.* Plant Cell Physiol 60: 2449-2463.

Kornberg MD, Sen N, Hara MR, Juluri KR, Nguyen JV, Snowman AM, Law L, Hester LD, Snyder SH (2010) GAPDH mediates nitrosylation of nuclear proteins. Nat Cell Biol 12: 1094–1100.

Kovacs I, Lindermayr C (2013) Nitric oxide-based protein modification: formation and site-specificity of protein S-nitrosylation. Front Plant Sci 4, 137; https://doi.org/10.3389/fpls.2013.00137

Lee TY, Chen YJ, Lu TC, Huang HD, Chen YJ (2011) SNOSite: exploiting maximal dependence decomposition to identify cysteine S-nitrosylation with substrate site specificity. PLoS One 6, e21849; https://doi.org/10.1371/journal.pone.0021849

Lee YI, Giovinazzo D, Kang HC, Lee Y, Jeong JS, Doulias PT, Xie Z, Hu J, Ghasemi M, Ischiropoulos H, Qian J, Zhu H, Blackshaw S, Dawson VL, Dawson TM (2014) Protein microarray characterization of the *S*-nitrosoproteome. *Mol Cell Proteomics* 13: 63–72.

Lindermayr C, Saalbach G, Bahnweg G, Durner J (2006) Differential inhibition of *Arabidopsis* methionine adenosyltransferases by protein *S*-nitrosylation. J Biol Chem 281: 4285–4291.

Lindermayr C, Sell S, Muller B, Leister D, Durner J (2010) Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. Plant Cell 22: 2894–2907.

Liu Z, Cao J, Ma Q, Gao X, Ren J, Xue Y (2011) GPS-YNO2: computational prediction of tyrosine nitration sites in proteins. Mol BioSyst 7: 1197-1204.

Liu JZ, Duan J, Ni M, Liu Z, Qiu WL, Whitham SA, Qian WJ (2017) S-Nitrosylation inhibits the kinase activity of tomato phosphoinositide-dependent kinase 1 (PDK1). J Biol Chem 292: 19743–19751.

Lopes-Oliveira PJ, Oliveira HC, Kolbert Zs, Freschi L (2021) The light and dark sides of nitric oxide: multifaceted roles of nitric oxide in plant responses to light. J Exp Bot 72: 885–903.

Lozano-Juste J, Colom-Moreno R, León J (2011) *In vivo* protein tyrosine nitration in *Arabidopsis thaliana*. J Exp Bot 62(10): 3501-3517.

Lubega J, Umbreen S, Loake GJ (2021) Recent advances in the regulation of plant immunity by *S*-nitrosylation. J Exp Bot 72: 864–872.

Forrester MT, Thompson JW, Foster MW, Nogueira L, Moseley MA, Stamler JS (2009) Proteomic analysis of *S*-nitrosylation and denitrosylation by resin- assisted capture. Nat Biotechnol 27: 557–559.

Marino SM, Gladyshev VN (2010) Structural analysis of cysteine *S*-nitrosylation: a modified acid-based motif and the emerging role of trans-nitrosylation. J Mol Biol 395: 844–859.

Méndez AAE, Mangialavori IC, Cabrera AV, Benavides MP, Vázquez-Ramos JM, Gallego SM (2020) Tyr-nitration in maize CDKA;1 results in lower affinity for ATP binding. Biochim Biophys Acta Proteins Proteom 1868, 140479; https://doi.org/10.1016/j.bbapap.2020.140479

Mitchell DA, Marletta MA (2005) Thioredoxin catalyzes the *S*-nitrosation of the caspase-3 active site cysteine. Nat Chem Biol 1: 154–158.

Mitchell DA, Morton SU, Fernhoff NB, Marletta MA (2007) Thioredoxin is required for *S*-nitrosation of procaspase-3 and the inhibition of apoptosis in Jurkat cells. Proc Natl Acad Sci U S A 104: 11609–11614.

Nakamura T, Wang L, Wong CC, Scott FL, Eckelman BP, Han X, Tzitzilonis C, Meng F, Gu Z, Holland EA, Clemente AT, Okamoto S, Salvesen GS, Riek R, Yates JR, Lipton SA (2010) Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death. Mol Cell 39: 184–195.

Nakamura T, Lipton SA (2013) Emerging role of protein-protein transnitrosylation in cell signaling pathways. Antiox Redox Signal 18: 239-249.

Naseer S, Ali RF, Fati SH, Muneer A (2021) iNitroY-Deep: Computational identification of nitrotyrosine sites to supplement carcinogenesis studies using deep learning. IEEAccess 9, 73624-73640; https://10.1109/ACCESS.2021.3080041

Ng JY, Boelen L, Wong JWH (2013) Bioinformatics analysis reveals biophysical and evolutionary insights into the 3-nitrotyrosine post-translational modification in the human proteome. Open Biol 3, 120148; https://doi.org/10.1098/rsob.120148

Nilamyani AN, Auliah FN, Moni MA, Shoombuatong W, Hasan MM, Kurata H (2021) PredNTS: Improved and robust prediction of nitrotyrosine sites by integrating multiple sequence features. Int J Mol Sci 22, 2704; https://doi.org/10.3390/ijms22052704

Pawloski JR, Hess DT, Stamler JS (2001) Export by red blood cells of nitric oxide bioactivity. Nature 409, 622–626.

Qu J, Nakamura T, Cao G, Holland EA, McKercher SR, Lipton SA (2011) *S*-Nitrosylation activates Cdk5 and contributes to synaptic spine loss induced by β-amyloid peptide. Proc Natl Acad Sci U S A 108: 14330–14335.

Qu Z, Meng F, Bomgarden RD, Viner RI, Li J, Rogers JC, Cheng J, Greenlief CM, Cui J, Lubahn DB, Sun GY, Gu Z (2014) Proteomic quantification and site-mapping of S-nitrosylated proteins using isobaric iodoTMT reagents. J Proteome Res 13: 3200–3211.

Radi R, Peluffo G, Alvarez MN, Naviliat M, Cayota A (2001) Unraveling peroxynitrite formation in biological systems. Free Radic Biol Med 30: 463–488.

Romero-Puertas MC, Laxa M, Matte A, Zaninotto F, Finkemeier I, Jones AME, Perazzolli M, Vandelle E, Dietz KJ, Delledonne M (2007) *S*-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell 19: 4120–4130.

Sabadashka M, Nagalievska M, Sybirna N (2021) Tyrosine nitration as a key event of signal transduction that regulates functional state of the cell. Cell Biol Int 45: 481-497.

Sainz M, Calvo-Begueria L, Pérez-Rontomé C, Wienkoop S, Abián J, Staudinger C, Bartesaghi S, Radi R, Becana M (2015) Leghemoglobin is nitrated in functional legume nodules in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent mechanism. Plant J 81: 723–735.

Sánchez-Vicente I, Fernández-Espinosa MG, Lorenzo O (2019) Nitric oxide molecular targets: reprogramming plant development upon stress. J Exp Bot 70: 4441–4460.

Santhanam L, Gucek M, Brown TR, Mansharamani M, Ryoo S, Lemmon CA, Romer L, Shoukas AA, Berkowitz DE, Cole RN (2008) Selective fluorescent labeling of *S*-nitrosothiols (S-FLOS): A novel method for studying *S*-nitrosation. Nitric Oxide 19: 295–302.

Santos AL, Lindner AB (2017) Protein posttranslational modifications: roles in aging and age-related disease. Oxid Med Cell Longev 2017, 5716409; https://doi.org/10.1155/2017/5716409

Serrato AJ, Romero-Puertas MC, Lázaro-Payo A, Sahrawy M (2018) Regulation by S-nitrosylation of the Calvin-Benson cycle fructose-1,6-bisphosphatase in *Pisum sativum.* Redox Biol 14: 409-416.

Seth D, Stamler JS (2011) The SNO-proteome: causation and classifications. Curr Opin Chem Biol 15: 129–136. https://doi.org/10.1016/j.cbpa.2010. 10.012

Siraj A, Chantsalnyam T, Tayara H, Chong KT (2021) RecSNO: Prediction of protein *S*-nitrosylation sites using a recurrent neural network. IEEE Access 9: 6674-6682.

Skelly MJ, Malik SI, Le Bihan T, Bo Y, Jiang J, Spoel SH, Loake GJ (2019) A role for *S*-nitrosylation of the SUMO-conjugating enzyme SCE1 in plant immunity. Proc Natl Acad Sci U S A 116: 17090–17095.

Smith BC, Marletta MA (2012) Mechanisms of *S*-nitrosothiol formation and selectivity in nitric oxide signaling. Curr Opin Chem Biol 16: 498–506.

Souza JM, Peluffo G, Radi R (2008) Protein tyrosine nitration- Functional alteration or just a biomarker? Free Rad Biol Med 45: 357-366.

Spadaro D, Yun BW, Spoel SH, Chu C, Wang YQ, Loake GJ (2010) The redox switch: dynamic regulation of protein function by cysteine modifications. Physiol Plant 138: 360–371.

Stamler JS, Toone EJ, Lipton SA, Sucher NJ (1997) (S)NO signals: translocation, regulation, and a consensus motif. Neuron 18: 691–696.

Stamler JS, Lamas S, Fang FC (2001) Nitrosylation. the prototypic redox-based signaling mechanism. Cell 106: 675–683.

Szabó C, Ischiropoulos H, Radi R (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 6: 662–80.

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X (2008) Plant immunity requires conformational changes of NPR1 *via* S-nitrosylation and thioredoxins. Science 321: 952–956.

Takahashi M, Shigeto J, Sakamoto A, Izumi S, Kozi A, Morikawa H (2015) Dual selective nitration in *Arabidopsis*: Almost exclusive nitration of PsbO and PsbP, and highly susceptible nitration of four non-PSII proteins, including peroxiredoxin II E. Electrophoresis 36: 2569-2578.

Takahashi M, Morikawa H (2019) Nitrogen dioxide at ambient concentrations induces nitration and degradation of PYR/PYL/RCAR receptors to stimulate plant growth: a hypothetical model. Plants 8, 198; https://doi.org/10.3390/plants8070198

Taldone FS, Tummala M, Goldstein EJ, Ryzhov V, Ravi K, Black SM (2005) Studying the *S*-nitrosylation of model peptides and eNOS protein by mass spectrometry. Nitric Oxide 13: 176–187.

Tanou G, Filippou P, Belghazi M, Job D, Diamantidis G, Fotopoulos V, Molassiotis A (2012) Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. Plant J 72: 585-599.

Tavares CP, Vernal J, Delena RA, Lamattina L, Cassia R, Terenzi H (2014) S-nitrosylation influences the structure and DNA binding activity of AtMYB30 transcription factor from *Arabidopsis thaliana*. Biochim Biophys Acta 1844: 810–817.

Terrile MC, Paris R, Calderon-Villalobos LI, Iglesias MJ, Lamattina L, et al. (2012) Nitric oxide influences auxin signaling through *S*-nitrosylation of the *Arabidopsis* TRANSPORT INHIBITOR RESPONSE 1 auxin receptor. Plant J 70: 492–500.

Torta F, Usuelli V, Malgaroli A, Bachi A (2008) Proteomic analysis of protein *S*-nitrosylation. Proteomics 8: 4484–4494.

Tsikas D, Duncan MW (2013) Mass spectrometry and 3-nitrotyrosine: Strategies, controversies, and our current perspective. Mass Spec Rev 33: 237-276.

van der Linde K, Gutsche N, Leffers HM, Lindermayr C, Muller B, Holtgrefe S, Scheibe R (2011) Regulation of plant cytosolic aldolase functions by redox-modifications. Plant Physiol Biochem 49: 946–957.

Van Meeteren U, Kaiser E, Malcolm Matamoros P, Verdonk JC, Aliniaeifard S (2020) Is nitric oxide a critical key factor in ABA-induced stomatal closure? J Exp Bot 71: 399–410.

Virág D, Dalmadi-Kiss B, Vékey K, Drahos L, Klebovich I, Antal I, Ludányi K (2020) Current trends in the analysis of post-translational modifications. Chromatographia 83: 1–10.

Vu LD, Gevaert K, De Smet I (2018) Protein language: post-translational modifications talking to each other. Trend Plant Sci 23: 1068-1080.

Wang YQ, Feechan A, Yun BW, Shafiei R, Hofmann A, Taylor P, Xue P, Yang FQ, Xie ZS, Pallas JA, Chu CC, Loake GJ (2009) *S*-nitrosylation of AtSABP3 antagonizes the expression of plant immunity. J Biol Chem 284: 2131–2137.

Wang H, Xian M (2011) Chemical methods to detect *S*-nitrosation. Curr Opin Chem Biol 15: 32–37.

Wang P, Du Y, Hou YH, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK (2015) Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1. Proc Natl Acad Sci U S A 112: 613-618.

Wihinen M (2012) How to evaluate performance of prediction methods? Measures and their interpretation in variation effect analysis. BMC Genomics 13, S2; https://doi.org/10.1186/1471-2164-13-S4-S2

Wu C, Liu T, Chen W, Oka S, Fu C, Jain MR, Parrott AM, Baykal AT, Sadoshima J, Li H (2010) Redox regulatory mechanism of transnitrosylation by thioredoxin. Mol Cell Proteom 9: 2262–2275.

Xie Y, Luo X, Li Y, Chen L, Ma W, Huang J, Cui J, Zhao Y, Xue Y, Zuo Z, Ren J (2018) DeepNitro: prediction of protein nitration and nitrosylation sites by deep learning. Genom Proteom Bioinf 16: 294-306.

Xu Y, Ding J, Wu LY, Chou KC (2013a) iSNO-PseAAC: predict cysteine *S*-nitrosylation sites in proteins by incorporating position specific amino acid propensity into pseudo amino acid composition. PLoS One 8, e55844; https://doi.org/10.1371/journal.pone.0055844

Xu Y, Shao XJ, Wu LY, Deng NY, Chou KC (2013b) iSNO-AAPair: incorporating amino acid pairwise coupling into PseAAC for predicting cysteine *S*-nitrosylation sites in proteins. Peer J 1, e171; https://doi.org/10.7717/peerj.171

Xu Y, Wen X, Wen L-S, Wu L-Y, Deng N-Y, Chou K-C (2014) iNitro-Tyr: prediction of nitrotyrosine sites in proteins with general pseudo amino acid composition. PLoS ONE 9, e105018; https://doi.org10.1371/journal.pone.0105018

Xue Y, Liu Z, Gao X, Jin C, Wen L, Yao X, Ren J (2010) GPS-SNO: computational prediction of protein S-nitrosylation sites with a modified GPS algorithm. PLoS One 5, e11290; https://doi.org/10.1371/journal.pone.0011290

Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou JM, Zuo J (2015) S-nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. Plant Physiol 167: 1604–1615.

Yeo W-S, Kim YJ, Kabir MH, Kang JW, Kim KP (2015) Mass spectrometric analysis of protein tyrosine nitration in aging and neurodegenerative diseases. Mass Spectrom Rev 34: 166-183

Ytterberg AJ, Jensen ON (2010) Modification-specific proteomics in plant biology. J Proteom 73: 2249–2266.

Yun BW, Feechan A, Yin M, Saidi NB, Le Bihan T, Yu M, Moore JW, Kang JG, Kwon E, Spoel SH, Pallas JA, Loake GJ (2011) *S*-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature 478: 264–268.

Zaffagnini M, Morisse S, Bedhomme M, Marchand CH, Festa M, Rouhier N, Lemaire SD, Trost P (2013) Mechanisms of nitrosylation and denitrosylation of cytoplasmic glyceraldehyde-3-phosphate dehydrogenase from *Arabidopsis thaliana*. J Biol Chem 288: 22777-22789.

Zhan N, Wang C, Chen L, Yang H, Feng J, Gong X, Ren B, Wu R, Mu J, Li Y, Liu Z, Zhou Y, Peng J, Wang K, Huang X, Xiao S, Zuo J (2018) S-nitrosylation targets GSNO reductase for selective autophagy during hypoxia responses in plants. Mol Cell 71: 142-154.

Zhang J, Zhao X, Sun P, Ma Z (2014) PSNO: predicting cysteine S-nitrosylation sites by incorporating various sequence-derived features into the general form of Chou's PseAAC. Int J Mol Sci 15: 11204-11219.

Contributions:

ZsK: Conceptualization; Analyses (Bioinformatics); Writing - original draft; Writing - review & editing.

CL: Conceptualization; Analyses (Bioinformatics); Writing - original draft; Writing - review & editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: